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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 39th Annual Meeting of the Society of Toxicology, held at the Pennsylvania Convention Center, Philadelphia, Pennsylvania, March 19-23, 2000.

An alphabetical Author Index, cross referencing the corresponding abstract number (2), begins on page 423.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 448.

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1 SUNRISE MINI-COURSE: INTRODUCTION TO PROTEOMICS.

D. C. Liebler. *University of Arizona, Tucson, AZ.*

The proteome consists of all of the expressed proteins in any particular cell and is thus the functional expression of the genome. Research in proteomics focuses on the expression of proteins and their post-translational modification by endogenous or exogenous agents. Proteomics approaches open new avenues to investigate mechanisms by which environmental chemicals affect living systems through identification of specific protein targets and characterization of effects on cellular protein expression and post-translational modification. Modern proteomics and genomics approaches offer an ability to observe changes in whole pathways and signalling networks as opposed to individual genes and proteins that code for single, cellular components. This short, basic course will introduce participants to methods used in proteomics, peptide sequence analysis and identification of proteins using databases. Applications of the techniques will also be discussed. This course should be useful to all toxicologists involved in mechanistic research.

2 SUNRISE MINI-COURSE: METABONOMICS—A NEW APPROACH TO DRUG TOXICITY SCREENING USING NMR SPECTROSCOPY, PATTERN RECOGNITION, AND EXPERT SYSTEMS.

D. G. Robertson[1] and J. K. Nicholson[2]. [1]*Parke-Davis Pharmaceutical Research, Ann Arbor, MI and* [2]*Imperial College, University of London, London, United Kingdom.*

The combination of NMR with computer pattern recognition, expert systems and other bioinformatic tools has given rise to a new "Metabonomics" approach to toxicological assessment. Metabonomics is complementary to genomic and proteomic approaches and also gives new biomarker or surrogate marker information on toxic effects of drugs, as well as providing insights into toxic mechanisms. New high throughput flow injection technology and expert system spectrometer control allows high throughput of biofluids for *in vivo* toxicological assessment at a much lower overall cost than other "rival" high technology screening tools. NMR spectroscopy of biofluids and intact tissues can be used to provide detailed biochemical information on the functional integrity of organisms following challenges with xenobiotics or other pathophysiological perturbations. This basic course on NMR spectroscopy and expert systems, and its application to toxicology, should appeal to those with interests in mechanistic problems, high throughput *in vivo* screening, and lead candidate selection.

3 ENVIRONMENTAL EPIDEMIOLOGY AND TOXICOLOGY: THE INTERFACE AND THE INTERACTIONS.

R. A. Parent[1], C. Schonwalder[2], I. Hertz-Picciotto[3], M. Longnecker[2], G. Matanoski[4] and R. Santella[5]. [1]*Consultox, Limited, Damariscotta, ME,* [2]*NIEH, Research Triangle Park, NC,* [3]*University of North Carolina School of Public Health, Chapel Hill, NC,* [4]*Johns Hopkins, School of Public Health, Baltimore, MD and* [5]*Columbia University, New York, NY.*

Assessing human health hazards from environmental exposures is the common goal of both epidemiology and toxicology. Few investigations have successfully blended these disciplines. Knowledge and understanding of exposures, associations with effects and mechanisms of toxicity are the underpinning of risk assessment and risk management. This course will outline several perspectives on the need for multidisciplinary cooperation between toxicologists and epidemiologists. The goal is to help toxicologists understand how their biological science can provide valuable tools for human population studies of health effects of environmental exposures. Environmental epidemiology basics will be presented and integrated with concepts and examples of how biologic understanding can strengthen the search for both association of exposure and disease and proof of cause/effect. Lectures will demonstrate the use of biomarkers of exposure and effect in epidemiology; the integration of toxicological understanding into epidemiological study design; and the importance of understanding exposure and dose/response. Participants will be better able to work with those planning population studies for chemical exposure risk discovery by understanding study design, exposure evaluation and outcome analysis.

4 PULMONARY IMMUNOTOXICOLOGY.

M. D. Cohen[1], J. T. Zelikoff[1], G. L. Finch[2], M. W. Frampton[3], M. H. Karol[4], R. B. Schlesinger[1] and R. L. Sherwood[5]. [1]*New York University School of Medicine, New York, NY,* [2]*Lovelace Respiratory Research Institute, Albuquerque, NM,* [3]*University of Rochester Medical Center, Rochester, NY,* [4]*University of Pittsburgh, Pittsburgh, PA and* [5]*IIT Research Institute, Chicago, IL.*

Pulmonary immunotoxicology has been active over the past decade in seeking to elucidate how environmental and workplace agents can modify immune function in the lungs so as to allow for indirect alterations in respiratory health and, subsequently, overall host health. This course will review recent advances in pulmonary immunotoxicology for investigators in the field as well as to those about to enter it. The first speaker will describe normal respiratory system structure, function, and immunology, as well as disposition of inhaled particles/gases. The second presenter will describe the major pathologies which may arise from immunomodulation. Each remaining presentation will deal with a major class of pulmonary immunomodulating agents and will provide an overview of exposure, mechanisms by which immunomodulation evolves, and the potential risk to human health from exposure to each class of agents. Because of the wide scope of agents to be discussed, this CE course will not only be of great interest to researchers in the fields of pulmonary toxicology and immunotoxicology, but it will be informative to scientists involved in regulatory matters and to those doing research in food safety, neurotoxicology, metals toxicology, and mechanisms.

5 MOLECULAR GENETICS, METABOLISM AND CELL SIGNALING IN RENAL CARCINOGENESIS: A LESSON IN SYNERGISTIC TOXICOLOGY.

M. A. Davis[1], G. Hard[2], S. S. Lau[3] and C. Walker[4]. [1]*University of Maryland, Baltimore, MD,* [2]*American Health Foundation, Vahalla, NY,* [3]*University of Texas, Austin, TX and* [4]*UTMD Anderson Cancer Center, Smithville, TX.*

Renal carcinogenesis provides an example of the synergetic relationship between metabolism, genetics, proliferation and cell death in mediating renal toxicity. The objective of this basic course is to provide a review of cellular responses and other physiologically relevant aspects of the kidney that are important in mediating renal carcinogenesis. The presentations will provide the attendees with a useful review of several currently emerging topics that are being integrated in mechanistic investigations. The information provided in this course will be of general use to individuals involved in safety evaluations, risk assessment and those that examine mechanisms of renal toxicity.

6 MOLECULAR APPROACHES TO A COMPREHENSIVE UNDERSTANDING OF CARDIOTOXICITY.

Y. J. Kang[1], J. P. Kehrer[2], J. Robbins[3] and K. B. Wallace[4]. [1]*University of Louisville, Louisville, KY,* [2]*University of Texas, Austin, TX,* [3]*University of Cincinnati, Cincinnati, OH and* [4]*University of Minnesota, Duluth, MN.*

Cardiotoxicity is a major environmental health problem and an important complication of clinical applications of a diversity of drugs including anticancer chemotherapeutics. However, we toxicologists have not explored this field to the same extent as we have in studying the toxicology of the liver, lungs, kidneys, or brain. As a result, cardiac toxicology research is extremely under-represented in the discipline of toxicology. Advances in molecular biology of the heart have provided tremendous opportunities to study molecular mechanisms of cardiotoxicity. Such studies are increasingly important for the molecular era of cardiac medicine. For example, myocardial gene therapy is likely to soon be in phase 1 trials and its associated cardiotoxicity must be studied. It is time for toxicologists to become more involved in this exciting field and to make our contributions to the advanced understanding of cardiovascular diseases. This CE course will provide: (1) an overview of toxic events in the heart; (2) a comprehensive discussion of myocardial energy metabolism and oxidative injury and the role of apoptosis in cardiotoxicity; (3) an overview of the most exciting advances in molecular biology of the heart; and, (4) a discussion of the molecular tools used to dissect cellular and molecular mechanisms of cardiotoxicity. The ultimate goal of this CE course is to promote cardiac toxicology research and teaching within our discipline.

7 ADVANCED NEUROTOXICOLOGY: BIOMARKERS AND MECHANISMS OF OXIDATIVE STRESS-INDUCED NEUROTOXICITY.

W. Slikker, Jr.[1], T. Guilarte[2], D. Cory-Slechta[3], S. G. Orrenius[4], T. Narahashi[5] and A. C. Scallet[1]. [1]*National Center for Toxicological Research, Jefferson, AR,* [2]*Johns Hopkins University, Baltimore, MD,* [3]*University of Rochester, Rochester, NY,* [4]*Karolinska Institute, Stockholm, Sweden and* [5]*Northwestern University, Chicago, IL.*

This advanced course will focus on oxidative stress-induced neurotoxicity and the several multidisciplinary approaches used to define this important mechanism of action of neurotoxicants. Researchers in the field of neurotoxicology rely on neuropathological, behavioral, neurochemical/molecular biological and electrophysiological approaches to define both the effects and mechanisms of neurotoxicity. The use of the multidisciplinary approaches to define a toxicological profile for an agent or class of agents is both a challenge and advantage to the practicing neurotoxicologist. The underlying theory and necessary techniques to define necrotic and apoptotic neural cell death will be presented. The behavioral consequences and neurochemical/molecular biological underpinnings of oxidative stress-induced toxicity will also be described. Toxicologists from academia, government and industry will all benefit from this in-depth description of oxidative stress-induced neurotoxicity and the multidisciplinary approaches used to unravel its mechanism.

8 RODENT TOXICITY AND NONGENOTOXIC CARCINOGENESIS: KNOWLEDGE-BASED HUMAN RISK ASSESSMENT FROM MOLECULAR MECHANISMS.

R. A. Roberts[1], W. H. Farland[2], T. Dalton[3], J. I. Goodman[4] and H. Shertzer[3]. [1]*AstraZeneca Ltd., Macclesfield, United Kingdom,* [2]*USEPA, Washington, DC,* [3]*University of Cincinnati, Cincinnati, OH and* [4]*Michigan State University, East Lansing, MI.*

It is necessary to determine whether chemicals or drugs have the potential to pose a threat to human health. Chemicals that can damage DNA are detected in short-term assays but the detection of nongenotoxic carcinogens relies upon bioassays in laboratory animals. However, there are marked rodent-human species differences in response to nongenotoxic carcinogens, questioning the relevance of rodent data for human risk assessment. This course aims to provide useful background on rodent nongenotoxic carcinogenesis, then to illustrate, by example, how knowledge of the molecular mechanisms of rodent nongenotoxic carcinogenesis coupled with an understanding of species differences can assist in knowledge-based human risk assessment. The ability of nongenotoxic carcinogens to cause oxidative stress, induce cell proliferation, suppress apoptosis and activate nuclear receptors will be discussed. Finally, consideration will be given to incorporating mechanism-based information into risk assessment for regulatory purposes.

9 ADVANCES IN NON-INVASIVE MICROMETER AND NANOMETER SCALE CELLULAR/TISSUE VITAL IMAGING.

R. C. Burghardt[1], M. A. Philbert[2], R. Barhoumi[1], D. Kawahara[3], C. R. Meyer[2] and D. Piston[4]. [1]*Texas A&M University, College Station, TX,* [2]*University of Michigan, Ann Arbor, MI,* [3]*Xenogen Corporation, Alameda, CA and* [4]*Vanderbilt University, Nashville, TN.*

Non-invasive, real-time imaging tools employing nano-optical/magnetic biosensors and biomarkers to define the function of living cells are among the most significant emerging technologies in the life sciences for toxicology applications. This basic course will identify these new technologies and provide examples of applications ranging from *in vivo* toxicokinetic monitoring in intact living cells to the detection of gene expression *in vivo* to increase understanding of the mechanisms of cellular toxicity. Speaker 1 will discuss nano-optochemical sensors that can be used to assess metabolic substrates and ions within cells following toxicant perturbation. Speaker 2 will discuss the real time analysis of cellular responses in cultured cells and describe instrumentation developed to integrate data acquisition and analysis of toxicant effects on Ca^{2+}-mediated signal transduction. Speaker 3 will discuss a significant microscopy advance, multi-photon microscopy, which uses ultrafast infrared laser pulses to image fluorescence signals for the study of sub-cellular responses within living tissue. Speaker 4 will describe new technology using a non-invasive, real-time imaging system to monitor intact living animals for specific gene induction by compounds. Speaker 5 will discuss a mechanism to obtain experimental results with low variance by production of imaging-induced changes in single animals used as their own controls, including testing for changes on volumetric data sets such as autoradiography and MRI.

10 TIPS FOR EFFECTIVE RISK COMMUNICATION.

M. J. Miller[1], G. Gray[2], J. Bus[3], D. Hyman[4] and B. Shurdut[5]. [1]*Exxon Biomedical Sciences, Inc., Annandale, NJ,* [2]*Harvard School of Public Health, Boston, MA,* [3]*Dow Chemical Company, Midland, MI,* [4]*SOT Public Affairs, Reston, VA and* [5]*SOT Congessional Fellow, Washington, DC.*

This basic course provides members with a practical understanding of the importance and necessity of effectively communicating health and environmental risks. Effective risk communication is an essential part of the risk assessment/risk management process. Misguided perceptions of risk by the public can have significant contrary impacts on regulatory priorities, research initiatives, business development and public health. The first speaker will discuss the changing nature of risk communication and the importance of providing scientific information in context. The next speaker will provide specific tips on how to communicate risk-related issues to reporters and give an overview of the elements of a great news story. The third speaker will discuss the importance of communicating scientific issues to Congress, give an overview of the political process, and provide advice to members when visiting Congress. The last speaker will discuss the importance and most effective means of providing accurate risk-based information to the public and provide tips on available tools/resources to assist members in speaking to schools, local communities and special interest groups. A discussion panel including the speakers and invited guests from the press, Congress and USEPA will summarize the key messages from the course and field questions from the floor. The course should be of broad interest to members involved in risk assessment and regulatory issues.

11 ANTIBODIES AS REAGENTS TO EVALUATE TOXICANT-MEDIATED SIGNAL TRANSDUCTION PATHWAYS.

R. S. Pollenz[1], B. Abbott[2], G. H. Perdew[3] and T. R. Sutter[4]. [1]*Medical University of South Carolina, Charleston, SC,* [2]*USEPA, Research Triangle Park, NC,* [3]*Penn State University, State Park, PA and* [4]*University of Memphis, Memphis, TN.*

Signal transduction pathways mediate the effects of many toxicologically important compounds (i.e., dioxins, estrogens, peroxisome proliferators, etc.). To understand how these compounds mediate biological effects, it is necessary to understand the expression, distribution and concentration of the signaling proteins, their interactions, and the gene products they regulate. One of the most effective ways to carry out such analyses is with specific antibodies to each of the component proteins. This course will provide a spring board for beginning and established investigators to learn state-of-the-art techniques and strategies involved in the analysis of proteins both *in vivo* and *in vitro*. Completion of this course should benefit those interested in establishing the use of antibodies within the laboratory and will also provide enough detail to allow individuals to better critique studies that utilize antibody reagents. Topics to be addressed include: analysis of protein domain structure and design of antibody regents, production and purification of antigens and antibodies, use of enzyme activity and expression, immunoprecipitation to assess protein expression and interaction, use of protein tags, quantitative Western blotting, and immunocytochemical methods to assess protein expression and subcellular localization in tissues. Each session will be technique-oriented and presented in a problem solving format based on real experimental successes and failures.

12 PHOTOTOXICOLOGY: BASIS PRINCIPLES OF LIGHT, PHOTOBIOLOGY, AND REGULATORY ISSUES.

P. D. Forbes[1], P. C. Howard[2], F. G. Gerberick[3], A. C. Jacobs[4] and J. F. Nash[3]. [1]*Argus Research Laboratories, Inc., Horsham, PA,* [2]*National Center for Toxicological Research, Jefferson, AR,* [3]*The Procter & Gamble Company, Cincinnati, OH and* [4]*USFDA, Rockville, MD.*

The dermatotoxic and carcinogenic properties of solar light have been documented for several years. Increased leisure time in outdoor activities, use of tanning beds, and the use of drugs that are photoactivated have resulted in increased interest in phototoxicology. This basic course will assist investigators in understanding fundamental issues and techniques in phototoxicology. The first presentation will focus on light characteristics, sources, methods of

detection, quantification, and photochemistry. The reaction of biological systems to light will be the focus of the second and third presentations. Characteristics of photodynamic reactions, phototoxicity, photoirritation, photoallergy, photoaging, photogenotoxicity, photomutagenesis, and photocarcinogenesis will be presented. Regulatory issues in phototoxicology will be outlined including U.S., European, and ICH guidelines and the use of animal models, transgenic animals, and *in vitro* studies. The design of methods and protocol to address phototoxicology issues will be presented from both the regulatory and industrial positions. Toxicologists from industry, government and academia will benefit from exposure to the basic principles, biology and application of phototoxicology.

13 TOXICOKINETICS AND PHYSIOLOGICALLY-BASED TOXICOKINETICS IN TOXICOLOGY AND RISK ASSESSMENT.

R. Dixit[1], M. E. Andersen[2], K. Krishnan[3] and J. E. Riviere[4]. [1]*Merck & Co., Inc., West Point, PA,* [2]*Colorado State University, Fort Collins, CO,* [3]*University of Montreal, Medicine du Travail er Hygiene du Milieu, Montreal, Quebec, Canada and* [4]*North Carolina State University, Raleigh, NC.*

With the implementation of the International Conference on Harmonization (ICH) Guidelines on Dose Selection for Carcinogenicity Studies and Systemic Exposure, the conventional toxicokinetics (TK) assessment has become an integral part of safety assessment. Toxicokinetic models have received increasing application in dose selection, trans-species comparison of toxicity and in assessing human safety margins of pharmaceutical agents. Physiologically-based toxicokinetic models represent the kinetic uptake and disposition of chemicals using the rates of biochemical reactions, as well as physiological and anatomical characteristics. In the absence of human pharmacokinetics data, these models have been successfully used for extrapolation of toxicokinetics from high dose to low dose and from animals to humans. The objective of this basic course is to describe and compare conventional compartmental toxicokinetics models and physiologically-based toxicokinetics models. Emphasis will be placed on the application of these models in dose selection, ICH guidelines, interpretation of toxicity data and human health risk assessment.

14 METAL EXPOSURE AND TOXICITY OF THE RESPIRATORY TRACT.

D. L. Morgan[1], M. P. Waalkes[2], J. M. Benson[3], J. C. Bonner[1], S. H. Gavett[4] and T. Gordon[5]. [1]*NIEHS, Research Triangle Park, NC,* [2]*National Cancer Institute at National Institute of Environmental Health Sciences, Research Triangle Park, NC,* [3]*Lovelace Respiratory Research Institute, Albuquerque, NM,* [4]*USEPA, Research Triangle Park, NC and* [5]*New York University Medical Center, New York, NY.*

Environmental and occupational inhalation exposures to metal vapors, fumes, and particulates are associated with a number of diverse and debilitating respiratory diseases. The diverse nature of metal-induced pulmonary diseases is due in part to the wide range of physiochemical properties of metals and metal compounds. The molecular mechanisms by which metals cause pulmonary disease are not clear, but metals can induce a variety of effects ranging from aberrant gene expression, inappropriate signal transduction to enhanced or perturbed apoptosis. This course will focus on the mechanisms by which inhaled metals cause acute inflammatory lung diseases, such as metal fume fever, as well as acute/chronic respiratory diseases such as asthma pulmonary fibrosis, and cancer of the respiratory tract. This course will have wide appeal for investigators in the fields of metal and inhalation toxicology, mechanisms carcinogenesis, occupational medicine and immunotoxicology.

15 SAFETY PHARMACOLOGY AND RISK ASSESSMENT.

L. B. Kinter[1], A. S. Bass[2], J. L. Mattsson[3], D. J. Murphy[4] and R. D. Sarazan[5]. [1]*AstraZeneca, Ltd., Wayne, PA,* [2]*Pharmacia & Upjohn, Inc., Kalamazoo, MI,* [3]*Dow AgroScience, Indianapolis, IN,* [4]*SmithKline Beecham Pharmaceuticals, King of Prussia, PA and* [5]*Eli Lily & Company, Greenfield, IN.*

Safety Pharmacology (SP) identifies unanticipated drug effects on critical organ functions. These studies contribute directly to risk assessment/management in human volunteers/patients, particularly in the design of initial (Phase I) clinical trials. Recognizing these contributions, the International Conference on Harmonization (ICH) accepted SP in 1998 for standardization of international guidelines defining basic (core) evaluations and points to consider for design and conduct of SP studies. The instructors will focus upon the impact of the proposed ICH Safety Pharmacology guidelines for toxicologists, and will address in detail the likely core cardiovascular, respiratory, and central nervous system evaluations. The course will be of broad interest to both academic and industrial scientists engaged in animal research and will present a timely overview of safety pharmacology for toxicologists.

16 TOXICOGENOMICS IN THE TRENCHES.

T. R. Zacharewski[1], P. Saama[1], W. B. Mattes[2] and E. Nuwaysir[3]. [1]*Michigan State University, East Lansing, MI,* [2]*Pharmacia & Upjohn, Inc., Kalamazoo, MI and* [3]*NIEHS, Research Triangle Park, NC.*

Toxicogenomics has emerged as a new discipline that integrates genomics (the study of all genes encoded by an organism's DNA) and bioinformatics with toxicology to assess and elucidate the mechanisms of action of known and suspected toxicants. The suite of bioinformatics tools and resources that are essential for this research will be covered in the first talk. Hybridization of mRNA to hundreds of complementary cDNAs/expressed sequence tags (ESTs) provides a method to monitor the totality of gene expression following chemical exposure; design, generation and use of such arrays of cDNAs on membrane filters and glass slides will be the topic of the second and third talk, respectively. The final presentation, which is more advanced, will examine the difficult nature of analyzing the data generated from such experiments in a rigorous statistical fashion. This course will be of particular interest to those investigators who are considering incorporating genomic technologies into their research programs.

17 THE APPLICATION OF PHILOSOPHY TO RISK ASSESSMENT, MANAGEMENT AND COMMUNICATION.

M. Saner[1] and M. Gillis[2]. [1]*Consultancy for Environmental Science and Policy, Ottawa, Ontario, Canada and* [2]*Department of Philosophy, University of Florida, Jacksonville, FL. Sponsor:* J. T. Zelikoff.

The debate generated by public concerns over modern science and technology has become increasingly polarized between "hard-core scientists" and the "hysterical public." Such polarization threatens the autonomy of science, as well as the development of useful technology. The ability to clearly conceptualize the relation between science and values and to systematically express this distinction in a dialogue are the best ways to address this problem. This basic course, using the context of human and environmental toxicology, will help participants influence and effectively participate in this important debate through better understanding of environmental ethics, a discipline within applied philosophy providing a systematic account of the moral relations between human beings and our natural environment. It incorporates topics ranging from animal welfare to the justification of environmental protection goals. The goal of this course is to provide the participants with a balanced assessment of the role of environmental ethics within risk evaluation, management, and communication. Participants will be provided with an overview of the interface between science and values (ethics), an introduction into environmental ethical theories, and examples of application of this material to concrete issues within risk evaluation, management and communication.

18 HUMAN HEALTH AND ECOLOGICAL IMPACT OF HARMFUL ALGAL BLOOMS.

R. C. MacPhail[1] and P. S. Spencer[2]. [1]*USEPA, Research Triangle Park, NC and* [2]*Oregon Health Sciences University, Portland, OR.*

Harmful algal blooms (HABs) refer to a broad array of toxic organisms that can attack both wildlife and humans. HABs can be found in ponds, lakes, streams, estuaries and oceans. Interest in HABs research has been sparked by the observation that HAB events are increasing world-wide in both frequency and spatial extent, thereby increasing exposure potential and risks to vertebrate and invertebrate species alike. HABs can produce toxins, of remarkable molecular complexity, that can damage a number of organ systems. Moreover, many HABs produce neurotoxins whose mode of action is so specific they have proved to be valuable research tools to probe the nervous system and its functions. Other HAB toxins (e.g., *Pfiesteria spp.*), on the other hand, are still poorly understood. Most of what is known about HAB toxicity has been derived from acute poisoning episodes and/or laboratory exposures; the pos-

sibility of chronic effects resulting from acute, episodic or long-term exposure is largely a matter of speculation. This symposium will elaborate our current understanding of HABs, the toxins they produce, their target organs, and their mode(s) of action in both humans and wildlife.

19 BREVETOXINS ARE HUMAN NEUROTOXINS THAT ARE ACTIVE ORALLY, BY INJECTION, AND BY INHALATION.

D. G. Baden. *University of North Carolina, Wilmington, NC.*

The polyether brevetoxins (PbTx) produced by the marine dinoflagellate *Gymnodinium breve* are amongst the most potent non-proteinaceous neurotoxins known. They interact with site 5 of voltage-gated sodium channels in neurons and produce a spectrum of effects including a shift of the activation potential to more negative values, prolonged mean open time of sodium channels, inhibition of channel inactivation, and induction or visualization of multiple subconductance states. These effects would appear to account for the signs and symptoms associated with massive fish kills, neurotoxic shellfish poisoning in humans, and other wildlife illnesses associated with Florida red tides. The respiratory effects of brevetoxins are less clearly understood and appear to represent a series of toxicologic signs and symptoms that do not always align with specific sodium channel depolarization. Following a large manatee epizootic event in 1996 associated with major Florida red tide, a series of maladies associated with phagocyte accumulation of toxin, and toxic shock syndrome-like events, pointed to a newly recognized type of toxicity associated with blooms of *G. breve*. These effects, and their significance in the context of other recognized deleterious brevetoxin effects, indicate that red tide poisoning may produce long-lasting or chronic effects on wildlife and human health.

20 HUMAN-HEALTH AND ECOLOGICAL IMPACT OF CYANOBACTERIA.

W. W. Carmichael. *Wright State University, Dayton, OH.*

Cyanobacteria are freshwater organisms responsible for algal blooms in lakes, ponds, rivers and reservoirs. The increasing prevalence of cyanobacteria blooms is due to nutrient overload (eutrophication) of waters, climate change, and enhanced scientific awareness. Many cyanobacteria, including Anabena, Aphanizomenon, Microcystis and Oscillatoria, produce toxins that can threaten the health of many forms of wildlife and humans. Toxins such as saxitoxin and anatoxin-a are well-known for their neurotoxic effects in a variety of species. In addition, the neurotoxin anatoxin-a(s) is a naturally occurring organophosphate (OP) whose toxic effects resemble those produced by many commercial OP insecticides. Cyanobacteria also produce microcystins and nodularins which have been shown to be hepatotoxic as well as hepatic carcinogen promoters. In addition, a recently identified alkaloid hepatotoxin, cylindrospermopsin, has been found in several Florida lakes undergoing incresed eutropliction. Reports of toxic cyanobacterial blooms date from at least the last century, and have killed numerous livestock and domestic pets in addition to many wildlife species. Human toxicity produced by cyanobacterial toxins has long been suspected, but never confirmed. Recently, however, microcystins have been implicated in the death of at least 52 humans, and liver failure in exposed survivors, undergoing renal dialysis in Brazil. While the risks of acute exposure to cyanobacteria have been well established, growing evidence suggests chronic effects may also result from contact with cyanobacteria blooms.

21 DOMOIC ACID (DA): ENVIRONMENTAL EXPOSURES, NEUROTOXIC EFFECTS AND MECHANISMS OF SUSCEPTIBILITY.

J. S. Ramsdell. *National Ocean Service, Charleston, SC.*

Domoic acid is a tricarboxylic acid produced by certain species of the diatom genus *Pseudo-nitzchia*. It was the causative agent of the amnesic shellfish poisoning in 1987. Since that time the toxic algae has been determined to have a worldwide distribution. Domoic acid binds competitively to kainic and AMPA-subtype glutamate receptors, and leads to rapid elevation of cytosolic free calcium in hippocampal pyramidal cells, which requires activation of NMDA receptors. Domoic acid adminstered to mice causes a dose-dependent sequel of acute signs, including hyperactivity, stereotypic scratching, seizures and status epiletictus. Domoic acid also causes a does-dependent induction of the calcium-sensitive immediate response gene c-fos in brain tissue primarily in hippocampus, olfactory bulb and area postrema/nucleus solitarius. Long-term effects of domoic acid in mice include decreased performance in working memory in the absence of apparent structural damage. Neonate mice are forty times more sensitive than adults to domoic acid due to poor clearance of the toxin. Increased prevalence of toxic species of *Pseudo-nitzchia* suggests that incidents of exposure of wildlife and humans will likely continue to occur.

22 HUMAN-HEALTH AND ECOLOGICAL IMPACT OF *PFIESTERIA* TOXIN(S).

J. Burkholder. *NCSU, Raleigh, NC.*

Pfiesteria piscicida is a recently discovered toxic dynoflagellate found in estuaries in North Carolina and Maryland. Current evidence also suggest there may be multiple species of *Pfiesteria* found throughout estuaries in the Southeast and Gulf of Mexico. Research on *Pfiesteria* has provided strong linkages between environmental deterioration, fish kills (that are sometimes massive), and subtle but serious adverse effects on human health. *Pfiesteria* displays: (1) a strong attraction to live fish; (2) production of toxin(s) that cause fish distress, disease and death; and (3) toxicity triggered by the presence of live fish. Laboratory research has shown that *Pfiesteria* will kill numerous species of fish and bivalved mollusks. *Pfiesteria* is also remarkable in its complex life cycle, with multiple amoeboid and flagellated stages and rapid transitions between these stages. Progress in toxin isolation indicates a hydrophilic fraction destroys certain mammalian tissues and the nervous system of fish, and a lipophilic fraction that produces edema in fish. Human-health effects of *Pfiesteria* toxin(s), from contact with water or by inhalation, include irritation, nausea and headaches, erratic behavior, and learning impairments. Evidence also suggests some signs of toxicity in humans may persist for years after exposure.

23 MOLECULAR MECHANISMS OF CHEMICAL TERATOGENESIS.

P. G. Wells. *University of Toronto, Toronto, Ontario, Canada.*

Although 50 years have passed since the thalidomide tragedy left thousands of infants born with severe malformations, only recently have a variety of biochemical and molecular biological approaches begun to reveal the underlying mechanisms and risk factors for chemical teratogenesis. This symposium provides a timely update of four approaches which, while diverse in technical repertoire and mechanistic thrust, all focus upon teratological mechanisms in the conceptus, providing both corroborating results and novel insights. First is the role of reactive oxygen species and oxidative macromolecular damage in teratogenesis, and the contribution of embryonic antioxidative enzymes and DNA repair in modulating teratologic risk. The second investigates the teratological significance of alterations in gene expression and signal transduction pathways regulating conceptal cell cycle checkpoint activation, DNA repair and apoptosis. A subsequent approach focuses on the signaling pathways modulating apoptotic cell death in the developing embryo, and how their perturbation by xenobiotics may lead to teratogenesis. Finally, recent comprehensive techniques in molecular biology, including DNA microarrays, are used to examine the complex pattern of changing gene expression in developing embryos, and the role that xenobiotic-initiated alterations in these expression patterns may play in teratogenesis.

24 REACTIVE OXYGEN SPECIES AND OXIDATIVE DAMAGE.

R. R. Laposa, P. G. Wells, C. J. Nicol and T. Parman. *University of Toronto. Ontario, Canada.*

We examined xenobiotic bioactivation and free radical-initiated, reactive oxygen species (ROS)-mediated oxidative molecular target damage, and DNA repair in mouse and rabbit models. Phenytoin and related teratogens were bioactivated by purified prostaglandin H synthases (PHSs) to free radical intermediates identified by electron spin resonance spectrometry. PHS-2 was high in mouse embryos, PHS-2 knockout mice were resistant to phenytoin teratogenicity, and PHS inhibitors (aspirin, etc.) reduced teratogenicity (phenytoin, thalidomide). Bioactivation, DNA/protein oxidation and embryotoxicity occurred in embryo culture (benzo[a]pyrene (B[a]P), phenytoin), indicating embryonic determinants. Treatment with antioxidative enzymes (superoxide dismutase (SOD), catalase) increased embryonic activities, blocked DNA oxidation and inhibited teratogenicity (phenytoin, B[a]P). Phenytoin- and

thalidomide-initiated oxidation of embryonic cellular macromolecules, glutathione (GSH) and teratogenicity were reduced by the free radical trapping agent phenylbutylnitrone and/or antioxidants (caffeic acid, vitamin E). ROS involvement *in vivo* was further implicated by hydroxyl radical formation (3-hydroxylation of salicylate), and increased teratologic susceptibility with mice deficient in glucose-6-phosphate dehydrogenase, or inhibition of GSH peroxidase or GSH reductase. PHS inhibitors or SOD inhibited micronucleus formation initiated in cultured fibroblasts by B[a]P and phenytoin. DNA may be an important target, since p53 knockout mice with deficient DNA repair were more susceptible to phenytoin and B[a]P embryotoxicity. (Support: Medical Research Council of Canada).

25 SIGNAL TRANSDUCTION PATHWAYS IN THE CONCEPTUS.

B. F. Hales and T. R. S. Ozolins. *McGill University, Montreal, Quebec, Canada.* Sponsor: P. G. Wells.

The signal transduction pathways mediating insult to the conceptus by most teratogens are unknown. The consequences of teratogen exposure depend on the ability of the embryo to respond as well as on the nature of the insult. Complex mechanisms, ranging from alterations in gene expression to DNA repair, cell cycle checkpoint activation, or apoptosis, detect an insult and trigger an embryo-protective response. Many stressors alter gene expression by perturbing regulation of the immediate early gene activator protein-1 (AP-1) transcription factor. To test the hypothesis that AP-1 is a significant determinant of embryonic susceptibility to insult, we investigated the regulation of AP-1 activity in rat embryos during organogenesis. AP-1 DNA binding activity is induced during transient oxidative stress in rat embryos *in vitro*. Supershift assays indicate that basal AP-1 binding in the embryo is due primarily to JunD, while in the yolk sac both c-Jun and JunD are important. Under oxidative stress, c-Fos and c-Jun contribute to AP-1 binding. Oxidative stress-induced changes in AP-1 activity in embryos are not due to activation of the extracellular signal-regulated kinases1,2 (ERKs-1,2) or stress activated protein kinases (SAPKs). Interestingly, UV irradiation and heat shock stimulate SAPKs in the yolk sac, but only heat shock is effective in the embryo, suggesting that SAPK pathways are poorly developed. Immunodepletion of redox factor 1 (Ref-1), a nuclear factor that promotes AP-1 binding, eliminates AP-1 activity from embryonic nuclear extracts. Therefore, in the conceptus, where AP-1 phosphorylation changes are not apparent, Ref-1 is a critical determinant of AP-1 activity. These data suggest that Ref-1 plays a pivotal role in the embryonic stress response. (Supported by the MRC of Canada.)

26 APOPTOTIC SIGNALING PATHWAYS.

P. E. Mirkes. *University of Washington, Seattle, WA.* Sponsor: P. G. Wells.

It is well recognized that environmental agents capable of disrupting normal development (i.e., teratogens) often induce excessive cell death as an early event in teratogenesis. Teratogen-induced cell death is also specific, i.e., some cells die while the majority survive, and those that die often are associated with areas of programmed cell death. I will present data showing that 4 different developmental toxicants, hyperthermia (43°C), cyclophosphamide (DNA alkylating agent), sodium arsenite (heavy metal), and staurosporine (protein kinase c inhibitor), induce activation of caspase-3, cleavage of poly (ADP-ribose) polymerase (PARP), and internucleosomal DNA fragmentation. These same agents also induce the release of cytochrome c from mitochondria coincident with the activation of caspase-3. These results suggest mitochondria serve as targets for these developmental toxicants. We have also shown that cells of the heart from early postimpalntation mouse embryos are completely resistant to the apoptotic stimulus initiated by these 4 developmental toxicants. The failure to undergo cell death in response to exposure to these teratogens is associated with a failure to release cytochrome c as well as the downstream events such as caspase-3 activation, PARP cleavage and DNA fragmentation. My laboratory has also begun to probe known signal transduction pathways in an effort to understand the mechanisms by which developmental toxicants trigger apoptosis in early postimplantation murine embryos. We have now shown that hyperthermia rapidly, but transiently, activates the mitogen-activated protein kinases (MAP Kinases) belonging to the ERK, JNK, and p38 pathways. The activation of the ERK and JNK pathways precedes by several hours the activation of caspase-3; therefore, these signaling pathways may be part of the upstream signaling events required for the activation of the apoptotic pathway. In contrast, cyclophosphamide and staurosporine activate the p38 but fail to activate the ERK, JNK pathways. Thus, activation of MAP kinase pathways is teratogen-specific.

27 ALTERED GENE EXPRESSION PATTERNS TO PREDICT AND UNDERSTAND CHEMICAL TERATOGENESIS.

R. Finnell, G. D. Bennett and J. G. van Waes. *University of Nebraska Medical Center, Omaha, NE.* Sponsor: P. G. Wells.

The Human Genome Project is progressing towards completion, providing a wealth of information in the form of expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs) and physically mapped genes to the research community. As a result, toxicologists are now able to incorporate this information into their research programs, enabling them to easily efficiently perform studies that were once regarded as possible only by molecular geneticists. These new technologies have created the situation where many long sought after interactions between environmental toxicants and their gene targets can finally be addressed. Perhaps the most important development is the existence of expression chips or DNA microarrays. With these DNA chips, investigators can detect and simultaneously monitor the expression of ordered sets of hundreds or thousands of genes from small biological samples. The application of these experimental approaches to understanding the mechanisms underlying teratogen-induced congenital defects will be the focus of this presentation. Experimental examples will include both pharmaceutical compounds (valproic acid and phenytoin) and environmental contaminants (arsenic). Specific target genes include: cell cycle checkpoint genes, folate biosynthesis pathway genes, and selected transcription factors. Efforts to understand the implications of the gene expression data will be fully explored, including experimental approaches to validate gene expression observations.

28 ARE THERE AUTOIMMUNE CONSEQUENCES OF TOXICANT EXPOSURE IN HUMAN POPULATIONS?

K. E. Rodgers[1] and M. A. Lynes[2]. [1]*University of Southern California, Los Angeles, CA and* [2]*University of Connecticut, Storrs, CT.*

Numerous studies have addressed the influence of environmental toxicants, medical devices, and ethical pharmaceuticals on the potentiation, initiation, and/or exacerbation of human autoimmune disease. While some pharmaceuticals have been shown to contribute to autoimmune-like disease that may be reversed when exposure to the drug is removed, the ability of environmental toxicants or medical devices to contribute to autoimmune disease is still a matter of considerable debate. Animal models of autoimmune disease have been successfully used as screens for the contribution of toxicants to the development of disease and to explore specific pathogenic mechanisms, but human epidemiological studies have been less successful in connecting toxicant exposure with autoimmune disease. This difficulty may arise from several facts. First, individual human autoimmune diseases are relatively rare, making the association of toxicant exposure with a specific disease more difficult. Symptoms associated with autoimmune disease also can vary widely between individuals, making diagnosis of a specific autoimmune disease less certain. Moreover, a variety of potential susceptibility genes segregate in the human population, adding an additional variable to this analysis. Finally, it is difficult to establish cohorts with consistent exposure for epidemiological evaluation. Panelists will address these issues from a variety of standpoints, providing insights into the methodologies used to assess these diseases, the types of ongoing studies that address elements of the issues described above, and the prospects for resolving these important areas of investigation. This roundtable should provide research scientists, clinicians, and epidemiologists with an opportunity to consider a range of issues related to toxicant-induced autoimmunity.

29 AUTOIMMUNE DISEASE AND ENVIRONMENTAL CHEMICALS.

N. R. Rose. *Johns Hopkins University, Baltimore, MD.* Sponsor: K. E. Rodgers.

Autoimmune disease results from an attack by the immune system on the host's own tissues. All of the human autoimmune disease show evidence of genetic predisposition. A number of heritable traits contribute to heightened susceptibility, but he best defined genes are members of the major histocompatibility complex, HLA. In toto, however, genetic predisposition accounts for less than half of the risk of developing and autoimmune disease. The remaining risk may reside in two factors. First are somatic variations in the immune system itself. Both T-cell and B-cell receptors are highly diverse in order to accommodate the large number of potential antigens. Second, external factors may contribute to the risk of developing an autoimmune disease. Infection and certain drugs are the most studied examples in human autoimmune disease, but circumstantial evidence and animal experiments have

implicated a number of environmental chemicals, such as mercury. The issue is in great need of careful epidemologic studies as well as experimental investigations of plausible cause-and-effect relationships between specific chemical and particular autoimmune disorders.

30 CONSEQUENCES OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES IN THE DEVELOPMENT OF AUTOIMMUNE DISEASES.

D. M. Ozonoff. *Boston University, Boston, MA*. Sponsor: K. E. Rodgers.

It is well known that chemical agents (e.g., therapeutic drugs) can induce autoimmune disorders ("drug-induced lupus"). In addition, several workplace agents, especially organic solvents, have been implicated in autoimmune disorders (Goodpature's syndrome, Sevens-Johns syndrome, systemic sclerosis). This Roundtable presentation will discuss the extent to which we might expect autoimmune disorders to result from environmental/occupational exposures to chemical agents. The common chlorinated ethylenes trichloroethylene and percholorethylene (TCE and PCE) will be used as examples.

31 CRITICAL ISSUES FOR THE EVALUATION OF ENVIRONMENTALLY-RELATED AUTOIMMUNE DISEASES.

G. S. Cooper. *NIEHS, Research Triangle Park, NC*. Sponsor: K. E. Rodgers.

Some data from experimental and epidemiologic studies indicate that specific environmental exposures may contribute to the development of autoimmune diseases. However, the evidence for the most part can be described as somewhere between "scant" and "suggestive". Very few studies have been conducted, making it impossible to evaluate consistency between studies. First reports, even if they come from a well-conceived, well-conducted study, are often not confirmed by subsequent research. Another limitation is the difficulty in extrapolating from the high exposures typically found in certain occupational settings to the lower exposures that are more common in the general population. Although there is interest in silica dust as a cause of autoimmune disease, techniques for accurately assessing low-to-moderate levels of silica exposure, including likely sources of exposure in women, have not yet been validated and used in epidemiologic studies. A third limitation is the difficulty in generalizing across diseases: under what conditions or to what extent is what we learn about environmental associations with scleroderma applicable to systemic lupus erythematosus or rheumatoid arthritis or multiple sclerosis? Examples from the current literature will be discussed. It is important to build a case for the contribution of environmental or occupational exposures to the development or progression of autoimmune diseases, but this goal will be ill-served by premature judgements and by small, poorly-designed studies.

32 CHEMOPREVENTION OF TOBACCO SMOKE-INDUCED LUNG CANCER.

I. E. Espiritu, D. L. Uyeminami and H. P. Witschi. *ITEH, University of California, Davis, CA*.

In a series of experiments we investigated whether chemopreventive agents could inhibit the development of lung tumors in mice exposed to tobacco smoke. Male strain A/J mice were fed two chemopreventive agents: D-limonene at a concentration of 6.3 g/kg and XSC (1,4-phenylenebis (methylene) selenoisocyanate) at 20 mg/kg. They were exposed, 6 hours a day, 5 days a week for 5 months to a mixture of cigarette mainstream smoke (11%) and sidestream smoke (89%) at a concentration of 137 mg/m^3 total suspended particulate matter. After removing from smoke, they were allowed a 4 month recovery period in air before lung surface tumors were counted and multiplicity and incidence calculated. Animals exposed to tobacco smoke alone had an average of 2.8 tumors per lung, animals fed D-limonene 2.6 and animals fed XSC 2.4; the differences were statistically not significant. In a positive control experiment, animals injected with NNK had an average of 13 tumors/lung, animals fed D-limonene 7.8 and XSC 2.8 (differences highly significant), thus showing that the chemopreventive agents were effective against NNK. In addition to D-limonene and XSC, we have thus far evaluated the chemopreventive actions of green tea, phenethyl isothiocyanate (PEITC), aspirin, N-acetylcysteine and myo-inositol/dexamethasone. Of all these agents, only myo-inositol/dexamethasone successfully prevented smoke-induced lung tumor development. Chemopreventive agents effective against individual smoke constituents may thus often be not effective when evaluated against the real and complex mixture of full tobacco smoke (Supported by ES07908.)

33 A DIETARY ANTIOXIDANT PARADOXICALLY REDUCES TUMOR NUMBER BUT INCREASES MALIGNANCIES IN TRANSGENIC MICE TREATED TOPICALLY WITH BENZO(A)PYRENE.

K. R. Martin, C. Trempus, M. Saulnier, F. W. Kari, J. C. Barrett and J. E. French. *NIEHS/NIH, Research Triangle Park, NC*.

Epidemiological studies support the protective role of dietary antioxidants in preventing cancer. However, emerging evidence suggests that dietary antioxidants may also exacerbate carcinogenesis. To test this hypothesis in a model that possesses genotypic characteristics of human cancer, we selected the FVB/N Trp53 haploinsufficient Tg.AC (v-Ha-ras) mouse as a short-term cancer bioassay model, because it contains an activated, carcinogen-inducible ras oncogene and an inactivated p53 tumor suppressor gene, which are frequent genetic alterations in human cancers. These mice rapidly develop chemically-induced benign and fatal malignant skin tumors, which can easily be quantified. Mice were fed basal diets with or without 3% N-acetyl-L-cysteine (NAC), a well-recognized antioxidant, prior to, during, and after topical application of the carcinogen benzo(a)pyrene (64 µg/mouse, 2x/week, 7 weeks). Mice continuously fed NAC demonstrated a significant 43% reduction ($p<0.05$) in tumor multiplicity compared to controls (5.9 vs. 10.4). Tumor incidence (papillomas, keratoacanthomas and carcinomas) exceeded 90% for both groups. Although total tumors per mouse were reduced, skin malignancies (squamous cell and spindle cell carcinomas) were increased 70% (0.54 vs. 0.92 tumors/ animal at risk) in animals fed NAC (n=13) compared to basal-fed controls (n=13). Immunohistochemical analysis of tumors showed consistent and widespread expression of both the v-Ha-ras oncogene and p53 tumor suppressor protein. Southern analysis demonstrated no loss of p53 heterozygosity involving the Trp53 locus suggesting an alternate mechanism for inactivation of p53 or other critical genetic events leading to increased malignancies. In summary, these data demonstrate that the prototypic antioxidant NAC paradoxically decreases total tumor burden but increases progression to malignancy.

34 CHEMICAL INDUCTION OF LIVER TUMORS AND PRESTAGES IN THE AVIAN *IN OVO* CARCINOGENICITY ASSAY (IOCA) IN LESS THAN 24 DAYS.

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Preneoplastic liver foci are widely accepted as reliable indicators of hepatocarcinogenesis. Our aim was to induce preneoplastic and neoplastic lesions in avian embryonic liver within the short time of embryonic development. To that end, turkey hatching eggs were injected with diethylnitrosamine or other hepatocarcinogens prior to incubation. After incubation for up to 24 days the embryos were removed from the eggs and liver samples were flash frozen or fixed for subsequent microscopic examination. Hepatocellular tumors were induced by the genotoxic carcinogen diethylnitrosamine at doses of or exceeding 4 mg per egg. Lower doses produced basophilic foci of altered hepatocytes that exhibit enzymatic alterations that have long been known as typical features of preneoplastic liver lesions in rat liver. These foci were closely associated with the occurrence of enlarged hepatocytes with karyomegallic atypical nuclei (KAN cells). Lower doses of genotoxic carcinogens that were insufficient for the induction of foci or tumors as well as non-genotoxic carcinogens like thioacetamide induced KAN cells, whereas toxic but not carcinogenic substances like KCN did not. There is histological evidence for a developmental sequence from KAN cells through foci to tumors. The IOCA is proposed as a rapid and inexpensive test for hepatocarcinogens.

35 THE USE OF A PHARMACOGENETIC MOUSE MODEL TO ASSESS MATERNAL AND FETAL SUSCEPTIBILITY TO THE CARCINOGENIC EFFECTS OF COMPLEX MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS.

K. A. Rozett[1], M. D. Mitchell[2] and E. H. Weyand[1]. [1]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ and* [2]*Searle, St. Louis, St. Louis, MO.*

Polycyclic aromatic hydrocarbons (PAH) have been demonstrated to effectively bind to the aryl hydrocarbon (Ah) receptor, thereby eliciting the expression of enzymes responsible for their metabolism. The development of mouse strains that differ genetically in their inducibility of these enzymes can be used to evaluate how differential enzyme expression can modulate PAH metabolism and therefore, their carcinogenic potency. Using the C57BL/6 X DBA/2 backcross model, both maternal and fetal susceptibility to the carcinogenic effects of a complex mixture of PAH was determined. B6D2F1/J females were mated with DBA/2J males and DBA/2J females with B6D2F1/J males. Pregnant females were given a gel diet adulterated with 0.3% coal tar beginning on day 13 of gestation (day 1 indicated by presence of a vaginal plug). Control females received an unadulterated gel diet. Dams and pups were sacrificed at the time of birth. Lung and liver tissues were removed from each pup and lung, liver, forestomach, intestine, and uterus from each dam. ^{32}P-postlabeling with multidimensional TLC was used to evaluate PAH:DNA adduct formation and RT-PCR utilizing a taqman fluorogenic probe was used to assess cytochrome (CYP) P4501A1 and P4501A2 expression. Total PAH:DNA adducts were relatively higher in the lung, forestomach, and uterus of B6D2F1/J dams when compared to DBA/2J dams, whereas the opposite was true of both liver and intestinal tissues. CYP1A1 induction was greatest in lung of both B6D2F1/J and DBA/2J dams, while CYP1A2 induction was greatest in liver tissue of both strains. Interestingly, no PAH:DNA adducts were detected in lung or liver tissues of pups born to dams of either strain, although induction of both CYP1A1 and CYP1A2 relative to controls was observed. This study shows that regardless of the genetic differences in maternal metabolism, both strains of dams were able to decrease the PAH burden on their pups to a level at which no detectable genotoxicity was observed. (EPRI Grant WO2963-06.)

36 ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR BY PAH *ORTHO*-QUINONES.

M. E. Burczynski and T. M. Penning. *University of Pennsylvania, Philadelphia, PA.* Sponsor: T. Monks.

Polycyclic aromatic hydrocarbons (PAH) induce their own metabolic activation by binding to the cytosolic aryl hydrocarbon receptor (AhR) which then translocates to the nucleus to activate CYP1A1 gene transcription via xenobiotic response elements (XREs). Surprisingly, although the AhR demonstrates a strict specificity for planar aromatics, the nonplanar PAH metabolite trans-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene (B[a]P-diol) also induced CYP1A1 expression in HepG2 cells. Induction occurred over a delayed time-course (~6-12 h), suggesting a requirement for B[a]P-diol metabolism. Inhibition of dihydrodiol dehydrogenase (DD) members of the aldo-keto reductase (AKR) superfamily blocked this effect, suggesting that benzo[a]pyrene-7,8-dione (BPQ), a planar PAH ortho-quinone metabolite generated by DDs, was the actual inducer. BPQ was found to be a potent and rapid inducer of CYP1A1 mRNA, with an EC50 value in HepG2 cells identical to that of parent B[a]P. Multiple PAH o-quinones caused induction of CYP1A1, demonstrating that this was a general property of AKR-generated PAH o-quinones. HepG2101L cells stably transfected with an XRE-luciferase construct showed that BPQ activated CYP1A1 expression at the level of transcription via an XRE-dependent mechanism. BPQ failed to induce CYP1A1 in either AhR- or ARNT-deficient murine hepatoma cell lines and confirmed that induction of CYP1A1 was AhR-dependent. Electrophoretic mobility shift assays demonstrated the specific appearance of BPQ-activated AhR in the nucleus, and immunofluorescence studies confirmed that BPQ mediated nuclear translocation of the AhR. Previous studies have shown PAH o-quinones to be genotoxic both *in vitro* and in living cells. The cytosolic co-localization of the AKRs and AhR, and the subsequent hijacking of the AhR pathway by PAH o-quinones, provide a potential mechanism by which these genotoxic metabolites may be specifically targeted to the nucleus and may have significant consequences for PAH carcinogenesis. (Supported by grants CA39504 and CA55711 to TMP.)

37 INCREASED CELL PROLIFERATION AND HEPATOCELLULAR CANCER IN POLYCHLORINATED BIPHENYL-EXPOSED SPRAGUE-DAWLEY RATS.

J. Whysner. *American Health Foundation, Valhalla, NY.*

The objective of this research was to evaluate the histopathological sections of rat livers from a two year bioassay with polychlorinated biphenyls (PCBs) exposures using immunohistochemical staining for cell proliferation and other parameters related to the development of the hepatocellular carcinomas found in this study. Interim sacrifice groups of male and female rats exposed to different concentrations of four different commercially used PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) for 26 or 52 weeks were evaluated and compared with the results of histological identification of tumors in the two year bioassay. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) among the various concentrations of Aroclors at 52 weeks showed dose-related increases and a correlation of increased cell proliferation with eventual tumor development. No increases in PCNA labeling were found at 26 weeks, and no increases in terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) were evident at either 26 or 52 weeks. Increases in glutathione S-transferase placental form (GSTP) positive foci were found at 52 but not at 26 weeks of PCB exposures. All altered foci that were identified by conventional staining also stained positive for GSTP. The results of this study show that increased cell proliferation was related to tumor development according to the potencies of the Aroclor types and dosages and that the time course of increased cell proliferation coincided with the development of GSTP positive foci.

38 BIOCHEMICAL BASIS FOR THRESHOLDS IN PCB-MEDIATED CARCINOGENESIS.

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Parallel, multidose, chronic (2 yr) bioassays of Aroclors 1016, 1242, 1254 and 1260 in 35 groups of *ad libitum* fed male or female Sprague Dawley rats showed that PCBs could increase the incidence of liver tumors, except at the lower doses, and decrease those in other organs (Mayes *et al, Toxicol. Sci.* 41, 62-76, 1998). In order to identify the biochemical basis for the observed inhibitions and thresholds, we first determined the tissue accumulations (i.e., internal doses) of total PCBs (mostly non-coplanar), and total TEQ (mostly from coplanar PCBs). This showed that the hepatotumorigenesis could be induced by either the coplanars (in females only), the non-coplanars (in both sexes), or by non-PCB factors (especially in males). Determinations of other biochemical parameters then showed that regardless of mode of induction, hepato-tumorigenesis was closely correlated with cytosolic production of superoxide by redox cycling. This process, which became important in older animals, was found to be mediated by soluble, low molecular weight quinonoids. The resulting superoxide, or derived peroxide, is known to cause tumor promotion. The intracellular antioxidant found correlated with control of this route to tumor promotion, and hence production of the observed dose thresholds for hepatotumorigenesis, was glutathione. This is known to be upregulated by lipid oxidation products (increased even at the lower PCB doses), albeit not sufficiently to block rat liver tumorigenesis at high doses.

39 INFLUENCE OF NEONATAL STILBENE ESTROGEN EXPOSURE ON ESTROGEN RESPONSIVE GENES IN TESTIS OF HAMSTERS.

Q. Cai and D. Roy. *University of Alabama at Birmingham, Birmingham, AL.*

Exposure to stilbene estrogen (diethylstilbestrol, DES) to hamster neonates produces interstitial testicular cancer. However, the mechanism by which estrogen induces testicular cancer is not clear. In this study, we have examined the influence of neonatal stilbene estrogen exposure on estrogen responsive genes in testis. All neonates of Syrian golden hamsters received a single injection of DES (40 mg/kg) or corn oil (control) within 6 hours after birth. The animals were sacrificed at the age of 65 and 90 days. The testis was removed and total RNA was isolated. Using RT-PCR, the mRNA levels of estrogen receptor α (ER-α), estrogen receptor β (ER-β), aromatase, and inhibin/activin were determined. There was no difference in ER-α mRNA level between control and DES treated groups. The ER-β mRNA level was decreased after 65 days of DES treatment compared to control group, however there was no difference between control and 90 days DES treated groups. The aromatase mRNA level was decreased in the DES treated hamster testis after 65 days of DES treatment compared to control group, however there was no difference in aromatase level between control and DES treated groups after 90 days. We

also measured the levels of three subunits of inhibin/activin (α, β_A, β_B) in hamster testis. There was no difference between control and DES treated groups in all subunits. The results of this study indicate that neonatal DES treatment may result in the imbalance of intratesticular estrogen-androgen levels and their actions on the testis.

40 DRAMATIC SYNERGISM OF EXCESS SOYBEAN INTAKE WITH IODINE DEFICIENCY ON THE DEVELOPMENT OF RAT THYROID PROLIFERATIVE LESIONS.

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High intake of soy-based products is inversely associated with relative risk of breast, prostate, and gastric cancers in human. On the other hand, thyroid enlargement by excessive soybean feeding especially in children and women has been known since previous half a century. In the present study, the effects of soybean protein feeding and the combined influence with deficient iodine were investigated in female F344 rats with special reference to the specificity and dose response. First, rats were fed basal diet, iodine-deficient (ID) diet, 20% defatted soybean (S) diet or ID + 20% S diet for up to 10 weeks. Dietary soybean dramatically induced thyroid follicular hyperplasias synergistically with ID. Secondly, rats were fed basal diet, ID diet, or ID + 0.2, 1.0, 5.0 or 25% S for 5 weeks. Soybean feeding showed synergistic effects on the development of thyroid hyperplasias with ID only at a dose as high as 25%. Thirdly, rats were fed basal diet, 20% S, 0.025% sulfadimethoxine (SDM), 20% S + 0.025% SDM, 0.05% phenobarbital (PB) or 20% S + 0.05% PB for 5 weeks. Soybean intake did not exert any synergistic effects with SDM or PB. Our results thus indicate that soybean intake specifically exerts synergistic effects with iodine deficiency on the development of thyroid hyperplasias in rats only at high doses. (This work was supported in part by a Grant-in-Aid for Research on Environmental Health from the Ministry of Health and Welfare of Japan.)

41 EFFECT OF PERINATAL/JUVENILE EXPOSURE TO HEPTACHLOR ON ADULT IMMUNE, REPRODUCTIVE AND NEURAL FUNCTION.

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This study was performed to determine if persistent immunotoxic, reproductive toxic, and/or neurotoxic effects result from developmental exposure to heptachlor (HEP). Time-bred pregnant female Sprague-Dawley rats were dosed by gavage with HEP (0, 0.03, 0.3 or 3.0 mg/kg/d) from gestation day (GD) 12 to postnatal day (PND) 7, followed by direct dosing of the pups through PND 42. Separate groups of rats were used for testing immune, reproductive, and neurochemical parameters. The amount of HEP epoxide B found in milk, blood, fat and tissues was proportional to the dose of HEP administered. There were no untoward effects on the number or survival of pups born to HEP-exposed dams nor to pups exposed postnatally. There were no treatment effects for any of the reproductive endpoints examined. Gamma-aminobutyric acid (GABA)-stimulated ^{36}Cl flux was decreased in PND 21, but not PND 7 or 43 females, which suggests that HEP may transiently alter cortical $GABA_A$ function. The serum anti-SRBC IgM response of males, but not females, was significantly suppressed in a dose-dependent manner in rats 8-weeks-old. The percentage of $OX12^+OX19^-$ splenocytes (i.e., B cells and plasma cells) was reduced in the high dose males, suggesting that the suppressed anti-SRBC IgM response may be associated with the loss of these cells. The anti-SRBC IgM response was reduced only in males exposed to 0.3 mg HEP/kg/d in a second group of rats 21-weeks-old. At 25-weeks-old the anti-SRBC IgG response of these same rats was suppressed in all of the HEP-exposed males, but not females. These data indicate that perinatal exposure of male rats to HEP results in a persistent suppression of the primary (IgM) and secondary (IgG) anti-SRBC immune responses at a total dose of as little as 1.5 mg HEP/kg/rat. (This abstract does not reflect EPA policy. Partial support for this work came from the Hawaii Heptachlor Research and Education Foundation.)

42 DEVELOPMENTAL EXPOSURE TO TCDD AND MERCURIC CHLORIDE IN AUTOIMMUNE-PRONE MRL/*lpr* MICE.

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Autoimmune diseases affect 5-7% of the human population in over 40 different forms, characterized by inappropriate immune responses to self. Certain environmental contaminants, particularly estrogenic endocrine disruptors, may contribute to this aberrant immune function. We have examined the potential for increased incidence and/or exacerbation of autoimmune disease in MRL/*lpr* autoimmune-prone mice exposed prenatally to either tetrachlorodibenzo-*p*-dioxin (TCDD) or mercuric chloride (HgCl2). MRL/*lpr* mice exhibit severe renal necrosis (glomerulonephritis) and increased levels of circulating autoantibodies similar to that observed in human systemic lupus erythematosis (SLE), and females present disease symptoms approximately six to eight weeks prior to males. Assessment of autoimmunity via quantitation of urinary protein and serum autoantibody levels was compared in female versus male mice to determine if there were differential chemical-induced effects. Results in female MRL/*lpr* mice indicate a significant, dose-dependent increase in urinary protein in animals prenatally exposed to TCDD as compared to controls at six weeks of age. Further, TCDD-induced urinary protein levels were significantly elevated when compared to positive control mice exposed to HgCl2. Autoantibodies to ss-DNA were significantly increased in a dose-dependent manner in TCDD-exposed animals as compared to controls at ten weeks of age. In males, there was a dose-related trend in urinary protein amongst TCDD-exposed animals compared to controls and HgCl2 at six weeks of age, becoming statistically significant at eight weeks of age. Significant dose-dependent increases in autoantibodies to ss-DNA were noted in the male animals exposed to TCDD compared to controls at ten weeks of age. These findings suggest that developmental exposure to TCDD may accelerate the onset of SLE in both male and female MRL mice. Furthermore, TCDD may be a stronger inducer of SLE than the prototypical control HgCl2 in neonatal mice.

43 PRENATAL DIETHYLSTILBESTROL EXPOSURE ALTERS γ-INTERFERON LEVELS.

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Alterations in gamma-interferon, a T-helper 1-type cytokine, have been shown to have a profound impact on autoimmune and neoplastic diseases. In this study, we investigated whether gamma-interferon is modulated by estrogenic compounds. The levels of gamma-interferon mRNA and protein were increased in concanavalin-A-stimulated splenic lymphocytes from C57Bl/6 mice exposed to estrogen for 3-5 months. An estrogen-induced increase in gamma-interferon mRNA and protein was evident as early as 6 and 12 hours, respectively, after Con-A stimulation. Levels of gamma-interferon were also modulated by diethylstilbestrol (DES), a synthetic estrogen. The secretion of gamma-interferon by Con-A or anti-CD3 antibody-stimulated splenic lymphocytes from prenatal DES-exposed mice at 5-6 days of age was increased. This increase in gamma-interferon secretion was noticed despite decreased percentages of T cells. The increase in gamma-interferon in prenatal DES-exposed mice was transient, since by 2-3 months of age gamma-interferon levels approximated the controls. Preliminary studies on male mice show that secondary exposure to DES, 1 to 1.5 years after prenatal DES exposure, markedly increased the ability of splenic lymphocytes to secrete gamma-interferon after Con A stimulation. Our studies show that prenatal DES exposure alters the "cytokine programming" of lymphocytes and dysregulation of gamma-interferon-secretion may play a role in cancer and/or autoimmune diseases. (Supported by NIEHS:RO1 ES 08043-04.)

44 NEONATAL EXPOSURE TO PROPYLTHIOURACIL (PTU) INDUCES A SHIFT IN LYMPHOID CELL SUB-POPULATIONS IN THE DEVELOPING POSTNATAL MALE RAT SPLEEN AND THYMUS.

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It has been suggested that certain environmental toxicants can alter thyroid function in mammalian species. Such an effect may be particularly important in developing physiological systems involved in growth, reproduction, and immune function, all of which are influenced by thyroid hormones. The object of the present study was to determine the effects of PTU-induced hypothy-

roidism on the developing immune system by focussing on both the spleen and thymus gland. Male Sprague-Dawley rat pups were exposed to PTU through maternal milk by giving the mothers 0.02% PTU in their drinking water starting on the pups day of birth until weaning on day 28. Animals were sampled on days 14, 22, and 30. On days 22 and 30, PTU exposure increased the T-cells in the spleen as assessed by both the percentage of PanT positive and CD4 positive cells. PTU exposure also increased the percentage of CD8a positive cells in the spleen at d22, although no differences were detected at d14 or d30. PTU exposure decreased the percentage of CD45RA positive cells (B-cells) of splenocytes at d22 and d30 indicating that the relative B-cell population was decreased while the T-cell population was increased. PTU exposure decreased the NK lytic ability of cells at d22, but no functional differences were observed at d14 and d30, despite the increased percentage of CD161 positive cells (NK-cells) in PTU-exposed animals at each of the sampling points. PTU exposure increased the percentage of CD4+/CD8- in the thymus on d22 and caused a slight increase on d30. These data suggest that the effects of induced hypothyroidism on the cell populations in the spleen may result from changes in thymic T-cell development, although changes in B-cell development may also occur. The current data emphasize that thyroid hormones regulate not only the development of immune cells but also cell function and that both of these must be tested when investigating toxicological effects on the immune system.

45 NEONATAL EXPOSURE TO CADMIUM (CD) PRODUCES BOTH SHORT AND LONG TERM EFFECTS ON NK FUNCTION AND MITOGENIC RESPONSE OF RAT SPLENOCYTES AND THYMOCYTES.

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Heavy metals, including Cd, have been shown to modulate immune measures including blastogenic response and NK activity. Little is currently known regarding the sensitivity of the immune system to heavy metals at different stages of development. The present objective was to determine the effects of low doses of Cd via the maternal milk on NK activity of splenocytes and blastogenic response of both splenocytes and thymocytes. Male and female Sprague-Dawley rat pups were exposed to Cd through maternal milk by giving the mothers either 0 (controls), 10 ppb or 5 ppm $CdCl_2$ in the drinking water (d1-24). Rat pups were weaned on day 24 and sampled on days 28 and 63. Shortly after weaning (d28), male rats from the high dose group exhibited a lowered NK response. The NK response of all Cd-exposed females and the low-dose, Cd-exposed males did not differ from that of controls. The blastogenic response of splenocytes from the high dose group rats to con A resulted in a decreased stimulation index (S.I.), although the blastogenic response to PHA and LPS were not different from control rats. There were no differences in the blastogenic response of thymocytes. In 63 day-old rats, the high dose Cd group displayed a decreased S.I. of thymocytes to con A. However, no differences were observed in blastogenic response of thymocytes to PHA and no differences in the blastogenic response of splenocytes to any of the mitogens was observed. There were no effects of Cd exposure on NK response. In conclusion, short-term exposure of relatively low doses of Cd via the maternal milk can alter aspects of immune function in young rats and can result in effects throughout the life of the animal.

46 HEMATOTOXIC EFFECTS OF PRENATAL EXPOSURE OF MICE TO CHLORDANE.

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Chlordane, a member of the organochlorine insecticides, was used for structural preservation against underground termites until as late as 1990 when the EPA banned production of chlordane. The toxicity of chlordane is essential because more than 30 million homes were treated, and the half-life of chlordane is estimated to be 20 to 30 years in soil. Chlordane is readily absorbed through the skin, airway, and gastrointestinal tract. We used a murine prenatal exposure model to examine the hypothesis that prenatal exposure to chlordane affects the development of hematopoietic tissues and results in lifelong failure of normal leukocyte development. Nine-week-old pregnant Balb/C mice were administered chlordane (18 mg/kg/day) from day 10 to 18 of gestation. Colony forming unit (CFU) assays show that single cell suspensions from the fetal livers of chlordane-treated mice *in utero* had decreased colony formation in both myeloid and lymphoid cell lineages by at least 50% as compared to controls with the largest decrease observed for IL-7 stimulated colonies. The conditioned medium from chlordane-treated fetal liver stromal cells supported IL-3, GM-CSF, and M-CSF dependent myeloid cell lines in bioassays. *In vitro* proliferation assays stimulated by hematopoietic cytokines revealed differences in proliferative capacity of fetal liver cells when compared to cells from vehicle-treated embryos. These results suggest that chlordane continues to pose a serious health threat to exposed individuals and that fetal development is a critical window of exposure to this compound.

47 REDUCTION OF SPLEEN CELLULARITY BY LOW-DOSE MATERNAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN RATS.

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TCDD exerts adverse effects on immune functions. Fetus is known to be much more sensitive to TCDD than adult. In order to obtain a clue to elucidate the mechanisms of immunotoxicity by maternally-exposed TCDD, pregnant Holtzman rats were administered a single oral dose of 0, 12.5, 50, 200 or 800 ng TCDD/kg body weight on gestation Day 15, and the thymus and spleen cells of male offspring were investigated on Day 21, 49, 63 and 120 after birth. Maternal exposure to TCDD did not cause any significant change in thymus weight, cell number/tissue weight (cellularity), thymocyte population in terms of the percentages of $CD4^-CD8^-$ (DN), $CD4^+CD8^+$ (DP), $CD4^+CD8^-$ (CD4-SP) and $CD4^-CD8^+$ (CD8-SP) cells in pups on Day 21. No alteration was observed in spleen weight and cellularity. The percentages of T cells and $CD4^+$ T cells in splenocytes were found to be significantly reduced by 200 ng TCDD/kg. On Day 49, pups maternally exposed to TCDD showed decreased spleen cellularity in a dose-dependent manner with the significant reductions at 200 and 800 ng TCDD/kg. Since percentages of T cells in splenocytes were not changed between control and exposed groups, the decreased cellularity appeared to be due to the reductions of both T and B cell numbers. Thymus weight, cellularity, thymocyte population and spleen weight were not changed by TCDD exposure. On Day 63, significant decrease in spleen cellularity was observed only at 200 ng TCDD/kg. On Day 120, the spleen cellularity did not differ between control and exposed groups. In summary, the present study revealed that a low-dose maternal exposure to TCDD significantly reduces spleen lymphocyte numbers with the most prominent effect around the age of puberty.

48 EMBRYONIC WINDOWS OF INCREASED VULNERABILITY TO PB-INDUCED IMMUNOTOXICITY DURING AVIAN DEVELOPMENT.

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The developing immune system of rodents has been shown to exhibit increased sensitivity to Pb-induced immunotoxicity compared with that of adults. However, little is known about potential windows of increased vulnerability during discrete periods of embryonic development. To pinpoint differential embryonic sensitivity to Pb-induced immunotoxicity, sublethal doses of Pb were introduced in fertilized Cornell K Strain While Leghorn chicken eggs via the air sac at one of three different stages of embryonic development (7, 9, and 12 day of incubation, designated as E7, E9 and E12, respectively). Blood Pb levels were determined at hatching and Pb-induced immunotoxicity was evaluated in 5-6 week old young chickens using a delayed-type hypersensitivity reaction (DHR) against BSA, an anti-BSA antibody ELISA and macrophage production of nitric oxide as immune indicators. While juvenile levels of antibody responses to BSA were unaffected by the *in ovo* Pb exposure, macrophage production of nitric oxide (measured as nitrite production) was significantly depressed ($p<0.05$) following Pb-exposure (measured for E7 and E9 exposure only). In contrast with this pattern, the DHR function was unaltered following the E7 and E9 exposures, but was significantly depressed ($p<0.05$) after E12 exposure to Pb. Since the E9 and E12 sublethal exposures included overlapping doses of Pb with overlapping blood Pb levels, the capacity of Pb to influence the DHR function appears to emerge between days 9 and 12 of *in ovo* development. The stability of Pb-induced developmental immunotoxicity was examined using virus challenge. When chickens were exposed at E12 to Pb and then subjected to infection with infectious bronchitis virus (IBV), Pb-induced depression of the DHR was similar in severity to that observed in uninfected chickens. Based on these results, it is hypothesized that Pb exposure during different windows of embryonic development is likely to result in different immunotoxic outcomes in the juvenile. Furthermore, Pb-induced embryonic immune alterations are likely to remain evident during at least some juvenile disease challenges. (Supported in part by grants from the NIEHS-Superfund and the USDA.)

49 CHARACTERIZATION OF AN APPROACH TO DEVELOPMENTAL IMMUNOTOXICOLOGY ASSESSMENT IN THE RAT USING SRBC AS THEANTIGEN.

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The objectives of this study were 1) to optimize and establish baseline values for the primary humoral immune response to SRBC using an ELISA or the plaque forming cell (PFC) assay and 2) to establish baseline values for splenic lymphocytes (B-cell, T-cell, T-helper and T-cytotoxic/suppressor cells) using flow cytometry for both 10- and 21-day old Crl:CD[1](SD)BR rats. Responses for each age group were compared both within and between litters. Due to the difficulty in administering SRBC by tail vein injection, 10-day old pups received SRBC intraperitoneally; but were still unable to generate an IgM response using either assay. The following mean absolute and relative (%) numbers of splenic lymphocytes were obtained for 10-day old rats, respectively: $0.18\pm0.08 \times 10^8$ (13 ± 5) $CD45^+$; $0.13\pm0.04 \times 10^8$ (9 ± 3) $OX12^+$; $0.07\pm0.01 \times 10^8$ (5 ± 1) $W3/25^+CD3^+$; $0.03\pm0.01 \times 10^8$ (2 ± 0.5) $OX8^-CD3^+$ cells. A splenic differential showed that ~80% of the 10-day old splenocytes were lymphocytes. Results with the ELISA showed that the SRBC-specific IgM antibody $\log_2$ titer for 21-day old rats was considerably less compared to young adult rats due to a high background response. Results with the PFC assay produced a response in 21-day old rats similar to that of adults. The following mean absolute and relative numbers of lymphocytes were obtained for 21-day old rats, respectively: $0.96\pm0.25 \times 10^8$ (48 ± 5) $CD45^+$; $0.77\pm0.16 \times 10^8$ (39 ± 3) $OX12^+$; $0.23\pm0.02 \times 10^8$ (12 ± 2) $W3/25^-CD3^+$; $0.14\pm0.02 \times 10^8$ (7 ± 1) $OX8^-CD3^+$ cells. Based on our data, the litter can be used as the unit of comparison for developmental immunotoxicology. Results suggest that it may not be possible to use a functional parameter in 10-day old rats; however, additional antigens need to be examined. Further studies are also needed to determine whether SRBC or other antigens can be integrated into studies with rat weanlings. (Supported by a grant from the Chemical Manufacturers Association.)

50 DEVELOPMENTAL IMMUNOTOXICOLOGY ASSESSMENT IN THE RAT: AGE, GENDER AND STRAIN CONSIDERATIONS.

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A combined Delayed-type Hypersensitivity Response (DHR) to KLH and anti-KLH IgG antibody ELISA were assessed as potential biomarkers for developmental immunotoxicity with age, gender, site of antigenic challenge and strain as variables. The heavy metal, Pb, was used as a known developmental immunotoxin. Five-week old Sprague-Dawley (CD) rats produced lower levels of antibody and a decreased DHR compared with adults. In the age comparison, only adult males had a significantly increased DHR while both male and female adults produced higher levels of antibody than both genders of weanlings. When examining site of KLH challenge, the DHR was greater in young animals when challenged in the footpad vs. the earlobe and there were gender differences in antibody levels. Females had optimum antibody levels with a DHR challenge in the earlobe whereas males had optimum levels when challenged in the footpad. In a strain comparison between weanling rats exposed *in utero* to control acetate or Pb acetate (250 ppm lead acetate in drinking water) and pups examined at five weeks of age, the KLH immunization protocol produced a higher antibody response in CD vs. F344 rats; in contrast, F344 rats exhibited an elevated DHR. Pb exposure *in utero* via the pregnant dams produced differential gender effects in the juveniles of both strains. Females had a significantly decreased Pb-induced DHR ($p<0.05$) whereas males did not. These results suggest that the DHR and antibody ELISA against KLH are suitable as biomarkers of developmental immunotoxicity and, based on the Pb results, assessment can be performed in juvenile rats. Furthermore, at the KLH concentration utilized, the balance of cell-mediated vs. humoral response differed among the two strains examined. Further studies are needed to define the optimum concentrations of KLH for each strain when immunized as weanlings. (Supported by grants from the Chemical Manufacturers Association and the NIEHS-Superfund.)

51 THE INTERGRATION OF IMMUNOLOGICAL ASSESSMENTS AS PART OF THE RODENT DEVELOPMENTAL NEUROTOXICITY SCREEN.

R. P. Jones. *Covance, Harrogate, United Kingdom.* Sponsor: J. Norris.

Immunological assessments have in recent years become common additions to regulatory sub-chronic toxicity studies of 4 and 13 Weeks duration. However at our laboratory we have recently received requests to perform such evaluations as part of the established developmental neurotoxicology screen (EPA OPPTS 870.6300). This screen requires the oral administration of the test substance to female rats from Day 6 of gestation to Day 10 of lactation. The offspring are reared until they are at least 60 days of age. These offspring are used for a variety of neurological evaluations including neuropathology, a sub-group being destined for brain weight evaluation. Immunological assessments are added to this subgroup. The assessments included conventional histopathology together with image analysis and immunohistochemistry of select primary and secondary lymphatic organs. In addition, haematological assessments including differential white cell counts, red cell parameters and lymphocyte sub-sets are undertaken together with selected clinical chemistry parameters such as the immunoglobulins. Consequently, a broad assessment of immune competance of these Day 60 offspring can be made with out impinging on the primary design of the neurotoxicological screen.

52 IDENTIFICATION OF ESTROGEN RECEPTOR ISOFORMS IN MAMMARY CELLS: MODULATION BY 1,1-DICHLORO-2,2-*BIS*(CHLOROPHENYL) ETHYLENE (DDE).

L. M. Zipper and R. T. Mulcahy. *University of Wisconsin, Madison, WI.* Sponsor: A. A. Elfarra.

Mammary cell estrogen receptors (ER) play an important role in certain breast cancers. Environmental contaminants can modulate ER levels and/or activation. DDE, for which some studies have indicated higher levels in breast cancer patients, is an environmental contaminant which is thought to modulate the ER. Human cell models are pivotal tools to determine contaminant-induced changes in ER (e.g. TCDD effects in MCF-7 cells). We have observed that estradiol is mitogenic in the non tumorigenic MCF-10A cell line, defined in the literature as being ER-negative. Isoforms (α and β) of the ER have been identified in recent years; the functional role of these isoforms in carcinogenesis is still unknown. The present study aimed to assess the presence of ER-α and ER-β mRNA in cancerous (MCF-7A and T47-D) and normal (MCF-10A and MCF-12A) mammary cell lines by double PCR using nested primers. Both isoforms were present in each of the cell lines; levels for MCF-10A and MCF-12A were lower than those for MCF-7 and T47-D (highest amount) cells. In MCF-7 cells, treatment (24 h) with o,p'-DDE or p,p'-DDE increased ER-α mRNA level. Both the ER antagonist tamoxifen and ER agonist 16-α-OH-estrone decrease ER-α mRNA levels. This suggests that DDE isomers may magnify estrogenic activity in mammary cells by increasing ER levels through a mechanism independent of receptor occupancy. Future studies will look at the modulation of the ER-α/ER-β ratios following exposure to these compounds in MCF-10A cells.

53 TRANSCRIPTION OF THE HEAVY AND LIGHT SUBUNITS OF γ-GLUTAMYLCYSTEINE SYNTHETASE (γGCS) IS PARTIALLY REGULATED BY THE MAP KINASES ERK1 & 2.

L. M. Zipper and R. T. Mulcahy. *University of Wisconsin, Madison, WI.* Sponsor: A. A. Elfarra.

Transcription of both the heavy and light subunits of g-glutamylcysteine synthetase (γGCS), the rate limiting enzyme in glutathione synthesis, is induced by various xenobiotics including Pyrrolidinedithiocarbamate (PDTC). The potential involvement of the Mitogen Activated Protein kinases (MAPKs) in γGCS gene induction was investigated as these kinases are known to be important intermediate proteins in a variety signal transduction cascades. HepG2 cells were treated with 100mM PDTC and activation of JNK, p38, and ERK were assayed with western blots and *in-vitro* kinase assays. Results showed weak JNK activation at 1 and 2 hours after PDTC treatment, whereas ERK1/2 and p38 were activated by PDTC treatment as soon as 15 minutes (ERK1/2) and 30 minutes (p38) and remained active for as long as 4-6 hours. To determine if PDTC induced MAPK activation correlated with γGCS gene induction, HepG2 cells were treated with MAPK inhibitors and/or dominant negative MAPK constructs. Results show that such treatments decreased accumulation of γGCS RNA by 50% and also reduced transcription from promoter-reporter constructs. Binding of specific transcription factors to the EpREs that regulate γGCS were also altered by treatment with inhibitors as

assayed by gel shifts. Results from these studies suggest that MAPK family members, specifically ERK1/2, are at least partially involved in the regulation of γGCS gene transcription. Studies investigating the effect of p38 inhibitors and dominant negative p38 constructs on γGCS RNA accumulation and promoter activity are currently underway. (This work was supported by ES09749, CA57549, T32-CA09471.)

54 A CCAAT/ENHANCER BINDING PROTEIN (C/EBP) SITE ALONG WITH CREB BINDING PROTEIN (CBP) PARTICIPATE IN NEGATIVE REGULATION OF RAT *GST-Ya* IN VASCULAR SMOOTH MUSCLE CELLS BY BENZO(A)PYRENE.

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Recent studies in this laboratory have established that a C/EBP-like site within the antioxidant/electrophile response element (ARE/EpRE) functions as a negative regulatory sequence in the rat GST-Ya promoter in vascular smooth muscle cells (vSMCs) and HepG2 cells. In the present study, electrophoretic mobility shift assays and transfection experiments were conducted to evaluate the influence of DNA/protein interactions on the ability of ARE/EpRE to function as a negative regulatory sequence in vSMCs. Evidence was obtained that C/EBP-β interacts with the C/EBP-like site of rat *GST-Ya*, and that the aryl hydrocarbon receptor (AhR) is present in the ARE/EpRE binding protein complex. To evaluate the role of these proteins in transactivation, expression vectors were co-tranfected with a rat *GST-Ya* reporter construct (1.6 CAT) in vSMCs. Overexpression of CBP nullified ARE/EpRE-mediated repression of rat GST-Ya in the presence of BaP. The specificity of this interaction was suggested by the finding that human adenovirus E1A protein abrogated co-transactivation potential of CBP, while an E1A mutant did not. In subsequent experiments we found that in the presence of CBP, overexpression of C/EBP-α or C/EBP-β decreased the expression of 1.6CAT, while AhR had the opposite effect. Collectively, our results suggest that negative regulation of rat *GST-Ya* in vSMCs by BaP results from limiting CBP protein levels, and that CBP serves as a mediator of functional interaction between C/EBPs and AhR in the regulation of this response. (Supported in part by NIH Grant ES 04849 and ES 09106.)

55 IDENTIFICATION OF A DOMAIN RESPONSIBLE FOR THE NEGATIVE FUNCTION OF RTARNTA IN AH RECEPTOR SIGNALING.

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The secondary structure of the PST-rich COOH-end of rtARNTa was evaluated by computer analysis (Motif database). Computer analysis revealed that the sequence was dominated by β-sheets, numerous turn regions, and a lack of helices. In addition, the last 20 amino acids contained a region that appeared to be strongly hydrophobic. To assess the impact of these regions within the PST-rich COOH-end, the rtARNTa protein was truncated and function evaluated by electromobility shift assay and complementation of P4501A1 induction in ARNT deficient Hepa-1 cells. Complementation studies revealed that truncation of the hydrophobic domain that spanned the final 20-40 amino acids of rtARNTa restored the protein's ability to induce P4501A1. Gel shift analysis confirmed that these results were related to recovery of DNA binding of the rtARNT•AHR heterodimer. Addition of the hydrophobic domain to mARNT or rtARNT resulted in proteins with reduced DNA binding and the inability to complement AHR-mediated signal transduction. These findings indicate that the hydrophobic domain is primarily responsible for the negative function of rtARNTa, suggesting that it's repressor function may be related protein misfolding or masking of the DNA binding domain. In addition, the Gal4 fusion approach was employed to identify possible transactivation domains within the truncated rtARNTa proteins. Gal4 analysis revealed the lack of transactivation function in the truncated COOH terminal domains of rtARNTa, in contrast to the QN-rich COOH-end of rtARNTb. The lack of transcriptional activity was not related to DNA binding as the Gal4 fusion proteins bound DNA. Collectively, these studies narrow the negative function of rtARNTa to the presence of a C-terminal hydrophobic domain, and illustrates how changes in the C-terminal domain can affect protein function. (Supported by ES08980 and EPA STAR # U915218010.)

56 THE IMMUNOPHILIN HOMOLOG XAP2 SPECIFICALLY STABILIZES THE AH RECEPTOR AND IS ASSOCIATED WITH THE CYTOSKELETON.

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The aryl hydrocarbon receptor (AhR) is a ligand-inducible transcription factor that mediates biological responses to halogenated aromatic hydrocarbons. The unliganded AhR is a cytoplasmic, tetrameric complex consisting of the AhR ligand binding subunit, a dimer of hsp90, and the hepatitis B virus X associated protein 2 (XAP2). The role of XAP2 as a member of the AhR core complex is poorly understood. XAP2 shares significant homology with the immunophilins FKBP12 and FKBP52, including a highly conserved, C-terminal, tetratricopeptide repeat (TPR) domain. XAP2 forms a complex with hsp90 and the AhR, but can also bind to both independently. This binding is mediated by the conserved TPR domain. Single point mutations in this region are sufficient to disrupt the association of XAP2 with both the AhR and hsp90 *in vitro* and in cells. Co-transfection of the AhR and XAP2 in COS-1 cells results in increased AhR levels compared with cells transfected with the AhR alone. Co-expression of the AhR with the TPR containing proteins FKBP52, PP5, or XAP2 TPR mutants deficient in binding to the AhR and hsp90, does not effect AhR levels. However co-expression of the AhR with the TPR domain of protein phosphatase 5 (PP5) results in AhR down-regulation. These results demonstrate that XAP2 is apparently unique among hsp90 binding proteins in its ability to enhance AhR levels. A yellow fluorescent protein (YFP)-XAP2-FLAG was constructed and biochemically characterized to demonstrate that no loss of function was detected. YFP-XAP2-FLAG was transiently transfected into primary human fibroblasts and was found to co-localize with cytoskeletal structures when visualized by fluorescence microscopy. Treatment of Hepa-1 cells with the hsp90 binding benzoquinone ansamycin geldanamycin (GA) and the macrocyclic anti-fungal radicicol resulted in AhR, but not XAP2 or FKBP52 turnover. These results suggest that XAP2/hsp90 and FKBP52/hsp90 complexes are similar yet exhibit unique functional specificity.

57 THE ARYL HYDROCARBON RECEPTOR/TRANSCRIPTION FACTOR (AHR) AND THE REL A NUCLEAR FACTOR-κB SUBUNIT COOPERATE TO TRANSACTIVATE THE c-*myc* PROMOTER.

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NF-κB/Rel is a family of dimeric transcription factors that regulates the promoter activities of many genes including those which control cellular proliferation, neoplastic transformation, and apoptosis. For example, the c-*myc* oncogene has two functional NF-κB elements. Rel factors have been found to interact, either as subunits or dimers, with other transcription factors. In particular, the RelA (or p65) subunit of NF-κB was shown to interact with the aryl hydrocarbon receptor/transcription factor (AhR), the latter mediating environmental carcinogen-induced malignant transformation. Recently, we have observed that AhR and NF-κB levels are highly elevated in breast cancer cells and that both transcription factors appear to be constitutively active. Herein, we demonstrate, with the help of co-immunoprecipitations that there is a physical association between RelA and AhR in breast cancer cells. Interestingly, the resulting complex activates c-*myc* gene transcription in these cells as detected by reporter gene analyses and electrophoretic mobility shift assays (EMSAs). Our results show that RelA and AhR proteins could be co-precipitated from the whole cell lysates of pre-malignant MCF-10F breast epithelial cells and from both cytoplasmic and nuclear extracts malignant Hs578T breast cancer cells. *AhR* and *RelA* gene co-transfection studies demonstrated cooperation between these respective gene products in transactivation of the c-*myc* promoter, and that this transactivation was dependent on NF-κB elements in the c-*myc* promoter. Furthermore, a novel AhR/RelA-containing NF-κB element binding complex was identified by EMSAs of nuclear extracts from *RelA* and *AhR* co-transfected Hs578T cells. Thus, the RelA and AhR proteins functionally cooperate to bind to NF-κB elements and transactivate the c-*myc* promoter, suggesting a new mecahnism whereby AhR/ NF-κB-dependent activation of the c-*myc* gene can promote proliferation and neoplastic transformation.

58 CROSSTALK BETWEEN THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA AND PROTEIN KINASE C SIGNALING PATHWAYS.

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The peroxisome proliferator-activated receptors (PPARs) belong to the steroid hormone receptor superfamily, a group of transcription factors regulated through both ligand binding and phosphorylation events. Protein kinases may directly activate PPARs, affect their ability to bind ligands, or activate signaling in the absence of ligand. Preliminary work with protein kinase C inhibitors by other groups has shown decreases in both phenotypic and transcriptional responses of PPARα, although the reason for this "cross-talk" between signal transduction pathways is not clear. The goal of our research is to determine the mechanism of PKC cross-talk with PPARα. The phorbol esters, phorbol-12-myristate-13-acetate (PMA) and phorbol 12,13-dibutyrate (TPA), were shown to activate transcription from a peroxisome proliferator response element-driven luciferase construct (PPRE-Luc), generated using the upstream element of the rat ACO gene. When cells were co-treated with phorbol esters and Wy-14,643, a PPARα-specific activator, an additive effect was observed. Isotype-specific PKC inhibitors were used to show PKCα activity is necessary for Wy-mediated induction of the ACO gene. Rottlerin, a PKCδ-specific inhibitor, was able to inhibit activation by both Wy-14,643 and phorbol esters. In addition, an *in vitro* phosphorylation assay illustrated direct phosphorylation of PPARα by PKCα and PKCβ. These results suggest a direct interaction between PKC and PPARα. (Supported by NIH ES07799 and DK49009.)

59 OPPOSING ACTIONS OF p38- AND p44/p42-MAP KINASE INHIBITORS ON LPS-INDUCED NF-κB-MEDIATED GENE EXPRESSION IN NORMAL RAT KIDNEY EPITHELIAL (NRK52E) CELLS.

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MAP kinases play a central role in propagating stress signals mediated by lipopolysaccharide (LPS) and other toxicants in various cell types. We evaluated the role of p38- and p44/42-MAP kinase signaling pathways in LPS-induced NF-κB activation and NF-κB-mediated transcriptional gene expression in kidney epithelial (NRK) cells, employing SB203580 (SB), a specific inhibitor of p38, and PD98059 (PD), an inhibitor of MEK, the upstream activator of p44/p42 MAP kinases. LPS induces rapid activation and nuclear translocation of NF-κB in NRK cells (TAP 154:219-227, 1999). Neither SB nor PD pretreatment affected LPS-induced NF-κB activation or NF-κB-DNA binding in this cell line. However, SB (25-50 mM) caused a dose-related decrease in NF-κB-mediated transcriptional activity when administered to NRK cells transfected with a NF-κB-driven luciferase reporter gene (pLuc-4xNF-κB) prior to LPS treatment. Conversely, PD (10-30 mM) treatment enhanced LPS-induced luciferase activity by as much as 2-fold compared to treatment with LPS alone. These results indicate that p38 promotes, whereas p44/p42 MAPKs down-regulate, LPS-induced NF-κB-mediated gene expression at the level of transcriptional activation in NRK cells. These findings suggest the integrated regulation of NF-κB-mediated gene expression by MAP kinase pathways in kidney cells. (Supported by ES04696 and ES07033.)

60 A METHOD FOR HARVESTING RESPIRATORY/ TRANSITIONAL EPITHELIAL CELL RNA FROM THE ANTERIOR NASAL PASSAGES OF RATS.

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The transitional and respiratory epithelia lining the lateral meatus and anterior septum of the rat nasal passages are a common site for injury from inhaled chemicals, including the rodent nasal carcinogen, formaldehyde. This anatomical target area is relatively small and presents a challenge when attempting to recover target cells for molecular biological studies. We developed a method for the isolation of airway lining epithelial cells from this site. Nine rats were euthanized and the head removed. Cells lining the nasal airway anterior to the septal window were separated from the underlying lamina propria by instilling 200μL (consistent with reported and our measured nasal volume for this region) of 2% type I collagenase into the anterior nares followed by incubation at 37°C for up to one hour. The head was maintained vertical with the nares down to confine collagenase to the anterior nose. The collagenase/cellular mixture was withdrawn, and the airways were flushed with an additional 200μL saline, and the cellular suspensions were pooled. Cell yield was quantitated microscopically by cytospin and differential stain, and total RNA was isolated from the remaining cellular extract. Approximately $1x10^5$ well preserved, ciliated, non-ciliated, and other epithelial cells were isolated per rat, with a yield of approximately 40μg of high quality total RNA. The heads were processed by routine methods for microscopic examination to verify the extent and success of the epithelial removal. This method of rat nasal epithelial cell isolation can be used to extract RNA, DNA, and proteins for molecular analyses within this important nasal epithelial cell target cell population.

61 MAGNETIC RESONANCE IMAGING (MRI), IMMUNOLOGY AND BIOCHEMISTRY OF MURINE NASAL AIRWAYS EXPOSED TO 2,4-TOLUENE DIISOCYANATE (TDI).

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Little is known about the effect of TDI inhalation, a known cause of occupational asthma, on the nasal passages. A MRI method for monitoring the morphological alterations in the upper airways due to irritant exposure has been developed and tested on mice exposed to chemicals with known pathophysiological changes in the nasal region. In the current study, we employed this technique, along with immunological and biochemical methods to determine the effectiveness of MRI as a technique to detect subtle changes in morphology resulting from TDI exposure and to gain insight into the mechanism of response. C57BL6 and Swiss Webster mice were exposed to 2,4-TDI for 2ppm hr and 1ppm hr, respectively. MR images were acquired before and at several time points after exposure. Nasal lavage, serum collection and fixation of the nasal passages were performed on subsets of mice at each time point. MRI detectable alterations were observed in C57BL6 mice after the 2ppm hr exposure while no detectable changes were seen in the Swiss Webster strain. Specific antibodies to a TDI-mouse serum albumin conjugate were found in sera from both strains at 2 and 4 weeks post exposure. Difference protein mapping of nasal lavage fluid as a function of time post exposure shows increases and decreases of a variety of proteins. Immunological and biochemical responses appear to occur at exposure levels where morphological changes are not detectable by MRI.

62 A SIMPLE MODEL FOR INTERSPECIES TISSUE DOSE COMPARISONS FOR VAPORS DEPOSITED IN THE NASAL CAVITY.

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A simple hybrid computational fluid dynamics (CFD) and physiologically based pharmacokinetic (PBPK) dosimetry model has been constructed to evaluate the tissue concentrations of a variety of vapors that may be deposited in the mammalian nasal cavity. The CFD-PBPK model incorporates a one-compartment description of the nasal cavity which serves as a portal of entry for inhaled vapors prior to distribution to systemic tissues. Although the model incorporates a dynamic description of mass transport across the air:mucus interface in the nasal cavity, it can also simulate 'C = 0' boundary conditions used for CFD simulations. Under these conditions, it accurately replicated CFD simulations conducted at a variety of flow rates with models of both the rat and human nasal cavity. In addition, the CFD-PBPK model accurately replicated experimental data on the nasal deposition of a wide variety of vapors under unidirectional flow in the rat nasal cavities. Comparison of the model predictions for the average rat and human nasal tissue concentrations following simulated inhalation exposures to a wide range of vapors (acid, alcohol, ketone, etc.) indicated that this ratio (often referred to as an 'interspecies extrapolation factor') was approximately one. Although the simple one-nasal-compartment CFD-PBPK model is useful for comparing average nasal and systemic tissue concentrations, the complex geometry of the mammalian nasal cavity may require more complex CFD-PBPK models to correlate regional tissue concentrations with the regional nasal lesions that often characterize inhalation exposure to toxic vapors.

63 BIOCHEMICAL AND PROLIFERATIVE CHANGES IN RESPIRATORY TISSUES OF RATS EXPOSED TO METHYL *tert*-BUTYL ETHER.

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Approximately 25% of gasoline sold in the United States is formulated with methyl tert-butyl ether (MTBE), a fuel oxygenate which is blended into gasoline at concentrations of 11-15% by volume. Health complaints suggestive of respiratory effects have been reported in areas where gasoline containing MTBE has been introduced. However, studies assessing MTBE's respiratory effects have been limited. Analysis of bronchoalveolar lavage fluid (BALF) and nasal lavage fluid (NLF) is considered to be a sensitive tool in assessing suspected respiratory toxicants. In this study, rats were exposed to MTBE (300 ppm for 6 hours) and sacrificed both immediately and 24 hours following cessation of exposure. Significant increases in sialic acid (231%), lactate dehydrogenase (106%), and acid phosphatase (246%) were seen in NLF of rats sacrificed 24 hours post-exposure to MTBE. No changes in gamma-glutamyl transferase or total protein were seen in NLF, either immediately following, or 24 hours post-exposure to MTBE. In addition, no changes in any of the above lavage fluid parameters were seen in BALF following exposure to MTBE at either time point, suggesting that toxicity is limited to the upper respiratory tract. Increased cell proliferation, as a repair mechanism, is also indicative of tissue injury. An increased level of proliferating cell nuclear antigen (PCNA) was also seen in respiratory mucosa of the nasal cavity, both immediately following (16%) and 24 hours following (61%) exposure to MTBE. No increases in PCNA were seen in the olfactory mucosa of the nasal cavity or the lung. The nose, especially the anterior respiratory mucosa, receives the highest dose of an inhaled xenobiotic. Biochemical changes in NLF suggestive of nasal irritation, mucous hypersecretion, cytotoxicity, and inflammation, and accompanying proliferative changes in respiratory mucosa indicate that MTBE may indeed be a nasal irritant and/or toxicant.

64 THE RELATIONSHIP OF PROTEIN CARBONYL LEVELS TO INFLAMMATORY RESPONSES IN BRONCHOALVEOLAR LAVAGE FLUID IN RATS, MICE, AND HAMSTERS FOLLOWING INHALED PIGMENTARY TITANIUM DIOXIDE.

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Protein carbonyls occur from the oxidative modification of amino acid residues and have been postulated to result from oxidants associated with inflammatory disease states. We hypothesized that elevated levels of protein carbonyl groups in the bronchoalveolar lavage fluid (BALF) may serve as a biomarker of oxidative stress in pulmonary disease. Exposures of laboratory rodents to high airborne concentrations of relatively noncytotoxic poorly soluble particles (PSP) leads to pulmonary inflammation, which may contribute to the production of protein carbonyls. The objective of the present study was to compare the BALF protein carbonyl levels in three rodent species following a subchronic particle exposure to pigmentary titanium dioxide (TiO_2), a well-studied PSP. Female Fischer 344 rats, B6C3F1 mice, and Syrian golden hamsters were exposed by whole-body inhalation to TiO_2 concentrations of 0, 10, 50, or 250 mg/m$_3$. Groups of animals were exposed for 6 h/d, 5 d/wk for 13 wk and allowed to recover for up to 26 wk. Inflammatory responses and protein carbonyl levels in BALF were determined at the termination of the 13-wk exposure then at 4, 13, and 26 wk postexposure. Carbonyl levels determined using an ELISA were significantly increased in rats exposed to 50 and 250 mg/m$_3$ of TiO_2, while mouse and hamster levels were elevated only following 250 mg/m$_3$. Elevation in carbonyl levels reflected similar patterns in BALF-associated cytologic and biochemical inflammatory indices. Inflammatory changes in all three species were limited to animals exposed to extremely high concentrations. These results suggest that the measurement of protein carbonyls in BALF may be a useful biomarker of particle-induced oxidant damage in conjunction with other oxidative end points. (Supported in part by the Chemical Manufacturers Association and the European Chemical Industry Council.)

65 CYTOTOXIC AND GENOTOXIC RESPONSES OF BUTADIENE SOOT DMSO EXTRACT (BSDE) IN CULTURED NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELLS.

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Flaring of low molecular weight volatile organic compounds (e.g., 1,3-butadiene) at petrochemical plants produces soot containing a complex mixture of polycyclic aromatic hydrocarbons (PAHs). These include not only established carcinogens (e.g., benzo[a]pyrene) but also a range of cyclopenta-fused PAHs that are suspected carcinogens. The toxicological properties of these mixtures have not been well established. We are characterizing the cytoxicity and genotoxicity of BSDE *in vitro* using NHBE cells, the main target cell type for the development of lung cancer. Single-dose acute exposure of NHBE cells to BSDE for up to 72 hr resulted in the following effects that were not detected in vehicle-exposed control cells: 1)dose-dependent cytotoxicity as determined by clonal growth assays; 2)extranuclear cellular fluorescence (Ex.=365 nm; Em.>420 nm) presumably from the uptake of aromatic compounds and/or the formation of oxidized molecular complexes; 3)dose-dependent morphological changes including cell swelling and plasma membrane blebbing which are indicative of oncosis; 4)loss of cell organelles, an increased number of intracellular vacuoles and accumulation of membrane-limited extracellular material, as visualized by transmission electron microscopy; and 5)significantly elevated levels of binucleated cells, a marker of genotoxicity. These results suggest that inhalation exposure to industrially-generated soots may be significant from both the public and environmental health perspectives. It would seem that further toxicological studies on BSDE and related complex mixtures are warranted.

66 PULMONARY TOXICITY STUDY OF LUNAR AND MARTIAN DUST SIMULANTS INTRATRACHEALLY INSTILLED IN MICE.

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NASA is contemplating sending humans to Mars and the Moon for further exploration. The properties of Hawaiian and Californian volcanic ashes allow them to be used to simulate Martian and lunar dusts, respectively. NASA laboratories use these dust simulants to test performance of hardware destined for Martian or lunar environments. Workers in these test facilities are exposed to low levels of these dusts. The present study was conducted to investigate the toxicity of these dust simulants. Particles of respirable-size ranges of lunar simulant (LS), Martian simulant (MS), TiO2 (negative control) and quartz (positive control) were each intratracheally instilled (saline as vehicle) to groups of 4 mice (C57BL, male, 2-3 month old) at a single treatment of 1 (Hi dose) or 0.1 (Lo dose) mg/mouse. The lungs were harvested at the end of 7 days or 90 days for histopathological examination. Lungs of the LS-Lo groups had no evidence of inflammation, edema or fibrosis. The LS-Hi-7d group had mild to moderate acute inflammation, and neutrophilic and lymphocytic infiltration; the LS-Hi-90d group showed signs of chronic inflammation and some fibrosis. Lungs of the MS-Lo-7d group revealed mild inflammation and neutrophilic and lymphocytic infiltration; the MS-Lo-90d group showed mild fibrosis and particle-laden macrophages (PLM). Lungs of the MS-Hi-7d group demonstrated mild to moderate inflammation and large foci of PLM; the MS-Hi-90d group showed chronic mild to moderate inflammation and fibrosis. To mimic the effects of the oxidative and reactive properties of Martian soil surface, groups of mice were exposed to ozone (3 hour at 0.5 ppm) prior to MS dust instillation. Lung lesions in the MS group were more severe with the pretreatment. The results for the negative and positive controls were consistent with the known pulmonary toxicity of these compounds. The overall severity of toxic insults to the lungs were TiO2< LS<MS<Quartz. For the mice in the 90-d study, blood samples were taken for immunotoxicity study. Antinuclear antibodies (such as those against Golgi, kinetichore, and centromere) were detected in serum in a greater extent in mice treated with LS than those with quartz, suggesting that LS, like quartz, has the potential of inducing antoimmune disease.

67 DEPOSITION AND DISPOSITION OF LARGE, HUMAN-RESPIRABLE PARTICLES IN THE LUNGS OF RATS.

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The predictions of a recently developed multiple-path computer model for lobar deposition of inhaled particles (S. Anjilvel and B. Asgharian, Fundam. Appl. Toxicol. 28, 41-50,1995) were evaluated in the rat for particles with an aerodynamic diameter larger than 2 μm. Two groups of 6 Long-Evans rats were exposed by endotracheal inhalation once for 30 min to an aerosol mixture of 3-, 6-, and 9-μm polystyrene latex beads. Approximately 24 hr after the exposure, the lungs were harvested, and each lobe was dissolved with strong base (TEAH) overnight. Particle counts were made using a hemacytometer viewed through a standard light microscope. The endotracheal inhalation exposure system was capable of delivering particles 3, 6, and 9 μm in diameter into the lungs of rats. The lobar distribution pattern of the 3-μm particles was fairly consistent among animals. The distribution of 3-μm particles in the alveolar region of the left lobe appeared to be underestimated by the dosimetry model with marginal significance. The model significantly overestimated the alveolar deposition of 3-μm particles in the right diaphragmatic and intercostal lobes. The 6-μm bead deposition pattern was very similar to the 3-μm bead deposition pattern. There were not enough 9-μm diameter particles present to compare among samples.

68 COMPARISON OF PULMONARY RESPONSE TO INHALED AND INTRATRACHEALLY INSTILLED DIESEL EXHAUST PARTICULATE.

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Diesel exhaust particulate (DEP) is a complex mixture of polyaromatic hydrocarbons, ash, transition metals, and carbon core. Exposure levels of DEP up to 2 mg/m3 has been documented in underground mines, railroad, construction, and auto repair industries. Such exposures have been associated with pulmonary inflammation, fibrosis, lung cancer, increased rate of respiratory infections, and enhanced allergic sensitization. The purpose of this study was to compare the effect of the inhaled (4hr exposure, 17mg/m3) and intratracheally instilled (IT) DEP (5mg/kg b. wt.) and carbon black (CB, 34 mg/m3) particulate on pulmonary inflammation, pulmonary associated lymph node cells (LNC) and alveolar macrophages (AM) in a Brown Norway rat model. Carbon black was used as a surrogate of the non-extractable carbon core of DEP. Cellular Cysteine (CYS) and glutathione (GSH) levels were analyzed by conjugation to monobromobimane and subsequent HPLC-fluorescent analysis. Alveolar macrophage IL-1 and TNF- production were also assessed three days post exposure. Both, DEP and CB were generated by nebulizing suspended particles through a diffusion dryer into a nose-only chamber. Greater than 90% of the particles generated were in the submicron range. The microscopic evaluation of the DEP and CB particulate revealed similarities in shape and size between them. Inhaled DEP caused a significant increase in the levels of glutathione and cysteine in the LNC, but not AM. LNC thiols were unchanged following IT DEP, but AM GSH was elevated. A significant decrease of AM TNF- of DEP exposed rats after ex vivo endotoxin challenge was also observed in both IT and inhalation groups. Instillation of DEP caused pulmonary inflammation as noted by the increases in neutrophils count, and lavage protein and LDH levels. Inhaled DEP did not produce measurable pulmonary inflammation. The cause of the differences observed between acute inhalation vs IT DEP in pulmonary inflammation and thiol content is not known, but may be due to dose deposited in the lung.

69 CHRONIC INHALATION OF ROOM-AGED CIGARETTE SIDE-STREAM SMOKE (RASS) AND DIESEL ENGINE EXHAUST (DEE) IN RATS - EFFECTS ON LEUKOCYTE SUBPOPULATIONS IN BLOOD AND BRONCHOALVEOLAR LAVAGE FLUID (BALF).

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The immuno-modulating effects of RASS, an experimental surrogate for environmental tobacco smoke (ETS), and DEE were investigated in a 24-month inhalation study in rats. The particulate matter concentrations chosen (3 and 10 μg/l) exceed the upper limit of typical ETS and DEE exposure concentrations for humans by approximately 100-fold. Leukocyte and lymphocyte subpopulations in blood and BALF were analyzed by microscopic and flow cytometric analysis. The following changes were observed in DEE-exposed, but not in RASS-exposed rats: In blood, there was a small increase in the (absolute and relative) number of neutrophils and a corresponding decrease in the percentage of lymphocytes, but no changes in blood lymphocyte subpopulations. In BALF, there was an increase in relative and absolute numbers of neutrophils from 1% or 1E5 cells in sham controls to up to 60% or 8E7 cells in the high DEE group. Corresponding lymphocyte numbers were 1.5% or 2E5 in sham controls, 8% or 3E6 in the low DEE group, and 5% or 6E6 in the high DEE group. These changes indicate a massive pulmonary inflammation. Within the BALF lymphocyte population, the T suppressor/cytotoxic cells were slightly increased. These results show that leukocyte subsets are more sensitive than lymphocyte subsets to chronic DEE inhalation. The major effects are restricted to the lungs as the port of entry and do not have relevant systemic consequences. RASS, which has an organic composition relatively similar to DEE, did not produce any immuno-modulating effects at the same particulate matter concentration. The immuno-modulating effects seen in DEE-exposed rats are considered as to be associated with the pulmonary accumulation of elemental carbon particles which was not seen in RASS-exposed rats. (Sponsored by Philip Morris USA.)

70 AHR AND PM10.

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Ah receptor mediated activity associated with PM10 extracts from the Paso del Norte airshed.The Paso del Norte airshed, which includes El Paso, TX, Ciudad Juarez, Mexico, and Las Cruces, NM, has historically high levels of PM10. The extensive use of wood and scrap material for fuel, an aged vehicular fleet, and rapid urban development associated with maquiladoras and NAFTA, have contributed to this problem, which is compounded by winter inversion layers. As a first step in an effort to identify biomarkers that reflect contaminants in this airshed, PM10 filters were collected from six monitoring sites located in El Paso and Ciudad Juarez. Filters from Jan. 1999 were extracted with dichloromethane for 24 hours in a Soxhlet apparatus and extracts were concentrated with a Kuderna-Danish per EPA method 3540C. To assess the biological effects of Ah receptor ligands associated with PM10, H4IIE rat hepatoma cells were exposed in 6-well plates to extracts containing the equivalent of 1 and 4 weeks of respirable air (air equivalents) for 24 hours and ethoxyresorufin-O-deethylase (EROD) activity was measured in the post-mitochondrial supernatant. Control activity was 1.55 pmol-1min-1mg prot; 1 μM b-naphthoflavone (BNF), the positive control, caused a 6½ fold increase. Variability in EROD activity was greatest in cells exposed to 1-week air equivalents from a brickmaking district, where as great as a 4 fold difference in enzyme activity (2.76-11.65 pmol -1min-1mg prot.) was observed on different dates. These differences are likely to be associated with varying patterns of activity in this cottage industry. Greater than 1-week air equivalents from this site inhibited enzyme activity by 50%. At sites primarily associated with car and truck traffic, EROD activity remained fairly constant (2.4-4.8 pmol-1min-1mg prot.) regardless of date. Filters from all winter months, are presently being analyzed for EROD activity and Ah receptor ligands with a luciferase reporter system.

71 DEPOSITION OF MONODISPERSE AEROSOLS IN YOUNG BEAGLE DOGS.

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The effects of inhaled particles are determined by chemical composition and sites of deposition. The shape, density and size of the particles determine not only sites of deposition in the respiratory tract but also the fraction of inhaled particles that are deposited. Deposition of monodisperse aerosols (1 to 9 microns Mass Median Aerodynamic Diameter (MMAD)) was measured in young unsedated 4 and 7 month old Beagle dogs using mouth-breathing and nose-breathing models. Iron oxide aerosols, with ^{99m} Technetium tracer, were produced using a spinning top aerosol generator (STAG). Particle size was controlled by varying the concentration of the iron oxide suspension and the speed of the STAG rotor. Satellite particles were removed by adjusting the exhaust flow. Animals were acclimated to the mouth-breathing and nose-breathing masks prior to aerosol exposures and were exposed to aerosols of approximately 1, 3, 5, 7 or 9 microns MMAD for up to ten minutes. Deposition was determined using gamma camera scintigraphy. Scintography images were taken immediately after exposure. Areas of interest in the images were delineated and data reported for both total and regional deposition as a function of particle size. For the 7 month old Beagle dogs lung deposition was

greater at 3 microns than at 1 micron, whereas for the younger dogs lung deposition was greatest at 1 micron. The deposition over the one to three micron range in the 7 month old male Beagles was comparable to that previously published by Cuddihy (1973) for adult males. Deposition in the 4 month old dogs was lower at all particle sizes than in the 7 month old males; deposition in the 7 month old females was intermediate in value. Research Sponsored by: B Stuart, Shering-Plough; R Wolff, Eli Lilly; H Marchner, Astra; D Alexander, GlaxoWellcome and C Leach, 3M Pharmaceuticals.

71A CLEARANCE OF DEPOSITED AEROSOLS FROM YOUNG BEAGLE DOGS.

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The effects of deposited particles are determined by the chemical composition of the particles, the sites of deposition and the rate of clearance from the respiratory tract. Measurements were made of deposition and clearance of monodisperse aerosols over the range 1 to 9 microns Mass Median Aerodynamic Diameter (MMAD) in young unsedated 4 months and 7 months old Beagle dogs using mouth-breathing and nose-breathing models. Iron oxide aerosols, with ^{99m}Technetium tracer, were produced using a spinning top aerosol generator (STAG). Animals were acclimated to the mouth-breathing and nose-breathing masks prior to aerosol exposures, and were exposed to aerosols of approximately 1, 3, 5, 7 or 9 microns MMAD for up to ten minutes. Deposition was determined using gamma camera scintigraphy. Scintigraphy images were taken immediately after exposure, and at approximately 1 hour, 2 hours and 4 hours post-exposure. Areas of interest in the images were delineated and clearance subsequent to deposition was monitored. All data were corrected for both attenuation by the body and radioactive decay during the measurements. Except for a peak in the gastrointestinal tract data for the nose-only exposed animals, presumed to reflect swallowing, the clearance data fell on a single curve for all animals. Thus the rate of clearance of the labeled iron oxide particles appeared to be independent not only of animal age and sex but also of particle size between 1 and 9 microns MMAD. (Research Sponsored by: B. Stuart, Shering-Plough; R. Wolff, Eli Lilly; H. Marchner, Astra; D. Alexander, Glaxo Wellcome; and C. Leaach, 3M Pharmaceuticals.)

72 AIR POLLUTION AND ELEMENTARY SCHOOL ABSENTEEISM.

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This study assessed the association between ambient air pollution and daily elementary school absenteeism in Washoe County, NV from 1996-1998. All 57 elementary schools in Washoe County were included. There was a total of 28,829 enrollments during the study period. The daily average absence rate was 5.09%. Air pollutant values including PM_{10}, O_3 and CO were obtained from seven air monitoring stations. Weather variables were collected from five of these stations and from the Western Regional Climate Center. The daily average concentration of PM_{10}, CO and O_3 was 32.44 μg/m^3, 2.73 ppm and 37.45 ppb respectively. The absence rate was regressed on the three air pollutants, weather variables and other confounding factors, using Autoregression analysis. Although the results showed that many factors could affect school absenteeism, after adjusting for the effects of weather variables, day-of-the-week, month and holiday indicators, and time trend, the results showed that CO and O_3 were statistically significant predictors of daily absenteeism in elementary schools. For every 1 ppm and 50 ppb increase in CO and O_3, the absence rate would increase 3.79% (95% CI 1.04-6.55%) and 13.01% (95% CI 3.41-22.61%) respectively. PM10 values, however, were negatively correlated with school absenteeism. (The support and cooperation of the Washoe County School District is gratefully acknowledged.)

73 PARTICULATE AIR POLLUTION AND HOSPITAL ADMISSIONS FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN RENO, NEVADA.

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This study assessed the association between ambient PM_{10} pollution and daily hospital admissions for Chronic Obstructive Pulmonary Disease (COPD) in Reno-Sparks, Nevada, for the period 1990-1994. All three hospitals in the region were included. There was a total of 3115 admissions for COPD during this period. Ambient PM_{10} values were obtained from one of seven air monitoring stations. Weather variables were also collected. The daily average concentration of PM_{10} was 36.55 μg/m^3. The Generalized Additive Model (GAM) was used in the analysis. After adjusting for the effects of weather variables, day-of-the-week, seasons and time trend, the results show that PM_{10} is a statistically significant predictor for daily hospital admissions for COPD. The relative risk (RR) of hospital admissions for COPD for an interquantile increase (26.6 μg/m^3) of the 24-h average level for PM_{10} is 1.049 (95% CI 1.011-1.087) which suggests that for every 26.6 μg/m^3 increase in the ambient PM_{10} level, there would be 31 more hospital admissions for COPD every year (95% CI: 7-55).

73A URBAN STRAY DOGS AS AN INDICATOR SPECIES FOR MONITORING CHRONIC EXPOSURES TO AIR POLLUTION: PREDICTIVE VALUE FOR CHILDREN? SHOULD WE BE CONCERNED?

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A complex mixture of air pollutants is present in the ambient air in Mexico City. People and animals are exposed chronically and sequentially to outdoor pollutants. The principal objective of this study was to evaluate the lung histopathology and heavy and trace metal concentrations in Mexico City (MC) young (<1 y) and old (>5y) dogs from two different geographic areas with marked differences in the concentrations of criteria pollutants. Control dogs were included. Clinically healthy 94 stray dogs (49M/45F) including 19 controls and 75 from Southwest and Northeast MC were studied. Control dogs had unremarkable histological findings. Lung lesions were observed in all MC dogs regardless of age, gender and geographical source. Major findings included peribronchiolar and perivascular clusters of macrophages loaded with particulate material, increased numbers of intra-alveolar particle-laden macrophages, hyperplasia and hypertrophy of nonciliated bronchiolar cells with luminal accumulation of macrophages and decreases in luminal diameters, and replacement of type 1 alveolar cells with type 2 cells. Heavy and toxic trace metal lung analysis revealed significant differences in metals such as Pb between SW(residential area) and NE(industrial area) dogs and accumulation of metals in young vs older animals. Rb,Br,V,Cr and Co were detected in MC dogs. Chronic exposure to a polluted environment is causing significant lung damage in MC dogs. The lesions described in naturally exposed dogs may be a model for the early steps in human fibrotic/emphysematous/neoplasic lung diseases. Deep concern exist for similar lung lesions and metal deposition in children with a lifelong exposure to the same environment. This experiment was conducted on tissues obtained from pound dogs euthanatized for reasons other than this study. Euthanasia was conducted in accordanace with established guidelines of the American Veterinary Medical Association (J. Am. Vet. Med. Assoc. 202:229-249, 1993) and in accordance with applicable animal care and use regulations. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

74 ASBESTOS AND NON-ASBESTOS FIBER CONTENT IN LUNGS OF KOREAN SUBJECTS WITH NO KNOWN OCCUPATIONAL ASBESTOS EXPOSURE HISTORY.

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To provide an estimation of asbestos exposure among normal Koreans and a criterion for determining occupational asbestos exposed diseases, pulmonary fiber contents of both asbestos and non-asbestos types were evaluated in 25 Korean subjects 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 males and females were 0.3×10^6 fibers/g of dry lungs and 0.15×10^6 fibers/g of dry lung tissue. Non-asbestos fiber contents found in males and females were 8.02×10^6 fibers/g of dry lungs and 7.85×10^6 fibers/g of dry lungs, respectively. There was no difference in asbestos content in lung among various age groups. Comparing these results with Japanese male (2.11×10^6 fibers/g of dry lung) and female subjects (1.38×10^6 fibers/g of dry lung), Korean male and female subjects had much less asbestos in their lungs.

75 DEVELOPMENT OF AEROSOL SAMPLING AND ANALYTICAL METHODS FOR MAN-MADE ORGANIC FIBERS.

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The purpose of this study was to compare the results of airborne organic fiber measurements using different collection modes and counting techniques. Atmospheres of p-aramid or cellulose respirable fiber-shaped particulates (RFP) were generated in an inhalation chamber. For each experiment, 30 filters (10 gold-coated polycarbonate filters, 10 methyl cellulose filters, and 10 methyl cellulose filters and corresponding cowls) were exposed to fibrous aerosols at concentrations ranging from 25 - 50 f/cc. Subsequently, attempts were made to count and compare each of the filters by three optical techniques, namely light microscopy (LM) (PCOM- NIOSH 7400) scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Preliminary results demonstrated that the gold-coated polycarbonate filters were not appropriate for counting by LM and TEM. In addition, washing the cowl onto the same filter did not contribute greater numbers of fiber counts and seemed to facilitate clumping. When comparisons were made between LM, SEM, and TEM counts on the same filters, the fiber numbers were not significantly different when evaluated via the different analytical techniques. These findings are in contrast with the preliminary data of other investigators who have reported a 2-5 x increase in the numbers of organic fibers when counted by SEM vs. PCOM. Studies are ongoing to resolve the differences between analytical methods for counting organic fibers.

76 COMPARISON OF HISTOPATHOLOGY IN RATS SUBCHRONICALLY EXPOSED TO SMOKE FROM CIGARETTES THAT BURN OR PRIMARILY HEAT TOBACCO.

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A subchronic nose-only inhalation study comparing the effects of smoke from a cigarette that primarily heats tobacco (Eclipse™ prototype 9-014), to that of smoke from a Kentucky 1R4F reference cigarette was conducted using Sprague-Dawley rats. Rats of each sex were exposed 1 hour a day, 5 days per week, for 13 weeks to mainstream smoke at 0, 0.16, 0.32, or 0.64 mg/liter of air. Endpoints were carboxyhemoglobin (HbCO), serum nicotine, clinical signs, body and organ weights, serum chemistry, hematology, and histopathology. Neither cigarette produced adverse changes in clinical signs, body and organ weights, serum chemistry, or hematology. HbCO and serum nicotine increased with both cigarettes in an exposure-dependent manner. Smoke related histologic changes were nasal chronic/active inflammation and epithelial hyperplasia, chronic/active inflammation of the larynx, squamous metaplasia of the ventral larynx, tracheal epithelial hyperplasia, increased goblet cells of the lung, and increased non-pigmented and brown/gold pigmented lung macrophages. Overall, the evaluation of histologic changes in rats exposed to smoke of Eclipse cigarettes indicated a reduction of biological activity compared to the smoke of the tobacco burning 1R4F cigarette.

77 EFFECTS OF THE ADDITION OF FLAVOR INGREDIENTS TO THE TOBACCO ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF CIGARETTE SMOKE.

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Cigarette mainstream smoke from American-blend test cigarettes with and without the addition of flavor ingredients was analyzed for its chemical composition. In total, more than 300 commonly used flavors were assigned to three different flavor mixtures. The flavors were incorporated into the cigarettes at two levels that exceed normal use levels. An unflavored cigarette served as control. The smoke constituents analyzed included those proposed by the US Consumer Product Safety Commission and additional smoke constituents classified as human or animal carcinogens by the International Agency for Research on Cancer. The results obtained for genotoxicity (Ames Assay), cytotoxicity (Neutral Red Assay), and inhalation toxicity (90 d, rat inhalation) of the smoke are compared relative to that of the control cigarette. The addition of the flavor ingredients caused an increase of approximately 20% in the yields of total particulate matter and tar per cigarette compared to the unflavored control. On a per mg TPM basis a general decrease was seen for most of the smoke constituents analyzed. This reduction was especially high for phenols (up to 70%), polycyclic aromatic hydrocarbons (50%), and N-nitrosamines (45%). Only a few constituents were increased: HCN, cadmium, formaldehyde, and resorcinol (approx. 30%). Neither the *in vitro* mutagenicity and cytotoxicity nor the *in vivo* inhalation toxicity were increased by the flavor ingredients added. The overall assessment suggests that the flavor ingredients do not increase the biological activity of the cigarette smoke, even at the exaggerated levels tested in the present series of studies.

78 THE INFLUENCE OF A MODIFIED PUFFING REGIMEN ON THE YIELDS OF SMOKE CONSTITUENTS FROM ELECTRICALLY HEATED AND CONVENTIONAL RESEARCH CIGARETTES.

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The influence of puff volume and frequency on the yields of a selection of toxicologically relevant constituents in the smoke of an electrically heated cigarette (EHC) was investigated and compared to that of the conventional reference cigarettes 1R4F (representative of the low-tar market segment) and 1R5F (ultra low-tar). For the reference cigarettes, the influence of vent blocking was also analyzed; the EHC has no vents. At a puff volume of 55 ml with 2 puffs/min, total particulate matter (TPM) yields increased 1.8-, 2.5-, and 3.5-fold over those obtained under ISO conditions (35 ml, 1 puff/min) for the EHC, 1R4F, and 1R5F, respectively. Very similar to these ratios are the median ratios (and 25th; 75th percentiles) obtained for the more than 20 smoke constituents determined, i.e., 1.5 (1.4; 1.6) for EHC, 1.9 (1.8; 2.1) for 1R4F, and 3.4 (3.2; 3.8) for 1R5F. Relative to the tar yield of these cigarettes, these median ratios were 0.92 (0.84; 0.96), 0.85 (0.82; 0.91), and 0.96 (0.90; 1.11). Additional complete vent blocking increased the TPM yields of the 1R4F by 3.1-fold and that of the 1R5F by 7.8-fold compared to ISO conditions. The corresponding median ratios of all smoke constituents analyzed were 2.3 (2.1; 2.6) and 5.4 (4.6; 7.5) and, relative to the tar yield, 0.90 (0.82; 1.03) and 0.83 (0.71; 1.19). There were only a few constituents that did not follow this general trend. The ratios obtained for the TPM and tar yields were generally higher than the median ratios for the individual smoke constituents. Thus, TPM and tar would seem to be critical and valid indicators of changes overall. Due to the construction principle of the EHC, the yields of almost all toxicologically relevant smoke constituents are relatively low compared to those of the reference cigarettes, and a modified puffing regimen can only result in limited changes.

79 COMPARISON OF TWO EXPOSURE REGIMENS IN RAT SUBCHRONIC INHALATION STUDIES WITH CIGARETTE SMOKE.

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Part of the testing of cigarette modifications is an *in vivo* subchronic mainstream smoke inhalation study in rats often performed in basic accordance with OECD guideline 413. The testing entails the determination of relevant and sensitive endpoints and has been shown to be able to detect modification-induced differences in biological activity. The aim of this study was to compare two different exposure regimens: 6 hours, 7 days/week (proposed in guideline 413) vs. 2x1 hour, 5 days/week (new). In order to do this, the biological activity of mainstream smoke from the Reference Cigarette 1R4F (about 9mg tar) and the 1R5F (about 2mg tar) was determined. Qualitatively, there was no difference between the findings obtained with the different regimens. Furthermore, the findings were similar to those reported in the literature (e.g., reserve cell hyperplasia, squamous metaplasia, and atrophy at some sites in the upper respiratory tract). Both exposure regimens discriminated two reference cigarettes with the same 15% difference. For equal weekly Cxt, grading of histopathological findings for the two cigarettes were higher in the shorter exposure regimen. In addition, the number of statistically significant differences to sham-exposed control was also higher in the shorter exposure regimen. This shorter exposure regimen provides a better basis for discrimination between test cigarettes. These results indicate that exposure regimens other than the one proposed in guideline 413 are suitable options for cigarette smoke inhalation studies. (Sponsored by Philip Morris USA).

80 AGE RELATED ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY LIPID PEROXIDATION AFTER EXPOSURE TO JP-8 + 100 BLEND JET FUEL.

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The standard operation of military aircraft requires the use of several potentially hazardous chemicals. JP-8 + 100 blend jet fuel is an obvious necessity for aircraft function; however, chronic exposure to JP-8 + 100 blend jet fuel is common among personnel working on the flight line, engine mechanics, and pilots. The age range of these personnel can range from 18 years to 55 + years of age. We have demonstrated that exposure to JP-8 + 100 blend jet fuel induces significant alterations in lung permeability and pulmonary functions in our mouse exposure model. Our mouse model of JP-8 + 100 blend jet fuel exposure historically has ranged in age from 3-4 months. We therefore have conducted studies to determine the age-related effects of JP-8 + 100 blend jet fuel inhalation on the pulmonary microenvironment in aged animals. We exposed aged (12-14 months) and young (3-4 months) mice to 1000 mg/m^3 JP-8 + 100 blend jet fuel for seven days for one hour per day. Animals were sacrificed 24-30 hours after the final exposure and pulmonary functions (dynamic compliance and pulmonary resistance), lung permeability, histological analysis, and bronchoalveolar lavage fluid (BALF) were analyzed. Aged mice exposed to JP-8 + 100 blend jet fuel demonstrated significant alterations in pulmonary functions ($p<0.05$), lung permeability ($p<0.001$), and BALF levels of inflammatory mediators (TNF-alpha) and lipid peroxidation products (PGE_2 and 8-iso-PGF_2) ($p<0.0001$) compared to aged controls and young mice exposed to JP-8 + 100 blend. Histological analysis of lungs from aged animals exposed to JP-8 + 100 jet fuel demonstrate significantly increased alveolar epithelial injury compared to aged controls and young mice exposed to JP-8 + 100 blend jet fuel. We conclude that JP-8 + 100 blend jet fuel-induced lung injury is exacerbated in aged populations. (This work supported by the Air Force Office of Scientific Research Grant # F49620-94-1-0297 and by the U.S. Department of Defense Training Grant [AASERT].)

81 ACUTE INHALATION TOXICITY OF *CIS* AND *TRANS* ISOMERS OF 1,2-DICHLOROETHYLENE IN RATS.

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Trans-1,2-Dichloroethylene (t-DCE) is used as an intermediate in the production of chlorinated chemicals and as a solvent, the cis isomer (c-DCE) occurs in some t-DCE processes. The acute toxicities of t-DCE and c-DCE were evaluated in Crl:CDBR male and female rats. The liver, kidney, heart, and lungs were evaluated histologically in rats that died during exposure or that survived a 14-day recovery period. The LC50s for the cis and trans isomers were 13,700 and 24,100, respectively. During exposures to each isomer above 12,000 ppm, the rats were prostrate and showed a lack of response to an alerting stimulus. Dose-related, centrilobular fatty changes were observed in the livers of male rats exposed to 13,500 and 15,700 ppm of c-DCE, but no compound-related effects were observed in the livers of rats exposed to t-DCE at concentrations up to 34,100 ppm. No effects were observed in the heart, kidney, or lung in rats exposed to either isomer. The lack of effects in the heart contrasts with an earlier report of cardiac effects with single exposures to DCE (unspecified isomers) in rats. It appears that c-DCE is approximately twice as toxic as t-DCE on an acute inhalation basis.

82 IS TOTAL MASS OR MASS OF ALVEOLAR-DEPOSITED AIRBORNE PARTICLES OF BERYLLIUM A BETTER PREDICTOR OF THE PREVALENCE OF DISEASE? A PRELIMINARY STUDY OF A BERYLLIUM PROCESSING FACILITY.

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Cases of chronic beryllium disease (CBD) and beryllium (Be) sensitization continue to be identified among Be industry workers. The currently accepted method for measuring exposure, which involves measuring the total mass of airborne Be per cubic meter, shows an inconsistent dose-response relationship with the prevalence of CBD. This study was conducted to evaluate which Be aerosol characteristics other than total mass may be more informative in understanding the dose-response relationship between exposure to Be and disease. Personal samplers (n=53) using Andersen impactors and area samplers (n=55) using microorifice uniform deposit impactors (MOUDIs) were used to collect airborne Be samples were collected in five furnace areas at a Be manufacturing facility where prevalence rates of CBD and Be sensitization had been previously studied among 535 employees with significant Be exposure. The concentrations were expressed in terms of total mass per cubic meter, and in forms of mass, number, and surface area of particles per cubic meter that are predicted to deposit in the alveolar region of the lung. Tests for linear trend of the relationships of the various exposure metrics to prevalence of CBD and sensitization showed a highly significant association between mass, number and surface area concentration of particles predicted to deposit in the alveolar region of the lung and CBD and sensitization. However, no other exposure parameters showed significant relationships with CBD or Be sensitization. These results suggest that the concentration of alveolar-deposited particles is be a more relevant exposure metric for predicting the incidence of CBD or sensitization than the total mass concentration of airborne Be.

83 ASSESSMENT OF PERSONAL EXPOSURE OF GAS ATTENDANTS TO METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT).

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Methylcyclopentadienyl manganese tricarbonyl (MMT) is an organic derivative of manganese (Mn) used in Canada as an antiknock agent and octane enhancer. In a recent study, we assessed the atmospheric concentrations of MMT at selected sites outdoor in Montreal. Results ranged from 1.8 ng/m^3 to 25 ng/m^3 (expressed as Mn) and do not reflect personal exposure levels. The highest values were obtained at a gas station (mean of 12 ng/m^3). Consequently, assessment of personal exposure of gas attendants constitute a promising research avenue. Moreover, the sampling method used in the previous study entails an uncertainty related to the possibility that the samples could include ultra fine particles which were able to pass through the filters used and the chemical analysis used (neutron activation) did not permit to discriminate Mn and MMT. The present study aims to assess personal exposure to MMT of gas attendants and to validate the sampling method as well as the chemical analysis. First, the average percentage of MMT recovery from spiked samples was 91.6% (SD=7.9). Then, exposure of 13 service station attendants was measured on 1 to 6 occasions each (n=32). The workers were asked to wear a portable pump for two hours. The air was pumped through two tubes in series filled with the absorbent Tenax® and glass wool plugs at both ends. The tubes were wrapped in aluminium foil and samples were analysed by GC-AED. The MMT concentrations, expressed as Mn, vary between 0.3 and 10.8 ng/m^3, with a mean of 3.8 ng/m^3, which is similar to the level of the environmental MMT contamination previously found.

84 ESTIMATING THE PROBABILITY OF SARIN VAPOR TOXICITY IN RATS AS A FUNCTION OF EXPOSURE CONCENTRATION AND DURATION.

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This study examined the effects of varying exposure concentration and duration on the probability of lethality occurring in rats exposed to Sarin (GB) vapor. Groups of male and female rats (Sprague-Dawley) were exposed to one of a series of GB vapor concentrations for 10, 30 90, or 240 minutes in a whole-body dynamic chamber. Separate concentration – exposure time values for lethality in 50% of the exposed population (LCt50) and corresponding dose-response slopes were determined for each exposure duration by the Bliss probit method. Contrary to that predicted by Haber's rule, LCt50 values increased with exposure duration (i.e., the CT for 50% lethality was not constant over time). Probability of lethality was approximated by a nonlinear function (toxic load model where C^(n)*t=constant). However, this approximation was only valid for a limited range of exposure duration in males as compared to females. Overall, female rats were more sensitive to GB vapor toxicity than male rats over the range of exposure concentration and duration studied. Miosis was the initial clinical sign noted following the start of GB vapor exposure. Pupil constriction was maximal at all concentration-duration combinations studied but progressed to mydriasis over several days before returning to pretreatment pupil diameters. Although blood cholinesterase activity was significantly inhibited by GB vapor exposure, no correlation between cholinesterase inhibition and exposure conditions or cholinesterase inhibition and severity of clinical signs was noted. No histopathological findings in surviving rats could be directly related to GB exposure.

85 LACK OF ADVERSE EFFECTS FROM SUBCHRONIC EXPOSURE OF RATS TO AEROSOLIZED PENTAERYTHRITOL ESTERS.

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Groups of Sprague-Dawley rats (15/sex) were exposed to an aerosol of mixed pentaerythritol esters at concentrations of 0 (untreated controls), 0 (sham-exposed controls), 50, 150, and 500 mg/m^3. Exposures were 6 hr/day, 5 days/wk, for 90 days. The average mass median aerodynamic diameter was 1.0 µm for each concentration and geometric standard deviation was ~1.8. Particles were well within the respirable size range. Exposure did not adversely affect clinical signs, body weight, parameters of serum chemistry or hematology, sperm morphology, or the number of sperm or spermatids. Among the 12 organs weighed during the sacrifice following exposure, the lungs had a minimal increase in weight after exposure to 0.50 mg/L. Histological changes were also minimal; the number of macrophages in the pulmonary alveoli increased slightly. An additional 10 male rats were included in each group for pulmonary function tests and analysis of pulmonary hydroxyproline following exposure. No treatment-related changes were noted in any of the parameters measured in these animals. Overall, the effects of subchronic exposure of rats to an aerosolized of this sample of pentaerythritol esters at concentrations as high as 500 mg/m^3 were minimal.

86 FURTHER CHARACTERIZATION OF A RAT MODEL OF AMIODARONE-INDUCED PULMONARY TOXICITY (AIPT).

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The antiarrhythmic drug amiodarone (AD) is limited in use by its pulmonary toxicity involving inflammation and fibrosis. The goal of this study was to further characterize an animal model of AIPT and to examine the possible mechanisms involved in the progression of toxicity. Male F344 rats were instilled intratracheally with AD (6.25 mg/kg with a 3.125 mg/mL solution) in sterile water or the sterile water vehicle on day 0 and again on day 2, a protocol that leads to the development of pulmonary fibrosis on day 28 in the AD-treated animals. To study the initial events leading to this endpoint, animals were killed on days 3, 5, 6, 7, or 10 and bronchoalveolar lavage (BAL) was performed. Recovery of alveolar macrophages and eosinophils was increased on days 3 and 5, while neutrophil recovery was significantly elevated only on day 3. Potential oxidant production of BAL cells (total cells x PMA-stimulated luminol-dependent chemiluminescence [total counts/10^6 cells/20 min]) was markedly elevated in AD-treated rats on day 3. BAL cells recovered from AD-treated and control rats on day 3 were cultured overnight. Cells from AD-treated rats produced more nitric oxide (NO) breakdown products in the culture media than cells from control animals, indicating an increased cellular production of NO as a result of AD treatment. These findings indicate that this model exhibits a transient pulmonary inflammation soon after the administrations of the drug with the potential for elevated oxidant production in the lungs. Therefore, oxidant mechanisms may be involved in the development of AIPT in this model. (Supported by NIH 5 T32 GM07039.)

87 FIVE-DAY INHALATION EXPOSURE TO STYRENE HAS TOXIC AND PROLIFERATIVE EFFECTS IN THE LOWER RESPIRATORY TRACT OF THE SPRAGUE-DAWLEY RAT.

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Styrene exposure occurs primarily by inhalation and is associated with respiratory toxicity, which is attributed to the toxic metabolite, styrene oxide. Our lab has previously shown that a single (6 h) inhalation exposure to 50 ppm (TLV) styrene altered enzyme activities in the respiratory tissues to influence the formation and removal of styrene oxide. The nasal mucosa had a higher concentration of styrene oxide than the lung accompanied by an increase in toxicity and proliferation, not present in the lung. We examined whether inhalation exposure to 50 ppm styrene for five days influenced its own metabolism to the toxic metabolite, styrene oxide, and whether this resulted in tissue specific toxicity in the respiratory tissues. Styrene oxide is formed by the cytochrome P450 monooxygenase system and is then hydrolyzed or conjugated to less toxic metabolites by microsomal epoxide hydrolase (EH) and glutathione transferase (GST), respectively. In the respiratory tissues, there was a general decrease in P450 activity following styrene exposure while in the nasal mucosa GST activity was increased, but was decreased in the lung. There was no change in the activity of microsomal epoxide hydrolase in either tissue. Although styrene inhalation resulted in an inhibition of P450 activity in both tissues, the concentration of free styrene oxide in the nasal mucosa was 108 +/- 3 ng/g tissue, while in the lung, the concentration of styrene oxide was below the limit of detection (10 ng/g tissue). The activities of marker enzymes and the level of protein were measured in the bronchoalveolar (BALF) and nasal (NLF) lavage fluid. Proliferating cell nuclear antigen (PCNA) was determined following styrene exposure to measure proliferation. The concentration of the toxic metabolite, styrene oxide, was higher in the nasal mucosa than the lung after the five day exposure. However, there was no evidence of toxicity or proliferation in the nasal mucosa. In BALF, the activities of gamma-glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), acid phosphatase (ACP) and the level of protein as well as the level of PCNA in the lung were significantly increased following the five day exposure. The lung appears to be more sensitive to a five day inhalation exposure to styrene than the nasal mucosa. The free styrene oxide levels do not correlate with respiratory toxicity suggesting that styrene oxide may be bound to cellular macromolecules in the lung and not be detectable. Another explanation is that early production of styrene oxide during the five day exposure results in delayed pulmonary toxicity that is not evident until after the five day exposure. Cellular damage and proliferation occur in the lower respiratory tract after five days of exposure to levels of styrene equivalent to that experienced by occupational workers perhaps leading to potential respiratory problems.

88 CORRELATION OF *IN VITRO* AND *IN VIVO* PULMONARY RESPONSES TO DIFFERENT CELLULOSE MATERIALS.

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In vitro studies were conducted with bleached cellulose pulp (BC), microcrystalline cellulose (MC) and cellulose insulation derived from recycled newsprint (CI). The *in vitro* responses were then compared to markers of pulmonary response for the same three materials after intratracheal instillation in rats. Immortalized rat alveolar type II epithelial cells (RLE-6TN) and alveolar macrophages (NR8383) were dosed with varying concentrations and for different periods of time, to create dose-response and time-response curves for the end points of LDH, pro-inflammatory and anti-inflammatory cytokines. TiO2, SiO2 and asbestos, were also studied in these two *in vitro* systems to provide comparisons with responses seen with nuisance and fibrogenic particulate materials. The *in vivo* studies in rats ranked the overall toxicity of these three cellulose materials as CI>MC>BC, as determined by analysis of biochemical markers, differential cell count and histopathology. Bronchial alveolar lavage samples (BAL) were analyzed for LDH, lysosomal acid hydrolases, alkaline phosphatase, total protein, and cell differentials at 3, 7, 14, and 28 days after dosing. The measures of response for the three dif-

7, 14, and 28 days after dosing. The measures of response for the three different cellulose materials as determined in the *in vitro* systems provided an accurate representation of the responses determined *in vivo*, with the strongest association between lung enzyme responses and proinflamatory cytokines. Conversion of *in vitro* doses of the three cellulose materials to that used in the *in vivo* studies, based on μg/m3, indicated the degree of response in the different measured parameters as being of similar magnitude at approximately the same *in vitro* versus *in vivo* doses. With further exploration of these *in vitro* techniques, the mechanisms of response for some materials depositing in the lung may be determined, as well as providing tools for the better design of *in vivo* pulmonary experiments.

89 XENOBIOTIC-METABOLIZING ENZYME ACTIVITIES IN PRIMARY CULTURES OF RAT TYPE II PNEUMOCYTES AND ALVEOLAR MACROPHAGES.

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Because of the evidence for an involvement of xenobiotic bioactivation in pulmonary toxicity and carcinogenesis, it is important to improve our understanding of the xenobiotic-metabolizing enzymes in isolated and cultured specific pulmonary cell populations. Phase I and phase II xenobiotic-metabolizing enzyme activities and intracellular reduced glutathione (GSH) level were studied in primary cultures of rat type II pneumocytes (TII) and alveolar macrophages (AM). 7-Ethoxyresorufin (EROD), 7-pentoxyresorufin (PROD) and 7-benzyloxyresorufin (BROD) O-dealkylation, NADPH and NADH cytochrome c reductase, glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase (QR) activities were assessed in sonicated cells, before plating (100% activity) and 24h and 48h after plating in TII, and 3h after plating in AM. In TII EROD activity was not detected at the protein concentrations used (0.2-0.5 mg cell protein per sample). In TII BROD and PROD activities decreased at 24h by 84% and 82%, respectively, and to lower levels at 48h. The activity of NADPH cytochrome c reductase, was stable but NADH cytochrome c reductase activity decreased at 24h (by 53%) and 48h (by 67%). The activity of GST decreased by 28% and 42% at 24h and 48h, respectively. GSH content increased with time in culture and at 48h was elevated by 130%. QR activity increased at 24h (by 55%), and then decreased to approximately the initial level at 48h. In freshly isolated AM, BROD activity was the only cytochrome P450-dependent alkoxyresorufin-O-dealkylase activity measured and it was 23 times lower than in freshly isolated TII. BROD activity decreased by 50% in attached AM. NADPH and NADH cytochrome c reductase activities decreased in the attached AM by 26% and 39%, respectively. No significant changes in GST and QR activities and GSH content were observed in attached AM. In conclusion, in conventional cultures of TII and AM, many of the metabolic capabilities of the cells were rapidly lost with time. Phase II-related enzyme activities were better kept than those of phase I.

90 THE EFFECTS OF AIRWAY CONSTRICTION ON AIRFLOW THROUGH THE UPPER HUMAN LUNG: A FLUID DYNAMICS MODEL.

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A fluid dynamic model was designed to examine whether a constricted region of the upper human respiratory tract (simulating an insult to the airways) will significantly alter airflow in subsequent airway branches, that in turn may have physiologic relevance and public health importance. We constructed two-dimensional representations of the upper human respiratory system (generations 2-7) to model normal and constricted airways. Finite element fluid dynamic simulations of inspiratory air flow were calculated for both airway models, and the resultant flow solutions were analyzed using visualization software. The unobstructed airway model simulation predicted minimal airflow decrease through the primary path, with more notable decreases in the periphery. However, for the constricted airway simulation, there was a dramatic decrease in airflow for both the main pathway beyond the restriction site and the peripheral passages. These results suggest that areas of localized edema or bronchial constriction (where the passage narrows significantly) would cause a dramatic decrease in downstream airflow. This reduction of airflow could further compromise pulmonary function, and also alter the deposition pattern of particulate matter in the lung.

91 EFFECTS OF EXPIRED CARBON DIOXIDE ON VENTILATION AND AEROSOL DEPOSITION IN GUINEA PIGS UNDERGOING BAROMETRIC PLETHYSMOGRAPHY.

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Barometric plethysmography is frequently used to evaluate changes in ventilation and breath structure in unrestrained (physically and chemically) animals in effort to determine the effect of inhaled pharmaceuticals or toxins on the airways. Discontinuous aerosol generation and delivery to the plethysmograph may require operation of the plethysmograph without a bias flow which is normally intended to refresh the plethysmograph air supply and remove expired CO_2. Continuation of the bias flow would artificially deplete aerosol concentration. However operation without bias flow results in CO_2 build up and subsequent stimulation of ventilation. Measurements of ventilation (f=breathing frequency, TV=tidal volume, and Ve=minute volume) and the flow-derived parameter Penh (enhanced pause) were performed on 11 guinea pigs using a commercially available 4.5 L (Buxco, Electronics) plethysmograph operating with and without a 900 mL/min bias flow. During these measurements a low flow (5 mL/min) microcapnometer was used to measure, continuously, CO_2 concentration in the plethysmograph. Without the bias flow CO_2 concentration in the plethysmograph increased steadily to an average 5.4% after 30 min, compared to 0.9% with bias flow. The buildup of CO_2 above 2% resulted in a moderate suppression of f, however TV, Ve, and Penh increased to 1.5, 1.4, and 1.7 of their baseline values, respectively. Theoretical changes in aerosol deposition in the extrathoracic, tracheobronchial, and pulmonary regions of the lung attributable to CO_2 induced elevation of TV were calculated for 300 mg/m3 concentration aerosols with MMAD's of 0.3, 1, 3, or 7 μm and sigma g's of 1.7.

92 SELENIUM-TREATED LUNG: VIBRATOME SECTIONING FACILITATION BY GELATIN INFILTRATION.

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The lungs of guinea pigs (n=5) treated intratracheally (ITr) with selenium (Se) as selenomethionine (SEM) (0.06 mg Se/100g body weight) or saline were perfused with phosphate buffered saline via the right ventricle of the heart and fixed with ITr infusion of 10% buffered formalin. The formalin-fixed lungs were washed overnight with running water and infiltrated with gelatin (Gurusingie and Ehrlich, 1986). It was observed that infiltration of the lung lobe with 5% followed by 12% gelatin for three days each (6-8 hours per day) at 37°C was sufficient for easy sectioning using the Vibratome set at a speed of 8.5-9.and an amplitude of ≈10 with a blade angle, ≤25 degrees. Saline treated lung was sectioned in its collapsed state. The sections (30-40 microns) were stained with hematoxylin and eosin. Evaluation of stained sections revealed histological clarity. Light microscopic images of sections of Se-treated lungs showed cellular infiltration in the interalveolar septal area. Revisitation of the procedure corroborated that Vibratome is a useful tool to section tissues such as the lung in its inflated or collapsed state. (This was supported by the University research funds # 23030-CR10; NIH and SC Summer Undergraduate Research Programs.)

93 BIOCHEMICAL AND RESPIRATORY CHANGES CAUSED BY BRIEF EXPOSURE TO HIGH CONCENTRATION OF CARBON MONOXIDE AND CARBON DIOXIDE IN AWAKE RATS.

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Carbon monoxide (CO) inhalation can cause mental disorders, physical decrements and even death. Carbon dioxide (CO_2) is a respiratory stimulant. High concentrations of these combustion gases are of concern in military operations. This study addressed respiratory changes resulting from brief (5 min) exposures to high concentrations of CO and CO_2 (0-12000 ppm CO; 0-10% CO_2), alone or in combination, in awake male Sprague-Dawley rats (250-450g). Respiratory measurements were made using partial body flow plethysmographs attached to a static exposure chamber. Minute ventilation volume was found to increase approximately 1.5 times in rats exposed to 500, 1000, 3000, and 6000 ppm CO when also exposed to 5% CO_2, compared to corresponding CO-alone values for minute volume. At end-exposure, mean carboxy- hemoglobin (COHb) values were approximately 7.5, 14.5, 29.7, 57.2 and 63.0% in the 500, 1000, 3000, 6000, and 12000 ppm CO groups, respec-

tively. Post exposure COHb values increased to 10.8, 20.4, 47.3, 61.8 and 68.2% for these groups, respectively, when rats were coincidentally exposed to 5% CO_2. Blood chemistry returned to normal within 4h. No lethality was observed, up to 24h after exposure, likely attributed to the short exposure period. These results clearly demonstrate significant enhancement of CO poisoning by CO_2, an important toxicologic interaction between these two primary combustion gases. (Supported, in part, by DOT&E, Department of Defense, Washington, DC.)

94 INTRATRACHEAL ADMINISTRATION OF A RECOMBINANT SP-C LUNG SURFACTANT PREPARATION TO CYNOMOLGUS MONKEYS: A COMPARISON OF DIFFERENT TECHNIQUES.

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The technique and procedure for the intratracheal administration of a rSP-C lung surfactant (Venticute®) preparation into the lung is a methodical challenge due to the requirements which have to be fulfilled: a) minimal animal burden, b) minimal influence of the administration technique and procedure on respiratory function, c) up to 4 administrations per day should be possible under comparable conditions, d) minimal artificial effects on the morphology of the respiratory tract. In order to comply with the above outlined requirements, two different administration techniques were established in a primate model: (1) administration via a pediatric bronchoscope and (2) administration via a surgically implanted intratracheal catheter. Both methods were used in 22 - 24 cynomolgus monkeys (Macaca fascicularis) weighing between 2.5 and 3 kg. For the administration via a bronchoscope, the monkeys were slightly sedated and additionally a local anesthesia was applied to the laryngeal region. For the administration via a surgically implanted catheter a specially constructed polyurethane tracheotomy spiral catheter was utilized. The implantation was performed 2-3 days before the first administration, the administration itself was done without prior sedation of the animals. The recombinant surfactant preparation was well tolerated. There were no serious complications by using both techniques up to 4 times a day. By comparing the behavior and appearance of the animals during and up to 30 minutes after the administration, it was evident that the procedure using a surgically implanted catheter offers substantial advantages over the alternative method of bronchoscopical application: i.e., minor bronchial irritation; undamaged tracheal mucous membranes; repeated anesthesia becomes unnecessary and the administration of a substance may be timed exactly eventually achieving continuous application.

95 INTRATRACHEAL ADMINISTRATION OF SYNTHETIC ZEOLITES AND ALUMINAS IN RATS.

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An intratracheal (IT) screening assay was performed on a series of synthetic aluminosilicate zeolites and alumina binders to compare their relative ability to cause pulmonary fibrosis (scarring) and related changes. Groups of 12 male rats were evaluated 6 months after instillation of 50 mg for lung volumes, pulmonary pressure-volume curves, pulmonary hydroxyproline (OHPro), lung weights, and histopathology. Negative controls were saline and glass beads; positive control was quartz. The test materials were organic-free ZSM-5 crystals, organic form of ZSM-5 crystals, alumina-bound ZSM-5, nickel/ZSM-5/Al_2O_3 binder, used nickel/ZSM-5/Al_2O_3 binder, nickel-tungsten/ZSM-5/Al_2O_3 binder, and Bayer pseudoboehmite. Particles were ground until at least 95% were less than 2.4 μm. Altered body weights or clinical signs were observed with nickel-tungsten/ZSM-5/Al_2O_3 binder, quartz, and alumina. At 6 months after dosing, quartz produced increased lung weight (wet and dry), increased pulmonary hydroxyproline, decreased lung volumes (total lung capacity and vital capacity), and microscopically observable granulomas with fibrosis. The severity of the pulmonary reactions was compared among the test materials. Based on these results, the effects of 4 aluminas (Bayer pseudoboehmite, Bayer γ-Al_2O_3, Ziegler pseudoboehmite, and Ziegler γ-Al_2O_3) were compared in additional groups. The IT assay proved to be a very useful tool for ranking the relative effects of this series of zeolites and aluminas.

96 APPLICATION OF STEPWISE DISCRIMINANT ANALYSIS PROCEDURE FOR THE EVALUATION OF TREATMENTS AGAINST PHOSGENE EXPOSURE IN ANESTHETIZED, VENTILATED, WEANLING SWINE.

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Phosgene (CG), a choking agent used during World War I, presents an industrial hazard today. CG induces pulmonary edema after an asymptomatic period of up to 24 hr. A 72 hr anesthetized, ventilated, weanling swine model was developed to assess the efficacy of treatments for respiratory casualties using blood gas, clinical chemistry, hematology, and respiratory parameters. Thirty-one pigs (sus scrofus) were anesthetized with isoflurane for surgical implantation of catheters into a femoral artery and vein. Following catheterization, anesthesia was maintained by slow infusion (~3 mL/hr) of pentobarbital sodium (65 mg/mL) and the animal was placed on a ventilator (Positive End Expiratory Pressure (PEEP) of 3-5 cm H2O; 30% O2). Animals were exposed to approximately 450 mg CG/m3 for 10 min, or 10 min of air. Three treatments were evaluated: 1) 2 ml of a 200 mg N-acetylcystiene (NAC)/ml solution administered intratracheally 30 min post-exposure and every 4 hr thereafter for 16 hr; 2) a loading dose of 45 mg ibuprofen (IBU)/kg administered intravenously 30 min post-exposure, followed by 22.5 mg IBU/kg every two hr for 24 hr; 3) O2 was increased to 45% immediately after exposure, along with incremental increases of 2-3 cm H2O PEEP every 30 min until the targeted level of 15 cm H2O PEEP was reached and maintained until end of study. A stepwise discriminant analysis procedure was used to select blood gas, clinical chemistry, and hematology parameters that explained the greatest variability between exposure/treatment groups and were also physiologically plausible. Parameters such as oxygen saturation, potassium, and blood urea nitrogen levels were identified as explanatory variables in the discriminant analysis. These parameters were consistent with respiratory damage and hyperkalemia associated with CG-exposure and possible renal effects associated with high IBU doses. (Supported by DAMD 17-89-C-9050.)

97 90 DAY INHALATION STUDY OF STAINLESS STEEL WELDING FUME IN SPRAGUE-DAWLEY RATS.

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In order to investigate welding fume exposure related occupational diseases such as nasal septum perforation, pneumoconiosis and manganese intoxication, welding fume exposure system including welding fume generator, exposure chamber and fume collector were built. Then the welding fume exposure system was monitored and validated. After an acute inhalation toxicity study, welding fume generated from stainless steel arc welding was exposed to male Sprague Dawley rats. The rats were exposed to the welding fume with approximately 70-80 mg/m^3 for 2 hrs in an inhalation chamber for 90 days. Animals were sacrificed at 0, 14, 30, 60 and 90 days after initial exposure. Upper respiratory tract including the nasal pathway, the conducting airway, and the gas exchange region including the +alveolar ducts, alveolar sacs and alveoli were investigated through histopathological examinations. The organ distribution of major metals in the welding fume including Mn, Cr, Fe, and Ni was measured in the blood, liver, testis, brain, kidneys, and lungs. The fume particles had various in their diameters ranging from 0.02-0.81μm and were distributed log normally with average diameter and geometric mean of 0.1±1.42. The average diameter 0.1μm resulted in fewer adsorption of the welding fume particles in the upper respiratory tract. The major sites for particle adsorption and deposition were the lower respiratory tracts including the bronchioles, alveolar ducts, alveolar sacs, and alveoli. No significant Mn increase was observed in the various organs during 90day exposure of welding fume. We could not find any significant welding fume exposure related pathological legion in the nasal region. The lower respiratory region, however, showed gradual mobilization and destruction of macrophages and marked interstitial infiltration of macrophages, and many granulomatous legions in interstitium and pluera. The histopathological examination of welding fume exposed lung suggests that the repeated exposure of welding fume may lead into progressive fibrotic changes.

98 GENERATION, SAMPLING AND ANALYSIS OF GB VAPOR FOR INHALATION TOXICOLOGY STUDIES.

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Generation, sampling and analytical techniques were developed to support inhalation studies on the lethal effects (LCt50) of varying exposure concentration and duration of sarine(GB)vapor in rats. This study tested and optimized various methodologies to generate, sample and characterize GB test atmospheres in an inhalation chamber. A syringe drive spray atomization system was used for GB vapor generation. Continuous chamber monitoring was accomplished using a phosphorus analyzer. Sampling methods included the traditional solvent bubbler technique as well as the development of an automated solid sorbent sampling system. All samples were quantitatively analyzed by gas chromatography for GB vapor. Concentrations derived from each sampling method were compared against each other and statistically evaluated. Results showed good correlation between the two methods (t-test >95%). In addition, stable GB test atmospheres (0.1–30 mg/m3) were generated over different exposure duration's (10, 30, 90, 240 min) with rapid monitoring capability. Future applications include the ability to generate and monitor GB levels approaching the TWA-TLV of 0.0001 mg/m3.

99 BENZENE EXPOSURE ASSESSMENT FOR USE OF A PETROLEUM NAPHTHA METAL PARTS CLEANER.

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The OSHA Hazard Communication Standard requires that a product's Material Safety Data Sheet identify all carcinogenic agents whose concentrations are at least 0.1% (or 1,000 ppm). This study assesses benzene exposures associated with the operation of a small metal parts cleaner using virgin recycled petroleum naphtha solvents containing 9 and 58 ppm benzene. Benzene air concentrations (personal, area, and grab) were measured using NIOSH 1501 and USEPA TO-14 methods during one hour periods of vigorous parts cleaning. Airborne benzene concentrations associated with the 9 ppm solvent were ≤ 33 ppb in the worker breathing zone and above the parts cleaning tank. Peak concentrations adjacent to the metal parts during cleaning averaged 140 ppb. Area samples taken around the tank were less than 5 ppb. Cleaning with the 58 ppm benzene spiked solvent produced an average operator breathing zone concentration of 440 ppb. Peak concentrations measured adjacent to the metal parts cleaner averaged 490 ppb. Average concentrations around the tank were 63 ppb. These data indicate that operator exposures associated with aggressive parts washing using a naphtha solvent containing benzene at concentrations less than 58 ppm are associated with low level benzene exposures. Performing parts cleaning for long periods or use of naphtha solvents with higher benzene concentrations but still below the 0.1% criteria are likely associated with exposures that approach current workplace exposure limits.

100 SEASONAL CHANGE IN MITE NUMBERS AND INDOOR ALLERGEN CONTENT IN A FINNISH OFFICE ENVIRONMENT.

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Allergy prevalence has been increasing over the last decades. House dust mites (HDM) together with cat and dog allergens have been considered very important indoor factors causing symptoms for affected individuals. The role of workplace as an exposure source has thus far been a minor consideration. This study was done in an office building. Desk chair and occasional floor samples were taken with a sampler connected to a vacuum cleaner. Mite samples were collected in May, August, November, and February, and the mites were identified microscopically. Indoor allergen samples (Der p 1, Fel d 1 and Can f 1) were collected in November and analyzed by two-site ELISA methods. Fourteen workers were tested with prick test for cat, dog, and mite (*Dermatophagoides pteronyssinus*, *D. farinae*, *Tyrophagus putrescentiae*, *Acarus siro* and *Lepidoglyphus destructor*) allergy. Out of 32 samples so far analyzed (May and August), 19 contained mites. Only one sample contained more than 100 mites/g dust. Majority of the mites were other than HDM, including Tarsonemus sp. and Mesostigmatids. No seasonal difference was obvious so far. Mite allergens were found only in two chairs out of the 14 studied and the concentrations were low (mean 0.009 μg/g). Cat and dog allergens, on the other hand, were found in every chair and in similar amounts (Can f 1 3.0-185.9 μg/g, geometric mean 24 μg/g, and Fel d 1 0.8-171.8 μg/g, geometric mean 22.7 μg/g). The allergen levels were relatively low compared to studies done at homes, but still in 50% of the samples allergen levels exceeded the proposed threshold levels for cat or dog sensitization. Six (43%) of the workers were prick positive to either cat (n=3, 21%), dog (n=4, 29%) and/or storage mites (n=3, 21%). No correlation between reported symptoms and positive skin reactions was found.

101 ASSESSMENT OF EPH A5 KNOCKOUT MICE FOLLOWING COCAINE ADMINISTRATION.

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Eph A5 belongs to the Eph family of receptor tyrosine kinases. Eph receptors and their corresponding family of ligands, the ephrins, have been implicated in the guidance of axons and formation of topographic projection maps. Eph A5 receptor is most prominently expressed in the hippocampus and is critical during development. As Eph receptors appear to have distinct but overlapping patterns of expression, they possess unique, yet redundant functions. Previous studies in our labs using in situ hybridization have shown that ephrin-B2 ligand is up-regulated following cocaine, which suggests that the ligand may also play a role in drug-induced plasticity. To examine the behavioral toxicity of cocaine, Eph A5 knockout mice were evaluated in the place preference conditioning (PPC) and locomotor activity tests following cocaine 0.1-10 mg/kg (i.p.). To assess learning, the water maze was conducted without drug. Relative to controls, knockout mice were more sensitive to cocaine in the PPC test and exhibited less motor activity following saline or cocaine. Results from the water maze revealed no significant differences in acquisition ability, but retention time was increased for knockouts after two months. Neurochemical analysis revealed that compared to wild type, knockout mice had higher levels of dopamine and dihydroxyphenylacetic acid (DOPAC) in the striatum, yet this effect was reversed in the nucleus accumbens. In the hippocampus, there were no differences in monoamine concentrations. Knockout mice had slightly higher levels of dopamine, DOPAC, and homovanillic acid in the frontal cortex. These results indicate that Eph A5 receptor may play a role in plasticity involved in neural and behavioral toxicity.

102 VITAMIN E DEFICIENCY INCREASES SUSCEPTIBILITY OF THE BALB/C MOUSE TO MDMA-INDUCED DOPAMINERGIC NEUROTOXICITY.

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The mechanism of 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity has been hypothesized to involve the formation of toxic free radical species. Since Vitamin E is a potent radical scavenger, we tested whether Vitamin E deficiency affects susceptibility to MDMA-induced dopaminergic neurotoxicity. Male BALB/c mice (3-4 weeks old) were kept on control (Vit. E, 50 IU/kg) and Vitamin E deficient (<10 IU/kg) diets for twenty weeks. Vitamin E levels in brain of deficient mice were reduced 75% compared to controls. Vitamin E deficient and control mice were randomly assigned to the following groups: saline 200 ul, (every 2 hrs X 4, s.c), or MDMA 5 mg/kg, (every 2 hrs X 4, s.c.) or 10 mg/kg (every 2 hrs X 4, s.c.). Rectal temperatures were assessed every 2 hours during the dose period. Vitamin E deficient animals exhibited MDMA-induced temperature modulation that was similar to control animals. Seventy-two hours following the first dose, animals were sacrificed and brains were dissected for determination of Vitamin E, glutathione, total antioxidant reserve, protein thiols, dopamine, DOPAC, HVA, and Glial Fibrillary Acidic Protein (GFAP), and livers were analyzed by histopathology. Animals given the Vitamin E deficient diet exhibited neurotoxic responses at the lowest dose of MDMA, while mice on the control diet were undamaged. Striatal dopamine was reduced by 47%, DOPAC by 44%, and HVA by 29%, while GFAP was elevated 3 fold. Neurotoxic responses were also observed at 72 hrs at the higher dose of MDMA, in both diet groups. In the Vitamin E deficient mice MDMA caused greater hepatic necrosis compared with controls. These data indicate that Vitamin E deficiency increases susceptibility to MDMA-induced neurotoxicity and hepatic necrosis and provide support for a free-radical mediated mechanism of toxicity.

103 NEUROPROTECTIVE ROLE OF SELENIUM IN METHAMPHETAMINE-INDUCED PEROXYNITRITE GENERATION AND DOPAMINERGIC NEUROTOXICITY.

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Methamphetamine (METH) has been known to produce neurotoxicity via generation of reactive oxygen and nitrogen species. In the present study, neuroprotective role of selenium (Se) in METH-induced dopaminergic neurotoxicity was evaluated using *in vitro* as well as *in vivo* system. For *in vitro* studies, PC12 cell cultures were exposed to 200 μM METH with or without 10 and 20 μM Se. METH exposure resulted in significant depletion of dopamine, and its metabolites DOPAC and HVA and significant formation of 3-nitrotyrosine (3-NT) in PC12 cell cultures. For *in vivo* studies, adult male C57BL/6N mice were supplemented with 0.5 mg Se/kg in the form of sodium selenite (Na_2SeO_3) in drinking water for one week. Animals received 4x10 mg/kg METH, i.p. at 2 hr. interval after one week of Se administration. Se supplementation continued for one more week before animals were humanely killed for the neurochemical analysis. Multiple injections of METH produced significant formation of 3-NT and depletion of dopamine and its metabolites in the caudate nucleus. Selenium supplementation attenuated the depletion of dopamine and its metabolites, DOPAC and HVA and the formation of 3-NT in PC12 cells as well as in mice caudate nucleus. These data suggest that METH-induced dopaminergic neurotoxicity is mediated by the production of peroxynitrite, and selenium plays a protective role in METH-induced neurotoxicity.

104 EFFECT OF *D*-AMPHETAMINE ON EXTRACELLULAR GLUTAMATE AND ASPARTATE LEVELS IN RAT AMYGDALOID MICRODIALYSATE.

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Amphetamine related compounds are used in the treatment of attention deficit hyperactivity disorder, weight control/obesity and narcolepsy. In humans, it is known that seizures, as well as hyperthermia, result from overdose/poisoning from amphetamines and related compounds, and these seizures may contribute substantially to the lethality of amphetamines. Increased levels of extracellular glutamate are known to induce excessive neuronal stimulation, seizures and neuronal death. However, little is known about the effects of doses of d-amphetamine, ranging from those that produce stereotypic behaviors to those that induce seizures, on extracellular levels of glutamate in the amygdala. The goal of this study is to determine via brain microdialysis if extracellular glutamate levels are elevated in the amygdala after exposure to amphetamines. Male Sprague-Dawley rats (4 months old) were used. Extracellular brain levels of glutamate were determined after multiple doses (x3) 5.0mg/kg (elicits stereotypic behavior without seizures) or 15.0mg/kg (produce seizures) of d-amphetamine have been administered at 2 hr interval. Controls received ip injections of physiological saline. Glutamate concentration in the microdialysate averaged 1.84±1.1 μM prior to saline (basal level) and were 70% basal levels 2 hours after the 3rd saline dose. Glutamate levels were at about 90% of basal levels after the 3rd dose of 5.0mg/kg d-amphetamine. Robust increases in glutamate levels were also not observed after the 3rd dose in animals that received 15.0mg/kg d-amphetamine. In retrospect, glutamate increases in the amygdala were not as robust as those previously reported in the striatum. Extracellular amphetamine after the 3rd dose increased from 9 μM to over 40 μM in animals treated with 5 mg/kg and 15 mg/kg d-amphetamine, respectively. (Supported by NCTR/FDA and Intra Agency Agreement.)

105 INHIBITION OF γ-GLUTAMYLTRANSPEPTIDASE AT THE BLOOD-BRAIN BARRIER POTENTIATES METHYLENEDIOXYAMPHETAMINE NEUROTOXICITY.

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Systemic metabolites play an important role in methylenedioxy-amphetamine (MDA) neurotoxicity, although the nature of the neurotoxic metabolite(s) remains unclear. We have shown that 5-(glutathion-*S*-yl)-α-methyldopamine (5-GSyl-α-MeDA), an *in vivo* metabolite of MDA, is a serotonergic neurotoxicant. Brain uptake of 5-GSyl-α-MeDA is decreased by glutathione (GSH) coadministration, but sharply increased by pretreatment with a γ-glutamyltranspeptidase (γ-GT) inhibitor [acivicin (AT-125)], suggesting competition of intact 5-GSyl-α-MeDA with GSH for the putative blood-brain barrier (BBB) GSH transporter. γ-GT is enriched in BBB endothelial cells. Inhibition of endothelial γ-GT may enhance the uptake of 5-GSyl-α-MeDA into brain by increasing the pool of 5-GSyl-α-MeDA available for the putative GSH transporter, thereby potentiating neurotoxicity. We now show that pretreatment of male Sprague-Dawley rats with AT-125 (18 mg/kg, ip) produces a 60% inhibition of γ-GT activity in rat brain microvessel endothelial cells. AT-125 pretreatment potentiates MDA-mediated depletions of serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA). Thus MDA (10 mg/kg, sc) decreases 5-HT in the cortex, hippocampus, striatum, and hypothalamus by 15%, 13%, 11%, and 29% of control values respectively. Following AT-125 treatment, MDA (10 mg/kg, sc) significantly decreases 5-HT concentrations to 60%, 56%, 73%, and 54% of control levels in cortex, hippocampus, striatum, and hypothalamus respectively. 5-HIAA levels in the forebrain are also decreased from 80-93% to 61-77% of control levels. In addition, glial fibrillary acidic protein (GFAP) is induced 1.6 fold in the striatum of AT-125 and MDA treated rats, but not in rats treated with MDA alone. The results indicate that 5-GSyl-α-MeDA, and perhaps other thioether conjugates of α-methyldopamine, play an important role in MDA-mediated serotonergic neurotoxicity. (DA 10832, ES 07784.)

106 PERIPHERAL BENZODIAZEPINE RECEPTORS ARE TRANSIENTLY INCREASED AFTER METHAMPHETAMINE ADMINISTRATION.

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The peripheral benzodiazepine receptor (PBR) has been demonstrated to be a sensitive marker of neurotoxicity and has been characterized in brain injury induced by neurotoxicants producing different types of cellular damage (Guilarte *et al.* NeuroToxicol. 16: 441, 1995; Kuhlmann and Guilarte Brain Res. 751: 281, 1997; Toxicol. Sci. 48: 107, 1999). Neurotoxicant-induced increases in PBR levels are the result of glial cell activation and the effect is specific to the region of damage. Methamphetamine (METH) is a drug of abuse known to result in protracted changes in dopamine (DA) and serotonin (5-HT) axonal markers in the brain. METH exposure (10 mg/kg, 4 times every 2 hrs) in rats induced approximately 50% depletions of DA and 5-HT. Increased expression of PBRs was measured in the hippocampus (91.8%), cingulate (89.5%) and striate (94.5%) cortex, thalamus (107%) and dorsal raphe (87.3%) but not in striatum 5 days after METH administration. At 14 days after drug administration, PBR levels in METH rat brains were no longer different from controls. These findings suggest that PBR expression in the brains of METH-treated rats is transiently increased. Striatal changes in PBR levels may be detected at an earlier time after METH administration (Escubedo et al., Brain Res. 814: 120, 1998). The relationship between regional changes in PBR expression and DA and 5-HT axonal markers as well as microglia and astrocytic markers are under investigation. (Supported by ES07062 to TRG.)

107 OBESITY AS A POTENTIAL RISK FACTOR FOR SUSCEPTIBILITY TO NEUROTOXIC INSULT.

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Obesity is a major risk factor associated with a variety of diseases including cardiovascular disorders, diabetes and certain cancers. Little information exists, however, as to whether obesity modifies the response of the central nervous system (CNS) to neural injury. The purpose of the present study was to determine if obesity serves as a predisposing factor in modifying/mediating a neurotoxic response. Leptin deficient (ob/ob) mice were used as a model of obesity and the dopaminergic neurotoxicants, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine (METH) were used as neurotoxic insults. Tyrosine hydroxylase (TH) was used as an index of dopaminergic nerve terminal integrity and was assessed by a novel fluorescence-based ELISA and by immunoblot. Glial fibrillary acidic protein (GFAP) was used as an index of the glial reaction to terminal damage and was assessed by sandwich ELISA. Both MPTP and METH significantly decreased striatal TH levels in a time-dependent fashion in the striatum of wild and ob/ob mice. Basal levels of TH were 25% lower in ob/ob mice compared to wild type mice. By 48 hours post-dosing, MPTP reduced TH by approximately 70-75% in both wild type and ob/ob mice. METH resulted in nearly a 90 % decrease in ob/ob mice in comparison to 75% decrements in wild type controls. These data were in agreement with immunoblot results on the same samples. While both MPTP and METH induced a large (>600%) increase in striatal GFAP of wild-type mice, this effect was attenuated (not exacerbated) in ob/ob mice. Basal levels of GFAP also were lower (25%) in ob/ob mice.

The data indicate that METH-induced dopaminergic neurotoxicity (based on TH decreases) is enhanced in the obese (ob/ob) and that the leptin-deficient condition decreases the basal levels of TH and GFAP. The ob/ob mouse may not serve as an appropriate model for assessing the effects of obesity on toxic responses of the CNS.

108 GENETIC POLYMORPHISMS OF SUPEROXIDE DISMUTASE (SOD1, SOD2) AND PARKINSON'S DISEASE.

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Oxidative stress caused by exposure to toxic agents may be associated with the pathogenesis of Parkinson's disease (PD). Superoxide dismutases are involved in reducing oxidative stress by catalyzing superoxide radicals which may damage DNA. We developed genomic DNA and cDNA-based sequencing assays to identify genetic variants in the copper/zinc superoxide dismutase (SOD1) and manganese superoxide dismutase (SOD2) genes. These assays involve PCR amplification of specific regions of DNA or cDNA followed by sequencing of the PCR amplicons on an ABI 377. DNA from 94 subjects from a case-control study of PD (45 cases and 49 controls) were analyzed and no genetic variants were detected in the gene encoding SOD1. However, a previously described polymorphism was identified consisting of a C47T in exon 2 of SOD2, which results in an alanine to valine substitution. We subsequently analyzed 386 PD subjects (155 cases and 231 controls) for this polymorphism using a fluorogenic 5' nuclease assay and an ABI 7700. The allelic frequencies for the cases were: CC (35, 22.6%), CT (77, 49.7%), TT (43, 27.7%). The allelic frequencies for controls were: CC (51, 22.1%), CT (125, 54.1%), TT (55, 23.8%). No statistically significant difference was found in the distribution of the mutant T allele among cases and controls (odds ratio, 0.97; 95% CI 0.60-1.58). These results indicate that there is no association between the presence of this SOD2 mutation and PD. (Supported by ES04696 and ES07033.)

109 IS NITRIC OXIDE INVOLVED IN NEURODEGENERATIVE PROCESSES?

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The role of nitric oxide in nigrostriatal degeneration, the pathological hallmark of Parkinson's disease, was investigated using two animal models, i.e. mice injected with either MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) or methamphetamine. In MPTP-exposed animals, the nNOS (neuronal nitric oxide synthase) inhibitor 7-nitroindazole seemed to prevent neurotoxicity. However, additional experiments clarified that this neuroprotection was mostly due to inhibition of MAO-B (monoamine oxidase type B, the enzyme responsible for MPTP bioactivation) rather than NOS. Another NOS inhibitor, L-NG-nitroarginine, failed to exert any neuroprotective effect in the MPTP mouse model of nigrostriatal damage. In mice injected with methamphetamine, NOS inhibitors prevented both dopamine depletion and the loss of tyrosine hydroxylase-positive terminals in the striatum. The data indicate that the role of nitric oxide in nigrostriatal degeneration may vary depending upon the mechanisms involved in neuronal damage. Since NMDA (N-methyl-D-aspartate) receptor activation is thought to play an important role in methamphetamine neurotoxicity (but not that caused by MPTP), we postulate that the effects of nitric oxide may be an event downstream from excitotoxicity.

110 UPREGULATION OF PHOSPHORYLATED STAT3 PRECEDES GLIAL INDUCTION IN MPTP-MEDIATED NEUROTOXICITY.

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STAT3 is a transcription factor that is activated by the JAK family of kinases or by the growth associated MAP kinase (MAPK). As recent studies have shown that Stat3 regulates gliogenesis in the central nervous system (CNS) and astrogliosis is a unifying effect of diverse injuries to the CNS, we are interested in elucidating the temporal relationship among alterations in Stat3, MAPK and gliosis following neurotoxic insult. At various timepoints following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- (MPTP, 12.5 mg/kg, s.c.) treatment, female C57BL/6J mice were sacrificed by focused microwave irradiation to preserve steady-state phosphorylation. Target (striatum) and non-target (hippocampus) tissue homogenates were assayed for levels of glial fibrillary acidic protein (GFAP) and dopamine (DA), as well as alterations in Stat3 and MAPK protein phosphorylation. A 2-fold increase in striatal GFAP was measured in MPTP-treated mice, 48 hours after dosing. Striatal DA depletion occurred within hours following treatment. Alterations in activated Stat3 and MAPK, as detected by phospho-state specific antibodies, were observed as early as one hour following neurotoxic insult and persisted for weeks. The most dramatic increase in the phosphorylated form of Stat3 and MAPK (500% and 100%, respectively) was measured prior to GFAP upregulation (12 hr post-MPTP). As MPTP-induced neurotoxicity requires uptake of MPP+ into DA terminals via the dopamine transporter (DAT), DAT inhibition should block phosphorylation events linked to dopamine terminal damage. Pretreatment with the DAT inhibitor nomifensine (25 mg/kg, s.c.) completely blocked the MPTP-induced increases in GFAP and phospho-Stat3, without altering these levels in saline-treated mice. These data suggest that the activation of Stat3 and MAPK are early events in toxicant-induced glial activation. These kinase/substrate pathways may serve as potential targets for modulation of degenerative and regenerative responses to neural damage.

111 ENVIRONMENTAL RISK FACTORS FOR PARKINSON'S DISEASE: POTENTIATED EFFECTS OF COMBINED PARAQUAT AND MANEB.

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Parkinson's disease (PD) is a neurological movement disorder resulting from a loss of dopamine (DA) neurons in the nigrostriatal dopaminergic pathway. The absence of any compelling data for a solely genetic etiology for this disease has focused attention on environmental exposures as causative agents, or as factors which act in conjunction with a genetic predisposition. The herbicide paraquat and the fungicide maneb have been shown to impact DA systems. These compounds show highly comparable patterns of geographical use, suggesting overlap in human exposure as well. This situation raises the possibility of a multi-hit environmental model for PD. C57BL/6 mice exposed to paraquat and maneb in combination show significant decreases in locomotor activity compared to when these agents are administered alone. Potentiated effects of levels of DA, its metabolites and DA turnover accompany combined relative to when administered alone. Furthermore, combined but not single compound exposure resulted in significant decreases in striatal but not nucleus accumbens tyrosine hydroxylase density. Thus these chemical mixtures may be important etiologic agents for PD. These findings also question the adequacy of current risk assessment guidelines for such chemicals based on effect levels derived from exposure to single agents. (Supported by ES05727, ES05017, ES05903, ES01247.)

112 THE CYCLODIENE INSECTICIDE HEPTACHLOR ALTERS DOPAMINE HOMEOSTASIS.

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Epidemiological studies have demonstrated that exposure to pesticides is a risk factor for Parkinson's disease. We have previously shown that subchronic, repeated exposure to the cyclodiene insecticide heptachlor increases the expression of the plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) in mouse striatum (Neurotoxicology. 20:631-638, 1999). The purpose of this study was to determine if a single dose of heptachlor has similar effects. 8-10 month old C57BL/6 mice were injected with a single dose of heptachlor (9 mg/kg, i.p.). 24, 48, and 72 hr later, striata were harvested and subjected to HPLC-EC analysis of dopamine (DA) and metabolites and western blot analysis of DAT and VMAT2 expression. Administration of heptachlor did not significantly affect striatal dopamine levels at any timepoint (-1.0%, -8.5%, +1.6 for 24,48, and 72 hr, respectively). Western blot analysis revealed that heptachlor altered DAT expression (-13.0%, -1.7%, and +13.0% for 24, 48, and 72 hour treated mice, respectively). We hypothesized that the trend of DAT upregulation would increase susceptibility to the dopaminergic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); thus, MPTP was administered to mice that had been treated with heptachlor 72 hr earlier. HPLC and western blotting showed that the combination of heptachlor and MPTP was more toxic than either compound administered individually (DA +75% vs -28.6%; DAT -31.02% vs -51.9%; VMAT2 -19.4% vs -35.8% for MPTP vs heptachlor+MPTP, respectively). These results demonstrate that heptachlor increases susceptibility to MPTP and may be involved in the development of Parkinson's disease. (Supported by NIEHS 09248, 07784, and NINDS 37031.)

113 NIGROSTRIATAL DOPAMINERGIC TOXICITY INDUCED BY THE PYRETHROID INSECTICIDE DELTAMETHRIN.

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Recent studies suggest that the majority of cases of idiopathic Parkinson's disease involve an environmental component. Epidemiological studies have demonstrated that exposure to pesticides is a risk factor for Parkinson's disease. In this study we show that the pyrethroid insecticide deltamethrin significantly alters the levels of dopamine (DA), its plasma membrane transporter (DAT), and the vesicular monoamine transporter (VMAT2) in the striata of treated mice. 8-10 month old C57BL/6 male mice were given a single injection of deltamethrin (9mg/kg, i.p.) and sacrificed either 24, 48, or 72 hr later. Striata were harvested and DA levels analyzed by HPLC-EC and expression of the DAT and VMAT2 by western blotting. By 72 hr DA levels were reduced, while DAT and VMAT2 levels were increased (DA -27.2%, DAT +58.3%; VMAT2 +39.9%, respectively). Since DAT is thought to be the molecular gateway by which the active metabolite of the dopaminergic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) enters the dopamine neuron, MPTP was administered to mice that had been treated with deltamethrin 72 hr prior. Results indicate that animals treated with deltamethrin were more sensitive to the toxic effects of MPTP than animals treated with MPTP alone (DA +75.9% vs. -25.2%; DAT -31.3% vs. -76.8%; VMAT2 -19.6% vs. -62.9%, for MPTP vs. deltamethrin+MPTP respectively). A similar upregulation of DAT by deltamethrin (10 nM) was seen in neuroblastoma cells stably expressing human DAT (43.2% increase in DA uptake). These results suggest that disruption of dopamine function by pesticides such as deltamethrin may contribute to the etiology of Parkinson's disease by altering dopamine transport. (Supported by NIEHS 09248, 07784 and NINDS 37031.)

114 HYPERTHERMIA-ENHANCED SEROTONIN (5-HT) DEPLETION RESULTING FROM *d*-FENFLURAMINE (*d*-FEN) EXPOSURE DOES NOT EVOKE A GLIAL-CELL RESPONSE IN THE CENTRAL NERVOUS SYSTEM OF RATS.

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d-Fen-induced hyperthermia has been shown to coincide with an enhanced depletion of 5-HT and 5-hydroxyindole acetic acid (5-HIAA). Because these observations have relied on d-Fen exposure at multiple environmental temperatures, some have questioned the validity of the findings. Therefore, this experiment was designed to determined if the correlation between elevated body temperature and 5-HT depletion could be observed when d-Fen exposure occurred in one warm environment (28 degrees C) and to determined if a hyperthermia-enhanced glial-cell response could be evoked by d-Fen exposure. Hyperthermia-enhanced 5-HT and 5-HIAA depletion resulting from d-Fen exposure was dependent on body temperature during drug exposure. In the frontal cortex, 5-HT concentrations ranged from 3 to 45% of control values. Likewise, in the striatum and hippocampus, 5-HT concentrations ranged from 13 to 53% and 6 to 40%, respectively. The 5-HIAA concentrations had a wider range than the 5-HT concentrations for each brain region. In the frontal cortex, striatum and hippocampus, 5-HIAA ranged from 0 to 93%, 15 to 72% and 0 to 83% of control, respectively. In spite of the substantial reductions in 5-HT, there was no detectable glial-cell response. d-Fen-induced hyperthermia does not appear to cause generalized damage to neurons in the frontal cortex, striatum and hippocampus.

115 ACUTE EFFECTS OF METHYLPHENIDATE ON OPERANT BEHAVIOR IN THE RHESUS MONKEY II.

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In the present study, the stimulant methylphenidate (MPH) was administered to adult rhesus monkeys (N=8) previously trained on the five food-reinforced tasks of the NCTR non-human primate Operant Test Battery (OTB). Each task is designed to model a different aspect of brain function, including motivation (M), time estimation (TE), learning (L), color & position discrimination (CPD) and short-term memory (STM). MPH was administered acutely (i.v., 0.03 to 1.75 mg/kg, conducted over a 10 week interval) at 5.50 years of age and then again later at 9.25 years of age. Between these first and second acute administrations, MPH was also administered chronically (p.o., 2 times/day, 5 consecutive days/week, 0.1 - 2.0 mg/kg over 72 weeks and then 1.6 mg/kg for 81 weeks). Chronic administration of MPH produced no significant change in behavioral performance on any task of the OTB (The Toxicologist: 36, 63, 1997; 42, 304, 1998). However, when tested 15 minutes after acute MPH challenges, operant performance was significantly decreased on all tasks of the OTB (% task completed: for first acute administration, $F=14.23$ (M), 9.28 (TE), 9.59 (L), 5.55 (CPR) and 12.80 (STM), all $p<0.05$; for second acute administration, $F=16.83$ (M), 7.29 (TE), 26.56 (L), 12.16 (CPR) and 27.63 (STM), all $p<0.05$). Furthermore, the minimum dose for producing significant behavioral effects was lower for the second acute administration (% task completed, in mg/kg, first vs second: 1.0 vs 0.1 (L), 0.3 vs 0.1 (M, TE, STM); but 1.0 vs 1.0 (CPD)). It was concluded that, using a battery of operant behavior tests in a rhesus monkey model, the acute behavioral effects of the stimulant MPH can be modified by a preceding period of chronic MPH administration.

116 ROLE OF THE TSC-2 TUMOR SUPPRESSOR GENE IN QUINOL-THIOETHER MEDIATED NEPHROCARCINOGENICITY.

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Sustained compensatory cell proliferation following toxic insult contributes to the mechanisms by which non- or weak genotoxicants exert their carcinogenic effects. 2,3,5-Tris-(glutathion-S-yl)hydroquinone (TGHQ), a potent nephrotoxic metabolite of HQ, markedly induces cell proliferation within the outer strip of the outer medulla (OSOM), and increases the incidence of renal cell carcinoma in Eker rats carrying the mutant Tsc-2 allele (Tsc-2EK/+). To identify factors involved in TGHQ-mediated renal carcinogenesis, Tsc-2EK/+ and wild type (Tsc-2+/+) Eker rats were treated with TGHQ (2.5 μmol/kg, i.p., 5X/wk) for 1, 2, or 4 months. TGHQ markedly increased cell proliferation within the OSOM in both Tsc-2EK/+ and Tsc-2+/+ rats in regions in which the tumors arose. Elevated cell proliferation became more pronounced as chemical treatment continued. TGHQ increased renal tumor incidence in Tsc-2EK/+ but not in Tsc-2+/+ rats, suggesting that cell proliferation was necessary but not sufficient for tumor development. Consistent with this hypothesis, loss of the wild type allele in the Tsc-2 gene was detected in early preneoplastic lesions (dysplastic tubules) isolated using laser capture microdissection, and in renal cell carcinomas that arose in TGHQ treated Tsc-2EK/+ rats. We hypothesize that loss of tuberin (the Tsc-2 gene product) may lead to tumor development due to a requirement for tuberin function during compensatory cell proliferation, consistent with its proposed role as a negative regulator of the cell cycle. Loss of the normal Tsc-2 allele in tumors is associated with elevated levels of cyclin D1 and ERK activity, suggesting that these genes may be down-stream effectors of tuberin which drive tumor growth following loss of tuberin function. (GM39338, ES07784).

117 THE USE OF VARIANT GLUTATHIONE S-TRANSFERASE GENES TO CONFER PROTECTION AGAINST ALKYLATING DRUGS TO HUMAN HEMATOPOIETIC STEM CELLS.

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Past work in the field has shown that native glutathione S-transferase (GST) genes, when overexpressed in human cancer cells, can confer marked resistance to alkylating chemotherapy drugs as well as drugs known to generate reactive oxygen species. Previous work from our laboratory produced genetic variant GST genes with gain-of-function mutations that confer increased efficiency in conjugating and detoxifying nitrogen mustard derivatives. The current work presented here shows that expression of these gain-of-function mutants in human B-lymphocyte derivatives, Raji cells, results in greater resistance against the alkylating drug melphalan than that conferred by the wild-type GST. For these studies, both wild-type and gain-of-function GST variants were cloned into the pLg-IN retroviral expression vector. Retroviral particles containing these expression vectors were purified from PG13 packaging cells. In initial experiments, the retrovirus was used to infect Raji cells, and clones of GST-expressing cells were isolated. The initial experiments have shown that the gain-of-function GST variants, 11s1 and 11s4 confer greater resistance to these human B-lymphocyte derivatives than that conferred by the wild-type GSTs. Our current studies also include experiments to determine what limiting roles substrate availability, *i.e.* glutathione concentration, as well as reaction product concentration play in determining the over-

all GSH conjugation capacity of these B-lymphocytes. The concentration of GSH-conjugates formed in the cells is being modulated by expression of a recombinant MRP1 cDNA in the cells; this MRP1 membrane pump is responsible for exporting GSH-conjugates from mammalian cells. A full understanding of the role which GSH conjugation can play in modulating and controlling the toxicity of alkylating drugs in human cells is the underlying goal of all of these experiments. Such understanding will enable the use of these variant, gain-of-function GST proteins to protect human bone marrow cells in chemotherapy, and enable selection of bone marrow cells in routine gene therapy applications.

118 INACTIVATION OF PROTEIN DISULFIDE ISOMERASE WITH 1-CHLORO-2,4-DINITROBENZENE.

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Protein disulfide isomerase (PDI) is a multifunctional protein with a number of diverse functional roles in the cell. In addition to thiol-disulfide exchange, PDI catalyzes the glutathione dependent reduction of dehydroascorbate, assists in the oxidative disulfide formation of protein folding, binds Ca^{2+}, estrogen, and thyroid hormones, and serves as a subunit of prolyl hydroxylase and triglyceride transferase found in the endoplasmic reticulum (ER). Protein disulfide isomerase, a 57-kDa ER resident is one of the most active and abundant oxidative folding catalysts isolated to date, with concentrations estimated at near mM in the ER. PDI is a member of the thioredoxin superfamily, having two functional domains with significant sequence homology of the redox cofactor, thioredoxin. These two domains are responsible for the disulfide isomerase activity. 1,2-Chloro-2,4-dinitrobenzene (DNCB, MW= 202.6) is an alkylating agent used for depleting intracellular GSH and has also shown distinct immunomodulatory properties. DNCB has been shown as an irreversible inhibitor of thioredoxin reductase which also contains the thioredoxin functional domain. In an effort to determine the relative reactivity of the thiols of the thioredoxin family of proteins, we incubated isolated recombinant rat PDI for 20 minutes at 20°C with increasing amounts of DNCB (0.1 to 1 mM). Our results show that a concentration of 1 mM DNCB decreases PDI activity by 70% within 5 minutes as measured by the GSH-dependent reduction of insulin. MALDI-MS analysis of PDI incubated with 0.5mM DNCB showed an increase in molecular weight by 462±156 g/mole. This increase corresponds to possibly three alkylation sites per molecule of PDI. Further analyses of the disulfide isomerase activity corresponding to these alkylation sites are currently underway. (This work was supported by grants from the NIH [ES00040 and ES00210].)

119 CHARACTERAZATION OF THE INTERACTION BETWEEN GLUTAMATE-CYSTEINE LIGASE SUBUNITS.

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Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme in the glutathione (GSH) biosynthesis pathway. This enzyme is a heterodimer comprising a structural subunit (GCLS) and a regulatory subunit (GCLR). Although GCLS alone can catalyze the formation of L-γ-glutamyl-L-cysteine, this activity is partially inhibited in the cell due to low affinity for glutamate and high sensitivity to GSH inhibition. The presence of GCLR enhances the enzyme activity by lowering the Km for glutamate and increasing the Ki to GSH inhibition. Therefore, GCLR plays a pivotal role in maintaining GSH homeostasis *in vivo*. To characterize further the interaction between GCLS and GCLR, we have developed a system to study heterodimer formation between purified His-6-GCLS (or GCLR) and ^{35}S-labeled GCLR (or GCLS) by means of Ni-6-Histidine tag affinity chromatography. When a mixture of His-6-GCLS and radiolabeled GCLR was applied to Ni-NTA resin, retention of GCLR was dependent on the presence of His-6-GCLS. Both GCLR and the heterodimer were present on the non-reducing SDS-PAGE gel, whereas under reducing conditions, only GCLR was seen on the gel, indicating that the GCLS/GCLR interaction involves both non-covalent binding and disulfide bond. Preliminary evidence revealed that the disulfide interaction is enhanced in the presence of oxidized GSH (GSSG) but not affected by L-glutamate, ATP, or L-α-aminobutyrate. GCLR contains six cysteine residues, two of them being consecutive. Each of the cysteine has been mutated to alanine by site-directed mutagenesis. The importance of each cysteine residue in the disulfide formation is currently under investigation. In addition, we have constructed a series of truncated GCLR proteins in order to map the region of non-covalent interaction. (Supported in part by NIH Grant R01 AG09235.)

120 GENETIC POLYMORPHISMS IN TWO GLUTATHIONE ASSOCIATED GENES AND SUSCEPTIBILITY TO IDIOPATHIC PULMONARY FIBROSIS.

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Pulmonary fibrosis(PF) is characterized by excessive accumulation of connective tissue in the lung interstitium. Asbestos-related fibrosis has been suggested to be mediated by reactive oxygen species. Moreover, it has been shown that patients with idiopathic PF (IPF) have very low levels of glutathione(GSH) in their alveolar epithelial lining fluid. In addition, glutathione-S-transferase(GST) polymorphisms, especially class GSTM-1, have also been implicated in asbestos-related PF. Therefore we focused on two polymorphic enzymes which are crucial in detoxification of reactive oxygen species, the first and rate-limiting enzyme in GSH synthesis, glutamate-cysteine ligase(GLCL), and GSTM-1. GLCL is composed of two subunits, a catalytic subunit(GLCLc) and a regulatory subunit(GLCLr). GLCLc has a polymorphic trinucleotide repeat(TNR) in the 5'-untranslated region of its mRNA, just upstream of the GLCLc start codon. The GLCLc TNR genotypes of human fibroblast cell lines isolated from both normal (19) and IPF (21) lung tissue were characterized by PCR and automated DNA sequencing. We found 7, 8 and 9 TNR homozygosity, and 9/8, 9/7 and 8/7 TNR heterozygosity in both cell types. Although there was no significant difference in the frequency of any genotype, the 8 TNR was more frequent in fibrotic cells, whereas 9 and 9/7 TNR were more frequent in normal cells. Studies to determine the functional significance of those polymorphisms are being carried out. We also examined GSTM-1 genotypes. No significant difference between fibrotic and normal cells were found with respect to this genotype. (This work was supported by NIH grant ES 07033 and ES 04696.)

121 PCB-INDUCED EFFECTS ON GLUTATHIONE, AND GLUTATHIONE-RELATED ENZYMES AT SUBCHRONIC TIME POINTS.

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Although polychlorinated biphenyls (PCBs) have been banned from production in many countries since the early 1970s, they persist in our environment and accumulate in target tissues, such as adipose and breast tissues. PCBs may induce drug-metabolism and may be substrates for the induced enzymes. Metabolism of PCBs may lead to oxidative events within the cell. The goal of this study was to determine the influence of PCBs on glutathione and glutathione-related enzymes. Male and female Sprague Dawley rats received two i.p. injections per week of PCBs 3, 28, 38, 77, 153 or both 77 & 153 (100μmol/kg/injection) and were sacrificed at the end of 1, 2, or 3 weeks. Livers were surgically removed, flash frozen in liquid nitrogen, and stored at –80. Whole liver homogenates, cytosol and microsomes were prepared and glutathione, glutathione peroxidase, glutathione reductase and glutathione transferase were determined. Both glutathione reductase and glutathione transferase activities were significantly increased in both male and female rats receiving PCB 77, a ligand for the Ah receptor, as well as both 77 & 153. No significant trend was observed in the total glutathione level data. Interestingly PCB 77, as well as the combination of PCB 77 & 153, decreased glutathione peroxidase activity levels in both male and female rats. Using northern analysis, we confirmed this decrease of activity, in male and female rats treated with PCB 77, was due to a decrease in GPx1 transcript. (P42 ES 07380, DAMD 17-96-1-6162, and T32 ES07266).

122 TISSUE SPECIFIC CHANGES IN GLUTATHIONE AND THE EXPRESSION OF THE CATALYTIC AND REGULATORY SUBUNIT OF GLUTAMATECYSTEINE LIGASE IN MICE EXPOSED TO METHYLMERCURY: A TIME-COURSE STUDY.

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The tripeptide glutathione (GSH) plays a crucial role in protecting cells against oxidative stress. GSH is synthesized in a two-step process, the rate-limiting step being the binding of glutamate and cysteine, which is catalyzed

by the enzyme glutamate-cysteine ligase (GLCL). This enzyme is composed of two subunits, a large catalytic subunit (GLCLc) and a smaller regulatory subunit (GLCLr). The toxicity of methylmercury (MeHg) is known to be influenced by GSH status, and MeHg exposure often results in GSH depletion and induction of GLCL at the mRNA and protein levels. The objective of this study was to determine the effect of MeHg treatment (single dose of 6 mg/Kg by ip injection) on different mouse tissues. The experiment was designed as a time-course study in which 3 control and 4 treated C57 female mice were used for each time-point (6 h., 12 h. and 24 h.) and the tissues analyzed were liver, kidney, lung and small intestine. GSH levels were analyzed by Tietze assay, GLCL activity by HPLC, GLCLc and GLCLr protein levels by Western blot and GLCLc and GLCLr mRNA levels both by Northern blot and real-time fluorogenic 5' nuclease assay. There were no substantial changes in GSH levels or GLCL activity in the tissues examined. The more dramatic changes occurred at the mRNA level, with induction of both subunits in kidney, lung and small intestine at 12 h. Interestingly, the small intestine shows preferential upregulation of GLCLc, and the liver shows downregulation of both subunit mRNAs at 6 h. The changes in protein levels were not as substantial, but they reflect the changes in mRNA levels. These results show that different mouse tissues respond differently to MeHg treatment in regards to changes in GLCL, which suggests time- and tissue-specific regulation of the enzyme.

123 EFFECT OF INCREASING GSH SYNTHESIS ON RESISTANCE OF HEPA-1 CELLS TO DNA BREAKS CAUSED BY H_2O_2.

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GSH is important in defending against oxidative stress. The rate limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GLCL) which is composed of catalytic (GLCLC) and a regulatory (GLCLR) subunits. We developed cells with metallothionein promoter-regulatable overexpression of either GLCLC alone, GLCLR alone or the combination to test the hypothesis that increased GSH synthesis ability can protect cells from DNA breaks caused by H_2O_2 exposure. Microgel single cell electrophoresis assay (comet assay) was used to measure the DNA single-brand breaks after exposure to 100 mM H_2O_2 for one hour. The length of comet tails, and ratio of tail length to nuclear size were measured to quantitate DNA breaks. Compared with vector controls, cells overexpressing GLCLC and cells overexpressing GLCLR had significantly less DNA breaks as indicated by shorter tail length. Cells overexpressing GLCLC and cells overexpressing GLCLC + GLCLR were significantly lower in the ratio of tail length to nuclear size. These results show that increased synthesis of GSH helps to protect cells from DNA breaks caused by H_2O_2.

124 PREFERENTIAL INDUCTION OF THE G-GLUTAMYLCYSTEINE SYNTHETASE CATALYTIC SUBUNIT (GCSH) GENE BY DESFERRIOXAMINE OR HYPOXIA.

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The genes encoding the catalytic (GCSh) and regulatory (GCSl) subunits of g-glutamylcysteine synthetase, which catalyzes the rate limiting step in glutathione synthesis, can be induced by many agents including pro-oxidants and electrophiles. Typically both subunit genes are similarly induced in response to these agents. However, exposure of HepG2 cells to the iron-chelator, desferrioxamine (DFX), results in a robust induction of GCSh expression (~7x) accompanied by a relatively minor elevation (<2x) of GCSl mRNA levels. Induction by DFX is inhibited by both Actinomycin D and cycloheximide, suggesting that gene transcription and synthesis of new protein(s) are required. In contrast, catalase does not alter induction. DFX exposure also results in reduction of the GSH/GSSG ratio (decreased GSH; increased GSSG). DFX has been reported to activate gene expression via hypoxia responsive elements (HREs) by yet unknown mechanisms. Like DFX exposure, incubation of HepG2 cells under hypoxic (95% N2/5% CO2) conditions for 12 hours results in a significant elevation (>6x) of GCSh mRNA levels, but no change in GCSl expression. Differential induction persists following hypoxic incubation up to 24 hours. Consensus HREs have not been identified in the promoter of either subunit gene, but multiple potential HREs are present in the first intron of GCSh. In preliminary experiments DFX failed to induce expression of GCSh promoter/luciferae transgenes. Determination of enhancer activity in GCSh intron sequences is on-going. These results demonstrate that GCS subunit genes are not necessarily coordinately regulated and may be induced by independent mechanisms. (Supported by CA57549 and ES09749.)

125 EXPRESSION OF GLUTATHIONE REDUCTASE (GR), PEROXIDASE (GPX) AND TRANSFERASE (GST) ACTIVITIES IN NUCLEI AND NUCLEOLI OF LIVERS IN MALE AND FEMALE FISCHER 344 RATS.

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GR is an important component of cellular antioxidant defenses, serving to sustain cellular supplies of glutathione (GSH) and to limit accumulation of glutathione disulfide (GSSG), thereby limiting the oxidation and S-thiolation of proteins. Increased expression of GR can significantly enhance cellular resistance to oxidants, particularly when expression is increased selectively in mitochondria. In the present studies, we isolated and purified nuclei from livers of male and female F344 rats using discontinuous sucrose gradient centrifugation, and assayed GR activities, which were 12 and 15 mU/mg pro, respectively. Nucleolar fractions showed GR activities of 30 and 51 mU/mg of pro, respectively, $P<0.05$ versus nuclear activities. Male F344 rats showed increases in GR activities in both nuclear and nucleolar fractions after giving 0.1 mmol/kg diquat; female F344 rats given 0.2 mmol/kg diquat showed increased GR activities in the nuclear fraction and decreased GR activities in the nucleolar fraction. GPX activities in nuclear and nucleolar fractions were between 200 and 300 mU/mg pro, but no differences in fractional activities were observed, although minor effects of diquat were observed in female rats. GST activities towards 1-chloro-2,4-dinitrobenzene (CDNB) in nuclei were 850 and 470 mU/mg pro in males and females, respectively; activities in nucleolar fractions were less than 40 mU/mg pro in all samples, indicating that the GR and GPX activities observed in the nucleolar fractions are not attributable to incomplete separation of nucleoplasm in isolation of the nucleoli. Some minor differences in activities of these enzymes were observed in nuclei and nucleoli, as were some changes in response to administration of diquat, but none of the differences or changes offer a reasonable explanation for the initiation of hepatic necrosis in diquat-treated rats, or the marked differences in susceptibilities between male and female F344 rats. The data indicate GR, GPX, and GST in nuclei contribute to cell defense functions, but the functions served by selective expression of GR and GPX in nucleoli are less evident. (Supported by NIH GM 44263.)

126 CLONING AND EXPRESSION OF RAT KIDNEY MITOCHONDRIAL DICARBOXYLATE CARRIER EXHIBITING GLUTATHIONE TRANSPORT ACTIVITY.

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Total rat kidney RNA was reverse transcribed and amplified with forward and reverse primers based on the published, complete cDNA sequence for the mitochondrial dicarboxylate carrier (DCC) from the Norway rat (*Rattus norvegicus*; GenBank accession number AJ223355). The expected PCR products were identified and a 1056-bp product was ligated into a T-A cloning vector (pGEM T-Easy) and its identity was confirmed by automated DNA sequencing. The amplified cDNA was then cloned into a bacterial expression vector (pRSET B) that adds an *N*-terminal $(His)_6$ tag to the translation product. The $(His)_6$-DCC fusion protein was overexpressed in *E. coli* and isolated from inclusion bodies. A time-dependent increase in expression of a 35.5-kDa protein, representing the fusion protein, was observed. Additional purification was attempted with a nickel-affinity column. The presence of the expressed protein was confirmed by immunoblot analysis using the RGS-His antibody, which only recognizes proteins with *N*-terminal $(His)_6$ tags. A single, immunoreactive protein at 35.5-kDa molecular weight, was observed. Before assessment of the function of the expressed carrier protein, liposomes were reconstituted with the solubilized inclusion body fraction and the presence of the DCC protein was confirmed by SDS-PAGE. The reconstituted proteoliposomes demonstrated rapid uptake of both [^{3}H]-glutathione and [^{14}C]-malonate. Furthermore, addition of 10 mM butylmalonate inhibited both transport processes, demonstrating function of the DCC. These results are consistent with properties of the DCC previously observed with intact rat kidney mitochondria and with partially purified and reconstituted protein. This approach will allow us to explore the molecular regulation of mitochondrial glutathione status and to better assess the function of this transport process in renal proximal tubular cells.

127 ELEVATED GLUTATHIONE (GSH) POOLS AND γ-GLUTAMYL TRANSPEPTIDASE (GGT) ACTIVITY IN COUMARIN-INDUCED CLARA CELL TOLERANCE.

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Coumarin is metabolized to epoxide and aldehyde intermediates which are acutely toxic to mouse lung Clara cells. However, with repeated dosing, Clara cells become resistant to coumarin-induced injury and demonstrate cross-resistance to other Clara cell toxicants. The purpose of the present work was to determine whether Clara cell tolerance is associated with changes in pulmonary glutathione regulation. Female B6C3F1 mice were dosed orally with coumarin (200 mg/kg) or vehicle for 1 or 10 days after which GSH levels and GGT activity were measured in whole lung. GGT and Clara cell secretory protein (CC10) were also evaluated with histochemical and immunohistochemical methods, respectively. Constitutive GSH levels increased from 1.3±0.1 μmol/g in control mice to 1.9±0.1 μmol/g in mice dosed for 10 days with coumarin. At 2 hr after a single dose of coumarin, whole lung GSH decreased by about 30% relative to control, whereas lung GSH was not significantly reduced at 2 hr after 10 daily doses. A single dose of coumarin did not alter lung GGT activity, but with repeat dosing, GGT activity was induced 7-times control values (26±3 and 182±13 pmol/min/mg, respectively). Histochemically, the increased GGT activity was localized to bronchiolar epithelium. Concurrent with changes in GGT and GSH regulation, the abundance of CC10 was markedly reduced in tolerant Clara cells. These results indicate that Clara cell tolerance is characterized by the maintenance of elevated GSH concentrations which is mediated at least in part by increased GGT activity. The loss of CC10 expression also suggests a change in the differentiation state of tolerant Clara cells.

128 *IN UTERO* ETHANOL EXPOSURE PRODUCES DIFFERENTIAL MITOCHONDRIAL GLUTATHIONE DEPLETION IN THE RAT CONCEPTUS.

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Glutathione (GSH) is the most abundant low molecular weight thiol in the cell. Glutathione is synthesized and primarily located in the cytosol (85%) but is also found in the mitochondria (15%). Mitochondrial GSH content is critically dependent on uptake of cytosolic GSH. Alterations in mitochondrial GSH content have been shown to cause uncoupling of oxidative phosphorylation and opening of the redox-sensitive mitochondrial permeability transition pore. Ethanol (EtOH) causes teratogenicity in various animal models *in utero* and *in vitro* and modulates mitochondrial GSH content in adult tissues. This study was designed to determine the ability to deplete mitochondrial GSH in the early organogenesis stage rat conceptus following *in utero* exposure to EtOH. Dams were gavaged with 4.5 g/kg EtOH on the morning of gestational day 10. Purified mitochondria were prepared from embryos and visceral yolk sacs (VYS) harvested 12 and 24 hr later. Total cellular, mitochondrial, and cytosolic GSH content were measured by HPLC detection. Twelve hours after exposure to 4.5 g/kg EtOH, total cellular GSH content in the embryo was unchanged, yet embryonic mitochondrial GSH was depleted by 95% compared to control and cytosolic GSH was increased by 40%. At the 12 hr time point, VYS total cellular GSH content was depleted by 27%. Mitochondrial GSH content in the VYS decreased by 95%, and VYS cytosolic GSH was increased by 32%. After 24 hr, embryonic total cellular GSH was unchanged. Embryonic mitochondrial GSH showed a rebound recovery and cytosolic GSH was returned to control values. At the 24 hr time point, VYS total cellular GSH content was unchanged. However, VYS mitochondrial GSH was decreased by 43% compared to control, and cytosolic pools of GSH returned to control values. These results indicate that EtOH causes transient changes in total cellular GSH content while producing a dramatic redistribution of GSH from mitochondria to cytosol. These data suggest a mechanism of teratogenicity for EtOH through selective modulation of conceptal mitochondrial GSH status. (This research supported by ES07062, ES08846, and ES05235.)

129 *IN UTERO* AND *IN VITRO* COMPARISON OF ETHANOL EFFECTS ON THE ORGANOGENESIS STAGE RAT CONCEPTUS.

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Ethanol (EtOH) produces neural tube and craniofacial defects and mental and growth retardation in the organogenesis stage rat conceptus. Teratogenic effects of EtOH have been suggested to result from oxidative stress which can alter reduced glutathione (GSH) status. Altered GSH and ATP concentrations are indices of oxidative stress and were evaluated in the gestational day 10 rat conceptus exposed to EtOH *in utero* and *in vitro*. Exposure of the dam to EtOH (4.5 g/kg) for 30 min did not affect GSH concentrations in the embryo or visceral yolk sac (VYS). After 3 hr, GSH content was decreased by 40% in the embryo and was unchanged in the VYS. By 6 hr, embryonic GSH content showed a rebound increase to 125% control and VYS GSH content remained unchanged. Exposure to EtOH *in vitro* (18 mg/ml) for 30 min did not affect GSH concentrations in the embryo or VYS. Beginning at 3 hr, embryonic and VYS GSH content decreased significantly over time until the end of the exposure period. Although GSH content was unchanged at 30 min, there was a 35% decrease in embryonic ATP levels following *in utero* exposure to EtOH. After 3 hr, embryonic ATP was still decreased by 35%. Cultured embryos showed a 55% depletion in ATP content, and after 3 hr, embryonic ATP levels remained depleted by 30%. ATP content in the VYS, however, showed only transient changes after EtOH exposure both *in utero* and *in vitro*. These results demonstrate clear differences in the *in utero* and *in vitro* effects of EtOH. Cultured embryos are more susceptible to changes in GSH and ATP content and do not show the adaptive response to EtOH-induced GSH depletion seen *in utero* at 6 hr. This rebound may provide a protective mechanism against EtOH toxicity *in utero*. Differences between *in utero* and *in vitro* responses to EtOH have important implications for the interpretation of *in vitro* developmental studies. (This research supported by ES08846, ES05235, and ES07062.)

130 EVALUATION OF CYTOPROTECTIVE PROPERTIES OF EBSELEN (2PHENYL-1,2 BENZISOSELENAZOL-3-(2H)-ONE) AGAINST CISPLATIN AND DIETHYLDITHIOCARBAMATE TOXICITY IN RAT HIPPOCAMPAL ASTROCYTES.

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Ebselen (2phenyl-1,2 benzisoselenazol-3(2H)-one), a seleno-organic compound, has been shown to have cytoprotective properties. This has been attributed to its ability to act as a glutathione peroxidase (GSH/PX) mimic. It has been implicated in increasing intracellular glutathione (GSH), as well as expressing antioxidant properties due to its ability to act as a scavenger of organic hydroperoxides. Cisplatin is a widely used chemotherapeutic agent that causes the generation of reactive oxygen species (ROS) which may interfere with cellular antioxidant defense systems. Diethyldithiocarbamate (DDC) is a compound commonly found in fungicides. It has been found to impose its toxic effect by disrupting cytoskeletal proteins and decreasing intracellular GSH pools. The purpose of this experiment was to evaluate the cytoprotective effects of ebselen pretreatment in cultures of rat hippocampal astrocytes exposed to cisplatin and diethyldithiocarbamate. Rat hippocampal astrocytes grown in DMEM/F-12 were pretreated with 30μM ebselen followed by exposure to 500, 250,125, 62.5, 31.25,15.625 and 7.8 μM cisplatin or 35 μg/ml DDC at subconfluency. Cell viability was determined using an MTT assay, a system that measures the activity of living cells via alterations in mitochondrial dehydrogenases. Dramatic increases in viability were seen in cells pretreated with ebselen and exposed to 500 and 250μM cisplatin. No protective effects were noted in cells pretreated with ebselen followed with DDC treatment. This poses questions on the mechanism of action of ebselen since GSH protects against DDC cytotoxicity.

131 IMPACT OF GSH DEPLETION ON HEPATIC COASH AND COASSG FOLLOWING TREATMENT WITH 4,4'-METHYLENE DIANILINE.

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Methylene dianiline (DAPM) is an aromatic amine that specifically injures biliary epithelial cells (BEC). Previous studies indicate DAPM injury involves BEC, but not hepatic, mitochondria. DAPM also has only a minimal effect on hepatic glutathione (GSH) or glutathione disulfide (GSSG). Liver

Coenzyme A in its thiol form (CoASH) and as glutathione mixed disulfide (CoASSG), found predominately in mitochondria, equilibrates with GSH/GSSG and is used to indicate mitochondrial thiol/disulfide status. Our goal was to determine whether GSH depletion with both bromoheptane (BH, an alkylating agent) and butathionine sulfoximine (BSO, a GSH synthesis inhibitor) alters hepatic CoASH or CoASSG following DAPM treatment. Male SD rats were given BH (0.5 mmol/kg, po) at -1.5h, BSO (2.5 mmol/kg, ip) at -0.5h, and DAPM (50 mg/kg in 35% ethanol) at 0h. Liver was collected and freeze-clamped at 0, 3 and 6h. CoASH/CoASSG were determined by modification of an HPLC method developed by Rogers and Smith. BH/BSO treatment depleted GSH by 60-80% over 0-6h. While BH/BSO alone produced no evident injury, DAPM treatment alone produced moderate BEC injury. GSH depletion prior to DAPM treatment, however, caused pronounced BEC injury and mild hepatocellular injury. BH/BSO alone significantly increased CoASH and decreased CoASSG. DAPM alone decreased both CoASH and CoASSG at 3h, with no effect at 6h. Finally, treatment with both BH/BSO plus DAPM slightly decreased CoASH and dramatically increased CoASSG at 6h. Further, HPLC revealed a new peak eluting at a similar retention time as CoASH in samples from DAPM– and DAPM plus BH/BSO-treated rats. Identification of this component may indicate whether CoASH alkylation by DAPM/DAPM metabolite is occurring. Thus, GSH depletion enhanced the severity of DAPM toxicity while significantly increasing CoASSG. DAPM-induced changes in CoASH/CoASSG could be due to mitochondrial injury or to cell death. Nonetheless, this method provides a sensitive indication of mitochondrial involvement in DAPM toxicity. (Supported by NIEHS T32-ES 07254; NIEHS 06348.)

132 N-METHYLDITHIOCARBAMATE (NMDC) AND N,N-DIMETHYLDITHIOCARBAMATE (DMDC) DEPLETE GLUTATHIONE THROUGH INDEPENDENT MECHANISMS.

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Dithiocarbamates are used in agriculture, medicine and industrial applications. NMDC has been reported to be two orders of magnitude more cytotoxic to CHO cells than the dialkyl analog, DMDC. The chemistry for these structurally related compounds is expected to be similar with the exception that NMDC can decompose at physiological pH to sulfhydryl ion (HS-), a potent inhibitor of cytochrome c oxidase, and methyl isothiocyanate (MITC), an electrophilic compound capable of covalently modifying cellular nucleophiles. The relative contribution of these two decomposition products to the cytotoxicity of NMDC was assessed using isolated rat liver mitochondria and suspension cultures of primary rat hepatocytes. Respiration of isolated rat liver mitochondria was assessed by monitoring oxygen consumption with an oxygen electrode and intracellular and extracellular reduced and oxidized glutathione from hepatocytes was measured by HPLC. Isolated rat liver mitochondria incubated with either NMDC or DMDC indicated that neither state 3 nor state 4 respiration was significantly different from controls for either dithiocarbamate. Incubation of hepatocytes with either NMDC or DMDC resulted in severely depleted intracellular levels of reduced glutathione. Prior depletion of intracellular GSH levels using diethylmaleate in cultured rat hepatocytes resulted in increased cytotoxicity for both NMDC and DMDC whereas a modest cytoprotective effect for NMDC and DMDC was observed upon supplementation of intracellular GSH levels with the membrane permeable ethyl ester of GSH. Extracellular samples from NMDC treated cells had significantly lower levels of oxidized glutathione than DMDC treated cells. LC/MS analysis of extracellular and intracellular samples from hepatocytes treated with NMDC indicated the presence of an S-(methyldithiocarbamoyl) glutathione conjugate and the mixed disulfide containing GSH and NMDC. The results obtained from the present study support the conclusion that NMDC and DMDC deplete GSH levels through independent mechanisms and that MITC plays a greater role in the cytotoxicity of NMDC than does HS-. (Supported by NIEHS grants ES06387, ES00267 and ES07028..

133 MECHANISTIC STUDY OF THE ACUTE TOXICITY OF 2-CHLOROACRYLONITRILE.

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The acute toxicity of 2-Chloroacrylonitrile (2-CAN) in Sprague Dawley rats as a function of cyanide generation and glutathione depletion was investigated. 2-CAN was administered through i.p. injection at doses of 10 mg/kg, 20 mg/kg and 40 mg/kg. Tissues (liver, kidney, heart and lung) were harvested at 15 minutes, 30 minutes, 1 hour, 2 hours and 4 hours after 2-CAN administration. Results show significant glutathione depletion in the liver after 1 hour. Depletion in the lungs was also observed but to a lesser amount. Detectable levels of cyanide increased as the dose increased. The cyanide level in blood after one hour was approximately 0.1 mcg/ ml. This data suggests that increased cyanide levels in the blood along with glutathione depletion may have a critical role in the mechanism of the acute toxicity of 2-CAN. Depletion of glutathione may allow the cyanogen to pass through oxidative metabolism and thereby cause increased cyanide levels in the blood.

134 THE PROTECTIVE ROLE OF THE ALPHA-CLASS GLUTATHIONE S-TRANSFERASE AGAINST OXIDATIVE STRESS.

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Glutathione peroxidase (GPx) plays a major role in cellular defense against oxidative stress by reducing H_2O_2 and the lipid hydroperoxide (L-OOH) which propagate the autocatalytic chain of lipid peroxidation. Several Se-dependent GPx isozymes and the alpha class Glutathione S-transferases (GSTs) can reduce L-OOH through the GPx activity. However, the relative contributions of GSTs and the Se-dependent GPx isozymes in the reduction of L-OOH in cells is not known and needs to be investigated. During the present studies recombinant human alpha class GSTs A1-1 and A2-2 were prepared through their heterologous expression in *E. coli*. hGSTA1-1 and hGSTA2-2 catalyzed reduction of L-OOH and cumene hydroperoxide. The catalytic efficiency (k_{cat}/K_m) of hGSTA1-1 for phosphatidylcholine hydroperoxide (PC-OOH) and phosphatidylethanolamine hydroperoxide (PE-OOH) was found to be 181.3 and 199.6s^{-1} mM^{-1}, respectively, while the catalytic efficiency of hGSTA2-2 for PC-OOH and PE-OOH was 353 and 317.5 $s^{-1}mM^{-1}$, respectively. Immunotitration studies performed with the extracts of liver and testis from human and rat using the polyclonal antibodies against the alpha class GSTs showed that these antibodies immunoprecipitated about 75% and 46% of total GPx activities toward cumene hydroperoxide from human and rat liver, respectively. Likewise, about 64% and 56% of total GPx activities toward cumene hydroperoxide were immunoprecipitated by these antibodies from human and rat testis, respectively. Similar results were obtained when L-OOH were used as substrates in the place of cumene hydroperoxide. These results strongly suggest that the alpha class GSTs play an important role against oxidative stress in liver and testis through their Se-independent GPx activity. (CA27967)

135 GLUTATHIONE DEPLETION AND THE PRODUCTION OF REACTIVE OXYGEN SPECIES IN ISOLATED HEPATOCYTE SUSPENSIONS.

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Freshly isolated suspensions of rat hepatocytes were exposed to the reduced gluathione (GSH) depleting agents *N*-ethylmaleimide (NEM), diethyl maleate (DEM) and ethyl methanesulfonate (EMS). These agents induced lipid peroxidation and cell death in a time-dependent manner. Dose response curves for DEM and EMS indicate that GSH must be depleted to below detectable levels (1 nmol/10^6 cells) in order for large increases in lipid peroxidation to occur. Concentrations of DEM and EMS that induced lipid peroxidation also increased reactive oxygen species (ROS) production as measured by increases in dichlorofluorescein (DCF) fluorescence. The addition of antioxidants prevented lipid peroxidation and protected against cell death, suggesting that lipid peroxidation is involved in the sequence of events leading to cell death induced by DEM and EMS. Mitochondrial electron transport inhibitors increased EMS and DEM-induced lipid peroxidation and cell death while the mitochondrial uncoupler, carbonyl cyanide m-chlorophenylhydrazone (CCCP), blocked lipid peroxidation but not cell death. Trifluoperazine (TFP), in contrast, decreased EMS and DEM-induced lipid peroxidation and cell death. Cyclosporin A provided no protection suggesting that TFP is not protecting solely through inhibition of the mitochondrial permeability transition. EMS-treatment resulted in the significant loss of mitochondrial α-tocopherol shortly after its addition, and this loss preceded losses in cellular α-tocopherol levels. Our results indicate that GSH depleting agents can induce large increases in lipid peroxidation and ROS production following the loss of GSH, and the mitochondria are the major source for this ROS generation. (This work was supported by NIEHS/NIH grant # R01ES 05452.)

136 INTRACELLULAR S-GLUTATHIONYL ADDUCTS IN HUMAN BRONCHOEPITHELIAL CELLS AFTER EXPOSURE TO TOLUENE DIISOCYANATE.

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Toluene diisocyanate (TDI) is a recognized chemical asthmogen, yet the mechanism of this toxicity and the molecular reactions involved have not been elucidated. We have previously shown that TDI vapor forms adducts with the apical surface of the respiratory epithelium, and that it colocalizes with ciliary tubulin. *In vitro*, we have shown rapid reaction of TDI with glutathione (GSH) and transfer of the monoisocyanato-monoglutathionyl-TDI to a sulfhydryl-containing peptide. In addition, in culture we have evidence for TDI reacting with both low and high molecular weight intracellular thiols. GSH reactivity and transcarbamoylation could provide a mechanism for formation of TDI adducted hemoglobin which we have detected in circulating red blood cells. This study sought to identify intracellular GSH as the nucleophile reactant for TDI. We used the dye CellTracker Green (5-chloromethyl-fluorescein diacetate, CMFDA) for detection of glutathione. Dye activation and fluorescence requires functional intracellular esterases and glutathione S-transferase. Undifferentiated (1 day) and differentiated (6 day) air-liquid cultures of human bronchoepithelial cells were exposed to 100 ppb TDI vapor for 5, 15, or 30 min. Following exposure, cells were placed in medium containing 10 micromolar CMFDA. After 30 min, they were washed, then incubated for an additional 30 min with fresh medium prior to fixation with paraformaldehyde. Cells were subsequently imaged using a confocal microscope. Both one and six day cultures showed a decrease in intensity of the thiol staining as a function of the length of TDI exposure. For 1 day cultures, decrease in intensity was seen at the 30 min time period. For 6 day cultures, the decrease was evident in cultures exposed for only 5 min. This difference may be attributed to the difference in the amount of thiols in undifferentiated vs. differentiated cells. Differentiated cells contain 30-50% of the thiols found in undifferentiated cells. These results indicate that TDI enters pulmonary cells and reacts with intracellular GSH. This rapid reaction leading to the formation of S-glutathionyl adducts of TDI suggests the importance of cellular thiols in TDI-induced pulmonary disease. (Supported by Arizona Toxicology Center grant #ES06694 and NIEHS #05651).

137 2,3,5-TRIS-(GLUTATHION-S-YL) HYDROQUINONE INDUCES CELL TRANSFORMATION IN PRIMARY KIDNEY EPITHELIAL CELLS OF THE EKER RAT.

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2,3,5-Tris-(glutathion-S-yl)hydroquinone (TGHQ) is a nephrotoxic and nephrocarcinogenic metabolite of the non-genotoxic carcinogen, hydroquinone (HQ) in the Eker rat. The Eker rat possesses a germline insertion in the Tsc-2 tumor suppressor gene, which predisposes these rats to renal cancer. In the present study, we report that TGHQ is capable of transforming renal epithelial cells from Eker rats after a single treatment (100 μM or 300 μM for 4hr). Cell lines were subsequently established (QT-RRE1, 2 and 3) from transformed colonies. QT-RREs exhibit characteristics of transformed cells, such as aneuploidy and anchorage-independent growth. G-banding analysis demonstrated a broad range of numerical chromosomal alterations among the cell lines. No Tsc-2 gene transcripts nor expression of tuberin, the protein encoded by Tsc-2, was observed in these cell lines as determined by RT-PCR and western blot analysis. Thus, the remaining functional allele of the Tsc-2 locus is inactivated by TGHQ treatment, which likely contributes to the development of the transformed phenotype. ERK (extracellular-signal-regulated kinase) may be one of the downstream effectors of tuberin, and high ERK activity may contribute to the disrupted growth signaling pathways caused by the loss of tuberin. Indeed, all three cell lines exhibit five fold higher ERK activity than untreated kidney epithelial cell lines. The results suggest that the Tsc-2 tumor suppressor gene is a target for TGHQ-mediated transformation. Moreover, inactivation of Tsc-2 by TGHQ appears to disrupt growth related signaling pathways via up-regulation of ERK activity. (GM39338, ES07784).

138 EFFECT OF DIETARY EXPOSURE OF 2,4,6-TRINIROTOLUENE (TNT) ON BIOCHEMICAL PARAMETERS IN NORTHERN BOBWHITE QUAILS (*COLINUS VIRGINIANUS*).

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The widespread military use, manufacturing, loading, assembly and processing of TNT has resulted in regional contamination of soil, water and sediment at certain Army installations and waste disposal sites. This contamination has the potential of negatively impacting the wildlife. Currently, there are no toxicity data in birds to assess the ecological risk associated with the exposure to TNT. To address this issue, male and female birds weighing about 250-300 g (26-weeks) were fed on a diet containing 1047, 1660 and 2630 ppm TNT for 14 days, for which the calculated average consumed doses were approximately 75, 97 and 163 mg/kg/day, respectively. Upon completion of the feeding trial, liver samples were obtained and analyzed for microsomal cytochrome P450 and b5 content and the cytosolic glutathione antioxidant system. Dietary exposure of TNT in quail resulted in a significant increase in oxidized glutathione content in the 1660 and 2630 ppm diets, and a decrease in glutathione peroxidase activity in the 2630 ppm diet. However, there were no significant differences in reduced glutathione content, glutathione reductase and glutathione transferase activity or in cytochrome P-450 and b5 content. Results from this study show that none of the observed minimal treatment related changes had any toxicity relevance either on the glutathione antioxidant system or mixed-function oxidase components in birds. Therefore, these biochemical parameters may not be sensitive indicators for TNT exposure in birds. (Abstract does not reflect US Army policy).

139 DETERMINING OXIDIZED AND REDUCED GLUTATHIONE USING THE FLUOROMETRIC PROBE O-PHTHALALDEHYDE.

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Due to the importance of GSH and GSSG in cellular signal transduction, gene regulation, redox regulation and biochemical homeostasis, accurate determination of cellular glutathione levels is critical. Several procedures have been developed, but many suffer from overestimating GSSG or from cellular substances interfering or competing with GSH determination. Assays based on HPLC, with enzymatic reduction of GSSG by glutathione reductase and NADPH appears to be valid, but are limited in sample throughput and availability of equipment. The fluorescence probe o-phthalaldehyde (OPA, phthalic dicarboxaldehyde) is semi-specific for GSH and has a high quantum yield, yet its use has been limited due to unidentified interfering and fluorescence quenching substances in liver. Assay conditions have been developed under which these limitations are avoided. By using a phosphate-buffered assay at lower pH, interference with non-specific reactants is minimal. Since enzymatic reduction is not possible due to the reaction of OPA with NAD(P)H, dithionite was used to reduce GSSG. Other stronger reducing agents lead to an overestimation of GSSG levels. High sample throughput combined with sensitive (20 pmol limit of detection) and accurate determination GSH and GSSG using OPA are achievable using any monochromatographic spectrofluorometer. Sample preparation and storage conditions are described that return the same levels of GSH and GSSG for at least 4 weeks. (Supported by NIH RO1-AG09235 and P30-ES06096.)

140 FLUORESCENCE-BASED ASSAY FOR GLUTAMATE-CYSTEINE LIGASE ACTIVITY IN MICROTITER PLATES.

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Glutamate-L-cysteine ligase (GLCL; also known as g-glutamylcysteine synthetase) is the rate limiting enzyme in glutathione (GSH) synthesis. Traditional assays for the activity of this enzyme are based either on coupled reactions with other enzymes, or on high performance liquid chromatography (HPLC) assessment of glutamylcysteine (GC) product formation. We took advantage of the reaction of naphthalene dicarboxaldehyde (NDA) with GSH or GC to form cyclized products that are highly fluorescent. Hepa-1 cells which were designed to overexpress mouse GLCL were used to evaluate and compare the utility of the NDA method with our standard assay based on

monobromobimane derivitization and HPLC analysis with fluorescence detection. Cell lysates from CR17 cells (overexpress both the catalytic and regulatory subunits of GLCL) and Hepa-V cells (contain plasmid vector alone) were incubated in reaction buffer and the resulting products were derivitized with either MBB for HPLC analysis, or with NDA for microplate reader analysis. Microplates were assessed for fluorescence of the NDA-GC + NDA-GSH adducts using a laser scanning cytometer. Good agreement was found between GLCL activities measured by MBB-HPLC and NDA microtiter analyses. However, high background GSH levels can limit the sensitivity of the assay, and in these cases, prior dialysis of cell lysates greatly improved the signal to noise. This assay should be useful for high-throughput GLCL activity analsyses. (Supported by NIH grants ES04696, ES07033, ES07032 and CA74184.)

141 QUANTITATION OF MOUSE GLCL-R AND GLCL-C MRNA AND OTHER GLUTATHIONE-RELATED ENZYMES USING FLUOROGENIC 5' NUCLEASE ASSAYS.

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Fluorogenic quantitation methods are becoming more important for completion of large-scale molecular studies. An ideal mRNA quantitation assay should be efficient, economical, automatable, and consistent with traditional Northern blot data. We have developed real-time fluorogenic 5' nuclease assays to quantitate mRNA expression levels of glutamate-cysteine ligase regulatory (GLCL-R) and catalytic (GLCL-C) subunits in mouse tissues. This fluorogenic assay employs 96-well microtiter plates that references the quantitation to a high expressing reference sample standard curve, and normalizes to GAPDH expression. Specific PCR primers and the fluorogenic probe were designed using PrimerExpressTM. The probe contains a fluorogenic reporter dye (6-FAM) on its 5' end, a quencher dye (TAMRA) on its 3' end. The probe is 100% homologous to the intron/exon boundary of the cDNA which was determined by sequence analysis from a genomic clone of GLCL-R. Our results corresponded to mRNA measurements derived from Northern blot data examining tissues from a case control study of mice exposed to methyl mercury. This procedure allows for precise quantitation of mRNA through real-time analyses of PCR amplification of cDNA. In addition, these types of assays are highly specific, non-radioactive, and cost-effective. Other assays are being developed for the quantitation of mouse glutathione reductase, glutathione peroxidase, glutathione synthetase, gamma-glutamyltransferase, and glutathione S-transferase genes. (Supported by NIEHS Center ES-07033 and ES-04696.)

142 FORMATION AND EXPORT OF THE GLUTATHIONE CONJUGATE OF 4-HYDROXYNONENAL (4-HNE) IN FRESHLY ISOLATED HEPATOCYTES.

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Excessive alcohol consumption, iron overload and chlorinated hydrocarbons are believed to produce chronic hepatic liver injury as a consequence of uncontrolled membrane lipid peroxidation. The peroxidative destruction of polyunsaturated membrane lipids produces α-β unsaturated aldehydes including 4-hydroxynonenal (4-HNE). Aldehydic lipid peroxidation products readily form covalent adducts with cellular nucleophiles and accumulation produces diverse cytotoxic effects. Consequently, a number of enzyme systems have been characterized that detoxify these reactive aldehydes. Previous studies from our laboratory have characterized the hepatocellular capacity to oxidize, reduce and conjugate 4-HNE in suspensions of freshly isolated rat hepatocytes. Further, hepatoma cells have also been shown to rapidly conjugate and export the 4-HNE– glutathione (4-HNE– SG) adduct. In the current studies, suspensions of freshly isolated hepatocytes (10^6 cells/ml) were incubated with either low (25 μM) or high (250 μM) concentrations of 4-HNE and the formation of metabolites, including the 4-HNE– SG conjugate, quantified in the intra-and extracellular fractions by reverse-phase HPLC. Kinetic parameters of 4-HNE– SG formation and export were determined, as was the export of the reduced and oxidized metabolites. These studies demonstrate that suspensions of freshly isolated hepatocytes (10^6 cells/ml) rapidly eliminate 4-HNE. 4-Hydroxynonenoic acid (HNA), the oxidized metabolite of 4-HNE that is produced by aldehyde dehydrogenase (ALDH) appeared rapidly in the intracellular fraction and was rapidly exported. The glutathione S-transferase (GST) mediated conjugate, 4-HNE– SG, rapidly reached maximal intracellular concentrations and was eliminated by export to the extracellular fraction. Taken together these data demonstrate that oxidative and conjugative pathways are primarily responsible for elimination of 4-HNE in freshly isolated hepatocytes and that the metabolites are rapidly exported out of the cell. Further, transport of HNA and 4-HNE– SG appears to be an active process since both metabolites are exported against the concentration gradient. (Supported by NIH/NIAAH-09300.)

143 IMMUNOHISTOCHEMICAL STAINING OF LEAD BINDING PROTEIN IN HUMAN KIDNEY SECTIONS.

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Lead poisoning is a global problem that affects millions of persons. A number of studies have shown that individuals vary greatly in their susceptibility to lead toxicity suggesting intrinsic differences in the *in vivo* handling of this metal. Studies from this laboratory have demonstrated the presence of target tissue-specific lead-binding proteins (PbBP) in humans and other species. These proteins appear to markedly influence the intracellular bioavailability of Pb to sensitive cellular processes such as the heme biosynthetic and the renal gene expression pathways. The present studies were undertaken to examine the cellular distribution of diazepam binding inhibitor, one of the human PbBPs in renal tissue. To achieve this objective, polyclonal antibodies were prepared and purified from the serum of rabbits exposed to human PbBP. Immunohistochemical techniques were utilized to delineate which cell types in human kidney tissue contain the highest PbBP concentration. PbBP was detected in the cytoplasm of most of the proximal tubular cells of human kidney tissues. (Supported in part by USPHS training grant ES07299-05.)

144 CHRONIC POSTNATAL EXPOSURE TO LEAD (Pb) SIGNIFICANTLY ALTERS SERUM LEVELS OF TT4 AND TT3 IN RATS.

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Brain thyroxine is thought to be transported by transthyretin (TTR), a major CSF protein originated from the choroid plexus (CP). We have previously demonstrated that Pb accumulation in rat CP inhibits the synthesis and secretion of TTR. We further hypothesized that the depression of CP TTR production by Pb may impair brain development in young animals by depriving the CNS of thyroid hormones. This study was then designed to investigate thyroid hormone distribution in rat brain following postnatal Pb exposure. Male Sprague-Dawley rats, aged 21 days, were exposed to Pb in drinking water at doses of 0, 50, 100 or 250 μg Pb/ml (as Pb acetate) for 30 days. Total thyroxin (TT4) and total triiodothyronine (TT3) in frontal cortex, hippocampus, and cerebellum were partially purified by resin column and quantitated by radioimmunoassay (RIA). Serum TT4 and TT3 were determined directly by RIA. While the levels of TT4 and TT3 in tested brain regions did not seem to be significantly different in all dose groups, serum TT4 in the high-dose group (5.02±2.76SD, ng/ml) was significantly decreased compared to the controls (10.79±3.46 ng/ml) ($p<0.001$, $n=12$). Serum levels of TT3 in both mid-dose group (1.64±0.62 ng/ml) and high-dose group (1.60±0.42 ng/ml) were significantly lower than those in the control group (2.22±0.42 ng/ml) ($p<0.05$, $n=12$). The results suggest that chronic Pb exposure alters systemic homeostasis of thyroid hormones in postnatal rats. It would be interesting to know how this action may underlie Pb-induced toxicities in clinic. The study on the maternal Pb exposure and brain thyroxine status is currently under investigation. (Supported by NIEHS RO1 ES-08146.)

145 THE EFFECT OF PB^{2+} ON THE STRUCTURE AND FUNCTION OF OSTEOCALCIN.

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Lead toxicity is a major health problem in the U.S. Despite governmental regulations elevated blood Pb^{2+} levels have recently been found in younger children and older adults. Bone is the major reservoir of body Pb^{2+}. Lead has been shown to be detrimental to bone in studies with humans, animals and cells. The effect of Pb^{2+} at the molecular level is unknown. Osteocalcin is a noncollagenous bone protein which may play a role in bone turnover. We have used

Circular Dichroism, Nuclear Magnetic Resonance and a hydroxyapatite (bone mineral) binding assay to investigate the effect of Pb^{2+} on the structural and functional properties of osteocalcin. With circular dichroism (CD), we have shown that Pb^{2+} induces a similar percentage of a-helical structure in osteocalcin as Ca^{2+} but at 2 orders of magnitude lower concentration. To explain these results we have measured Kd's for both Pb^{2+}- and Ca^{2+}-osteocalcin using the CD titration data at physiological ionic strength. It was found that the Kd for Pb^{2+}-osteocalcin is .34uM which indicates more than 3 orders of magnitude tighter binding of Pb^{2+} than Ca^{2+} (Kd=1.25mM) to osteocalcin. The 1D NMR spectra show that much lower Pb^{2+} concentrations perturb the same resonances as Ca^{2+} indicating it binds at the same site and induces a similar conformational change but with some alterations. The hydroxyapatite binding assays show that Pb^{2+} causes an increased adsorption to hydroxyapatite, similar to Ca^{2+}, but also at 2-3 orders of magnitude lower concentration. Affinity constants for the protein-mineral complex as well as the number of sites occupied on the crystal were calculated for both Pb^{2+}- and Ca^{2+}-osteocalcin. The interaction of Pb^{2+} with osteocalcin may play a role in the observed low bone formation rates and decreased bone density observed in Pb^{2+}-intoxicated animals. Furthermore Pb^{2+} intoxication may exacerbate the bone loss observed in osteoporosis.

146 EFFECT OF ORAL SUCCIMER ON GASTROINTESTINAL LEAD ABSORPTION AND RETENTION IN MONKEYS.

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Out-patient succimer treatment of Pb-poisoned children may occur with ongoing Pb exposures, due to the scarcity of Pb-safe housing and uncertainties associated with identifying and remediating environmental exposures. The effects of succimer on the absorption and retention of Pb from exposures suffered concurrently with treatment are not well known. Here we utilized a juvenile non-human primate model of childhood Pb toxicity and a sensitive double stable Pb isotope tracer methodology to investigate the effects of oral succimer on GI Pb absorption and retention. Seventeen rhesus monkeys (*Macaca mulatta)* aged 15 months were fasted for 18h, and then dosed in rapid succession with ^{204}Pb (5 μg i.v.) and ^{206}Pb (72.6 μg orally), followed by treatment with vehicle (n=8) or succimer (n=9, 30mg/kg/day X 5 days). Blood and daily (24h) total fecal and urinary outputs were collected over treatment days -1 to 5, and analyzed for ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb using magnetic - sector ICP-MS. Data were analyzed using an ANOVA ($P<0.05$ significance). Results indicate (i) GI Pb absorption was not measurably altered in the succimer group (51% +/- 5 SE of dose) compared to the vehicle (60% +/- 10 of dose); (ii) Succimer did significantly increase the urinary excretion of i.v. administered ^{204}Pb, but not ^{206}Pb that had been orally administered; (iii) Whole body retention of the orally administered Pb dose was not measurably different between treatments; and (iv) Succimer reduced fecal excretion of i.v. administered Pb, in favor of increased urinary excretion. Notably, only 11 and 16% (mean vehicle and control, respectively) of the i.v. ^{204}Pb dose was recovered in urine and feces over the 5 days of treatment (89 and 84% retained), while only 41and 49% of the orally administered ^{206}Pb dose was recovered (59 and 51% retained). (Supported in part by NIEHS R01 #ES06918 and F32 #ES05870.)

147 SALIVA MONITORING FOR EXPOSURE TO LEAD UTILIZING REAL-TIME MICROFLUIDICS/ ELECTROCHEMICAL ANALYSIS.

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There is a growing need to develop reliable and portable analytical instruments for on-site monitoring of trace metals in individuals. Currently, such assays are carried out in central laboratories using separation techniques such as ion chromatography, atomic absorption spectroscopy, or inductively coupled plasma mass spectroscopy (ICP-MS). A portable microfluidics/electrochemical (MFE) system based on saliva analysis is being developed to more rapidly assess lead (Pb) levels in individuals. The MFE system detects Pb by utilizing Square Wave Anodic Stripping Voltammetry techniques. For Pb, saliva flows over an electrode surface. Pb+2 is reduced and accumulated, and the electric potential of the electrode is scanned. Results indicate a good linear response over a broad concentration range (1-200 ppb). Since the current Pb biological exposure indice (BEI) is based on measurements in blood (500 ng Pb/g blood), saliva Pb concentrations must be correlated with blood/plasma Pb concentrations. To evaluate the relationship between saliva Pb and blood Pb, F-344 rats were treated with a single oral dose of 0, 50, 100, 200, 500 or 1000 mg Pb/kg body weight, and 24 hours later were administered the muscarinic drug pilocarpine to induce salivation. Blood samples and approximately 1-2 ml of saliva were collected. Lead concentrations in saliva, whole blood, red blood cells (RBC) and plasma were quantitated by ICP-MS. A comparison of saliva Pb levels with blood Pb levels revealed a bi-phasic curve wherein the slope increased as RBC binding saturated and more Pb was available to be excreted in the saliva. The interpretation of this data indicates that a Pb level of 1 ng/g saliva corresponds with 500 ng Pb/g blood in the rat. ICP-MS analyses of the Pb samples (saliva and blood) were compared to analysis results using the portable MFE system and found to correlate well ($r2 = 0.87$). Ultimately, blood, saliva, and target tissue dosimetry will be evaluated using a physiologically based pharmacokinetic model for Pb that incorporates the salivary gland. It is anticipated that, once fully developed, this MFE device will be utilized as an important tool for real-time analyses of non-volatile environmental contaminants. (Supported by US DOE under Contract DE-AC06-76RLO 1830.)

148 BONE LEAD DRIVES CHANGE IN SERUM LEAD FROM PRENATAL TO POSTPARTUM PERIOD.

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Maternal blood lead level rises significantly through the third trimester of pregnancy into the post-partum period. Investigators have noted that decreased blood volume, weight, and organ size, as well as removal of a significant sink for maternal lead, the fetus, after delivery might account for much of the increased post-partum blood lead concentration. Most (>99%) blood lead resides in erythrocytes. The remainder, found in serum, contains an unbound fraction that is likely to be the most bio-available. We measured serum lead in third trimester and post-partum by thermal ionization mass spectrometry, and *in vivo* cortical (tibia) and trabecular (calcaneus) bone lead concentration by K shell x-ray fluorescence post-partum. The range of prenatal and postnatal whole blood lead levels were 0.6-14.2 μg/dL and 0.8-25.4 μg/dL, respectively. Prenatal and postnatal serum lead ranged from 12-284 ng/L and 14-215 ng/L, respectively. Serum lead concentration was 0.22% (median) of whole blood lead level, with less than one percent of the sample showing greater than 1.0% concentration, consistent with data from other laboratories studying non-occupationally exposed groups. Tibia bone concentration ranged from -19.2 to 35.5 μg/g. Postnatal serum lead level was significantly higher than prenatal serum lead ($p<0.01$). After accounting for the highly significant correlation between change in whole blood lead pre and post delivery and change in serum lead over the same period, tibia lead concentration independently and significantly predicted increased postnatal serum lead ($p<0.01$), accounting for an additional 6% of variance in change of serum lead in a multiple regression model. There was no significant relationship between calcaneus lead and post-delivery change in serum lead level. As tibia lead has a half-life measured in decades, lead exposure years earlier can affect circulating serum lead in post-partum mothers, and thus play a role in postnatal lead exposure of nursing infants through breast milk.

149 LEAD AFFECTS FIBROBLAST PROLIFERATION AND BIOSYNTHESIS OF ARYLSULFATASE A.

X. G. Chen and R. D. Poretz. *Rutgers University, New Brunswick, NJ.* Sponsor: K. R. Cooper.

Lead exposure causes cognitive and behavioral deficits in some affected children. We had proposed that the combined effects of a single nucleotide polymorphisms (SNP) found in the human pseudodeficient arylsulfatase A (ASA) gene which results in reduced levels of the enzyme, and lead concentrations which decrease ASA activity culminate in cellular enzymic activity that is below a critical threshold required for the maintenance of normal nervous system function. Previously, we demonstrated that human fibroblasts grown in the presence of lead acetate exhibit a more than 60% decrease in steady-state levels of cellular ASA enzyme protein and that treatment of cells from individuals with the SNP(s) of pseudodeficient ASA, but not those from subjects with the normal gene, results in a significant decrease in ability of the cells to catabolize sulfatide, the substrate of ASA. The present study examines the potential of lead to affect the biosynthesis and turnover of ASA in human fibroblasts. Fibroblasts, grown in 20 μM lead, display a 30-40% increase in the rate of proliferation as determined by cell number, cellular protein and total mRNA. Metabolic labeling with 35-S-methionine demonstrates that lead

treatment causes a decrease of approximately 35% in the accumulation of newly synthesized intracellular ASA. This difference, in comparison to control cultures, is not due to differential rates of intracellular degradation of ASA or to levels of ASA specific mRNA. Though lead exposure results in increased cellular proliferation, it appears to cause decreased steady-state levels of ASA by affecting the protein translation or intracellular processing/trafficking pathways of ASA biosynthesis. (Supported in part by grants from NIEHS, R01-ES08373, and the March of Dimes Foundation.)

150 EVALUATION OF LEAD BIOKINETIC MODELS FOR ADULTS.

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Responding to a need for a scientifically defensible approach for assessing human health lead risks at non-residential Superfund sites (where adults rather than children are the primary receptors), the U.S. EPA's Technical Review Workgroup for Lead (TRW) developed the Adult Lead Model (ALM) in 1996. Rather than incorporating a complex compartmental or PBPK type kinetic component, the ALM employs a simplified slope factor approach that relates lead uptake to blood lead. The need for immediate guidance limited the scope of the approach to a narrowly defined receptor population (i.e., adult worker at a commercial/industrial setting) and specific medium (i.e., soil/dust). Subsequently, the TRW committed to a more exhaustive effort to identify the most scientifically defensible methodology currently available to model non-residential lead exposures and risks. The TRW identified seven lead biokinetic models that have been used to assess the relationship between environmental lead exposures and blood lead concentration in adults. The models were evaluated and compared to the ALM based on the following general evaluation criteria: (1) completeness of exposure module, (2) kinetic performance, (3) utility of model output, and (4) ease of use and flexibility. Possible outcomes of the evaluation were: replace ALM with a superior model, modify ALM, or retain the existing ALM. Although no single model was judged to be a significant improvement over the ALM, various components from the different models were determined to be refinements in adult lead modeling. Specific model improvements included multimedia exposure modules, and highly versatile kinetic modules. Rather than invest in integrating beneficial components into a hybrid model, the TRW recommends retaining the ALM - while appreciating that certain site-specific risk assessments (e.g., short-term lead modeling) may benefit from analysis by alternative models - and supporting a research initiative to develop an all-ages biokinetic model that may adopt some of the more attractive features of existing models. It is noteworthy that the kinetic performance of the models evaluated produced similar estimates of quasi-steady-state blood lead when exposure parameters were normalized across models (i.e., all were set to approximate ALM inputs).

151 TOXICOSIS IMPACT OF LEAD ON OCCUPATIONALLY EXPOSED CHILDREN AND ADULTS.

M. M. Hussain. *Osmania University, Hyderabad, India.* Sponsor: S. M. Hussain.

27 Children and 28 Adults working as petrol sniffers, Printing Press workers and petty shop owners whose shops are stationed at the traffic junctions were chosen in this study. The workers are repeatedly exposed to lead emissions and the ambient air lead in their work environment ranged between 170-260 nanogram/cum. Blood samples from the test subjects and controls were assayed for blood lead, rbc and wbc count, haemoglobin, haemotocrit, mchc, delta aminolevulinic acid dehydratase, haemsynthatase, sgot, sgpt and bilirubin. 8 adults and 7 children showed blood lead more than 70 microgram / 100 ml blood and 50 microgram/ 100 ml blood respectively. Blood lead served reliable indicator reflecting adverse health effects. Decreased delta amino levulinic acid dehydratase was significant in all subjects. Subjects showed anemia with microcytic and hypochromic red blood cells. Reticulocytosis and basophilic stipling was also found. Genotoxicity of lead though uncommon was found in 2 adult cases as evident by scg test. 3 children showed blueish teeth, allergic dermatitis and greying of hairs. An attempt will be made to present and discuss certain markers more specific and suitable for assessing lead toxicity in occupationally exposed workers.

152 APPLICATION OF INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY TO ASSESS CELLULAR EXPOSURE TO TOXIC METALS.

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Certain compounds of metals are implicated as environmental or occupational respiratory toxins or carcinogens. We have investigated the effects of soluble sodium chromate and particulate lead chromate in two relevant cultured cell types, normal human lung fibroblasts and normal human lung small airway epithelial cells. We have used inductively coupled plasma mass spectrometry (ICPMS) coupled with a specific method for assessing both the cellular uptake of chromium and observed in cells treated with either soluble sodium chromate or detected a dose-dependent increase in DNA-associated lead. This antioxidants such as melatonin affect the uptake and adduct formation of chromium. Whereas previous methods of assessing metal exposure often required large volumes of body fluids (such as urine or blood) and involved the use of hazardous radioactivity, the ICPMS analysis requires only microgram quantities of DNA and does not involve radioactivity. The ICPMS technique can readily be adapted to determine the exposure of humans and animals to different metals present in occupational and environmental settings. Sponsored by NIEHS grant ES05304 to S.R.P., NIEHS grant ES05844 to J.S., and USDE grant DF-FG02-93ER14320 and NSF grants CHE-9505726 and CHE-951244 to A.M.

153 TERATOGENIC EFFECT OF TRIVALENT AND HEXAVALENT CHROMIUM IN RABBITS.

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Chromium is considered to be an essential nutrient that helps to maintain normal metabolism of glucose, cholesterol, and fat in humans. Studies in the literature indicate that hexavalent chromium (Cr-VI) is more toxic than trivalent chromium (Cr-III) as it is actively transported across the plasma membranes and is reduced via unstable reactive intermediate to Cr-III. Also, The published data indicate that exposure to Cr-VI decrease the number of viable fetuses and increase the number of dead ones. In the current investigation pregnant rabbits were exposed to Cr -III (chromium chloride) or Cr-IV (potassium dichromate) in drinking water at a dose of 500 ppm each during the organogenesis period. Exposure Cr-IV significantly increased the number of resorption sites and decreased the number of viable fetuses compared to Cr-III and control groups. Exposure to either chemicals induced dwarfism, kinky and short tails, lung hypoplasia, heart hypertrophy, intrathoracic hemorrhage and dilated nares and brain lateral ventricles. Furthermore, reduced ossification in parietal and inerparietal bones was significantly increased in the fetuses from the females exposed to Cr-VI. The results of the current investigation may indicate that both Cr-III and Cr-VI are teratogenic for the tested concentration. However, the severity of effects are higher with the exposure to Cr-VI.

154 ALLERGIC CONTACT DERMATITIS-BASED SOIL CRITERIA FOR HEXAVALENT CHROMIUM [CR(VI)].

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Cr(VI)-induced ACD has long been recognized as an adverse health effect in occupationally exposed people. However, such an effect from environmental exposure to chromium has not been systematically investigated nor used as an endpoint for setting soil exposure criteria. There are now data demonstrating the occurrence of ACD in people living in areas contaminated with chromium slag. Cr(VI)-induced ACD is a type IV, delayed, or cell mediated allergic reaction. Once sensitized to chromium, an individual is at risk of developing ACD whenever exposed to low concentrations of this chemical via the dermal, oral, or inhalation routes. Since the underlying mechanism for Cr(VI)-induced ACD involves the delicately balanced immune system, health-based cleanup goals for Cr(VI) in soil can be established using ACD as an endpoint. Cr(VI) has been clinically patch-tested at several varying concentrations by several groups in order to establish dose-response relationships and identify threshold concentrations. However, in all of these studies, the mass of Cr(VI) applied on a given area of skin (mg Cr(VI)/cm^2 skin) was not reported, thus prohibiting the use of these data for quantitative risk assessment purposes. A recent study based on a better dose delivery patch-test method determined the actual mass of Cr(VI) per square centimeter of skin that will illicit ACD in 10 %

of the Cr(VI) sensitized population. This applied dose (0.000089 mg/Cr (VI)/ cm^2 skin) was used with a skin soil adherence factor and a Cr(VI) dermal absorption efficiency to derive a Cr(VI) soil criteria of 175 mg Cr(VI)/kg of soil.

155 EFFECT OF SELENOCYSTINE METABOLITES ON METHIONINE ADENOSYLTRANSFERASE ACTIVITY *IN VITRO*.

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Recent studies on metabolism of selenocystine (CySeSeCy) as selenium-containing amino acid showed that selenocysteine glutathione-selenenyl sulfide (CySeSG) was produced from reaction between CySeSeCy and reduced glutathione (GSH) in small intestine of mice receiving a single oral administration of CySeSeCy. In liver of animals, the CySeSG was reduced by GSH and/or glutathione reductase yielding selenocysteine, which is decomposed enzymatically to hydrogen selenide. The hydrogen selenide is subjected to sequential methylation with S-adenosylmethionine (SAM) as a mtthyl donner, resulting in the formation of mono-, di- and tri-methylated derivatives. The methylation process has been regarded as a detoxification mechanism of selenium compound. Our previous studies demonstrated that selenium methylation ability was depressed by inactivation of methionine adenosyltransferase, SAM synthesizing enzyme, in mice after repeated oral administration of CySeSeCy at a toxic dose. This study was carried out to elucidate a chemical form of selenium-containing metabolite which inhibited the methionine adenosyltransferase activity. Selenium-containing metabolite (CySeSeCy, CySeSG, sodium selenide, monomethylselenol, dimethyl selenide or trimethylselenonium ion) was incubated with liver cytosol supplemented methionine and ATP for 20 min, and SAM content was measured using HPLC method. The methionine adenosyltransferase activity was strongly inhibited by sodium selenide. In addition, inactivation of the enzyme was recovered by addition of a large amount of dithiothreitol. These results suggest that the selenide produced from CySeSeCy at a toxic level inhibits the methionine adenosyltransferase activity.

156 EFFECTS OF INORGANIC AND ORGANIC SELENIUM ON CYTOKINE PRODUCTION AFTER CONTINUOUS SHORT-TERM ORAL EXPOSURE IN MICE.

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Selenium (Se) is an essential as well as a toxic trace element. The immune system is a target of Se intoxication. We compared the effects of inorganic and organic forms of Se on the murine immune system following a short-term oral exposure. Male BALB/c mice, 6-7 weeks of age were continuously exposed to 0, 1, 3 or 9 ppm of Se as sodium selenite or seleno-L-methionine in the drinking water for 14 days. Following the treatment period mice were euthanized, trunk blood collected and spleen, thymus, liver and kidney were aseptically removed and weighed. Exposure to 9 ppm of Se as sodium selenite resulted in a marked decrease in body weight gain and relative organ weights. The thymus was significantly reduced in the 3 ppm of sodium selenite group. Sodium selenite at 9 ppm of Se reduced splenic cellularity and increased erythrocyte counts. The basal rate of splenocyte proliferation was increased in this group. Sodium selenite induced a dose-dependent increase in phytohemagglutinin-P-induced lymphocyte proliferation. Sodium selenite at 9 ppm of Se increased the production of pro-inflammatory cytokines, tumor necrosis factor α and interleukin-1β, in lipopolysaccharide-stimulated splenic macrophages. Seleno-L-methionine in drinking water did not produce any changes in the parameters examined. Results indicated a disparity in the immune system toxicity of inorganic vs. organic forms of Se administered via drinking water, inorganic Se being more toxic. Additionally, this study suggests that splenic macrophages and lymphocytes are targets of Se intoxication.

157 PULMONARY RESPONSES OF RATS IN VIVO AND IN VITRO FOLLOWING EXPOSURE TO VANADIUM PENTOXIDE.

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This study was to characterize the acute pulmonary responses of male F344 rats to vanadium pentoxide (V_2O_5) *in vivo* or *in vitro*. For *in vivo* exposure, rats (bw; 200-250g; n of 6/dose) were intratracheally instilled with V_2O_5 (0, 0.63, or 6.3 mgV/kg; 1mL/kg in saline). On days 1 and 3, the bronchoalveolar lavage (BAL; ~7 ml/wash) was performed. The BAL fluid from the first wash (BALF) was analyzed for LDH, NAG, protein, and cytokines (TNF-α, IL-6, and MIP-2). Cell pellets from all six washes were combined for cytological evaluation. The cells were resuspended and incubated (37°C, 95% RH, 5% CO_2) with endotoxin (LPS) *ex vivo* for 24 hrs. For *in vitro* exposure, BAL were performed on naïve rats (bw; 200-250g; n of 3/dose), the cell pellets were incubated with V_2O_5 (0, 1, 5, 25, and 125 µgV/ml; in RPMI 1640). At 2 and 24 hrs, culture medium was recovered and analyzed for LDH and cytokines. At 2 hrs, the cells were incubated with LPS for additional 24 hrs. Following instillation, increased lung weights and acute pulmonary inflammation were observed in a dose- and time-dependent manner. Concentrations of LDH, NAG, and protein increased in BALF from the high dose group up to day 3. The MIP-2 in BALF was highly responsive to vanadium exposure, whereas TNF-α was not. An increase in IL-6 was noted in BALF from the high dose on day 1. Following *in vitro* exposure, all three cytokines in the culture medium decreased in a dose-dependent manner at 2 hrs with minimal LDH leakage. When stimulated with LPS, the overall levels were increased but the relative reduction in cytokines persisted. Therefore, while vanadium exposure *in vivo* caused a clear acute inflammatory responses in the lungs accompanied by increases in enzymes and cytokines, vanadium *in vitro* appeared to suppress cytokine releases. The discrepancy between *in vivo* and *in vitro* cytokine responses was speculated to be related to differences in the microenvironments where vanadium particles interact with various cells involved.

158 THE ASSOCIATION BETWEEN GENETIC POLYMORPHISMS OF COPROPORPHYRINOGEN OXIDASE (CPOX) AND AN ATYPICAL PORPHYRINOGENIC RESPONSE TO MERCURY.

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Mercury (Hg) exposure poses a high risk of toxicity to humans. Previous studies have documented the efficacy of urinary porphyrin profile changes as a biomarker of low-level Hg exposure in human subjects and have defined the etiology of this effect as Hg-inhibition of the heme enzymes uroporphyrinogen decarboxylase and CPOX in the kidney. The objective of this study was to define the potential association between polymorphisms in the CPOX gene and an atypical porphyrinogenic response (APR) to Hg, characterized by excessively high urinary porphyrin excretion irrespective of level of Hg exposure, that is seen in 12-16% of Hg-exposed subjects. Automated DNA sequencing-based assays were developed to examine the 7 exons and flanking intron-exon boundries of the human CPOX gene in subjects with low-level occupational exposure to Hg as Hgo (mean urinary Hg=3.4 + 2.2 µg/l). Genetic analysis of exon 1 revealed a possible C39A encoding a cysteine to a termination codon change in 3 of 17 subjects. This change was not associated with the APR. In contrast, a polymorphism in exon 4 encoding a N272H substitution was found in 4 of 6 APR subjects in this group (mean urinary porphyins=129.6. + 38.2 µg/l vs. non-APR porphyrins=32.4 + 18 µg/l). Analysis of exon 5 revealed possible genetic polymorphisms that were also potentially associated with the APR to Hg. These findings indicate a possible genetic predisposition to an altered biological response to Hg that could affect Hg disposition and health risks. The APR might therefore serve as a biomarker of susceptibility to Hg toxicity. (Supported by ES04696 and ES07033.)

159 SPERMATOTOXICITY OF MERCURIC CHLORIDE IN RATS.

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Sponsor: R. R. Dalvi.

Recently, we demonstrated in a reproductive and fertility study that mercuric chloride causes a dose dependent decrease in fertility, viability, pups per litter, and weaning indices in F_0 generation rats. The present study was undertaken to investigate the dose response and time course of alterations in semen quality. Samples were evaluated with a Hamilton-Thorne Toxicology Semen Analyzer (H-TTSA). Male rats were gavaged daily with 0.0, 0.5,1.0, and 1.5

mg $HgCl_2$/kg/day for up to 84 days. Interim measurements of semen quality were made at 14, 28, 56, and 84 days. Motile sperm percentages (MSP) were decreased in a dose dependent manner on Day 56. Also, MSP in the high dose group was significantly decreased when compared with the control on Day 56, and Day 84. Progressive motility was significantly decreased in the highest dose on Day 84. The parameters that described vigor of swimming sperm such as average path velocity (VAP), curvilinear velocity (VCL), and straight linear velocity (VSL) were significantly decreased in the mid and high treatment groups on Day 28. However, the parameters that described the sperm pattern such as lateral head displacement (ALH), beat cross frequency (BCF), and linearity (LIN) showed no dose response. The experimental results presented in this study suggest that mercuric chloride exposure in rats leads to significant reduction in motility and some velocity parameters. Therefore, reduction in reproductive and fertility indices in rats exposed to mercuric chloride may be related to alterations in semen quality.

160 DISTRIBUTION AND RETENTION OF MERCURY IN METALLOTHIONEIN-NULL MICE AFTER EXPOSURE TO MERCURY VAPOR.

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We studied the role of metallothionein (MT) in the distribution and retention of mercury in the brain, lung, liver and kidney of MT-null and wild-type mice after exposure to mercury (Hg)vapor. Mice were exposed to Hg vapor at 5.5 to 6.7 mg/cu m for 3 h and killed at 1, 24, 72, or 168 h after exposure. One hour after exposure to Hg vapor, there were no differences in mercury concentrations in these organs from MT-null and wild-type mice. However, the elimination rate of mercury from the organs, except the brain, were remarkably faster in MT-null mice than in wild-type mice. MT-I and -II levels in the lung and kidney were significantly increased in wild-type mice but not in MT-null mice at 24 h after exposure to Hg vapor. At this time point, over 65% of the mercury was retained in the MT fraction of the cytosol of organs from wild-type mice. In contrast, mercury appeared mainly in the high-molecular-weight protein fractions in the cytosol of organs from MT-null mice. In the brain, a large amount of mercury was bound to MT in both strains of mice immediately after exposure. No difference was observed in the elimination rate of mercury from brain between both strains of mice. Brain MT levels were elevated slightly in wild-type mice at 168 h after exposure but could not be detected in MT-null mice. These data suggest that no detectable MT-I and -II levels were found in the brains of MT-null mice and that mercury was apparently bound to MT-III. Using MT-null mice, we also showed that MT-III may play an important role in the retention of mercury in the brain.

161 ROLE OF GLUTATHIONE AND METALLOTHIONEIN IN RENAL TOXICITY OF INORGANIC MERCURY.

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We examined the sensitivity of GSH-depleted MT-null mice to the renal toxicity of inorganic mercury. Eight-week-old male MT-null mice and wild-type mice were given s.c. injection of mercuric chloride (1.0 or 30 umol/kg). L-Buthionine-SR-sulfoximine (BSO, 2.5 mmol/kg), an inhibitor of GSH synthesis, was s.c. administered to these mice at 4 h prior to the mercury injection. Blood and kidney were removed from each mouse under ether anesthesia, at 24 h after the mercury injection. Blood urea nitrogen and creatinine values in the serum, and histopathological change in the kidney were utilized as indicators of renal toxicity. Injection of mercuric chloride at dose of 30 umol/kg did not induce the kidney damage in the wild-type mice. In the MT-null mice, the renal toxicity was induced by injection of a mercury dose of 30 umol/kg but not at 1.0 umol/kg. When renal GSH level was reduced by BSO pretreatment in the both strains of mice, injection of mercuric chloride at a dose of 1.0 umol/kg caused the kidney damage in the both strains of mice, and the nephrotoxicity was much severer in the MT-null mice than in the wild-type mice. In the present study, we found that GSH and MT play cooperatively an important role in detoxification of severe kidney damage caused by inorganic mercury. In addition, GSH may act as a primary protective factor against inorganic mercury-caused nephrotoxicity, because GSH-depleted mice were more sensitive to inorganic mercury than MT-null mice.

162 METHYLMERCURY'S EFFECTS ON MITOCHONDRIAL DNA IN DEVELOPING RODENT MIDBRAIN.

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Mitochondrial DNA damage is a significant target of oxidative stressors and has been shown to be more extensive and persists longer than nuclear DNA damage. Mitochondrial DNA damage leads to impaired electron transport chain function and results in decreased ATP production and formation of oxidative oxygen species (ROS). This can cause further damage to mitochondrial DNA, proteins and lipids and lead to opening of mitochondrial permeability transition pore and apoptosis. Research has shown that MeHg can induce formation of ROS by disrupting electron transport chain. In our present study, Extra-long PCR (XL-PCR) methods were conducted using two different DNA polymerases and in combination with automatic hot start techniques to detect mitochondrial DNA damage. Our results show that the amplification of a 5.7kb mtDNA fragment was decreased in MeHg-exposed primary rat embryonic CNS cells with a dose-response relationship over 0, 1, and 2 μM MeHg. This result suggests that MeHg induced mtDNA damage in rat embryonic CNS cells. However, in the rat kidney, exposure to 10 mM MeHg *in vivo*, the amplification of the 5.7kb mtDNA fragment was increased. Such an increase in mtDNA following *in vivo* MeHg exposure may result from changed mitochondrial replication as has been shown that MeHg induced a dose-response related increase in mitochondrial numbers in our preliminary studies. Further studies will detect the specific oxidative mtDNA damage (mutations, deletions) following MeHg exposure in primary rat embryonic CNS cells and relate these to MeHg's effects on cell kinetics and viability. (This research was supported by US EPA grant R825358.)

163 METHYLMERCURY-INDUCED APOPTOSIS IN NEURONAL AND NONNEURONAL CELL LINES THROUGH MICROTUBULAR DISRUPTION.

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Methylmercury (MeHg) is known to interfere with cell cycle progression by disruption of microtubules. Depression of cell growth by MeHg eventually leads to cell death. Several studies have demonstrated MeHg-induced apoptosis, but the underlying mechanism has not yet been fully understood. In the present study, the relationship between the changes in cell cycle and the induction of apoptosis caused by MeHg was investigated in cultured mammalian cells. MeHg at growth inhibiting concentraions (3 to 5 microM) caused nuclear fragmentation and DNA ladder formation in rat pheochromocytoma (PC12) and mouse neuroblastoma cells exposed to MeHg for 48 h. Flow cytometric analysis revealed that the occurrence of apoptosis was preceded by the accumulation of cells in G2/M-phase after MeHg treatment. Exposure to colchicine, a well-characterized microtubule disruptor, also caused G2/M-phase arrest followed by the appearance of apoptotic cells. These results suggest that G2/M-phase arrest through the disruption of microtubules is an important event in the development of apoptosis by MeHg. Similarly, MeHg treatment led to G2/M-phase arrest followed by apoptosis in nonneuronal HeLa cells. p53 expression was not changed in either cell line. However, bcl-2 was phosphorylated by MeHg treatment in HeLa cells but not in PC12 cells. Thus, MeHg induces apoptosis via a p53-independent pathway in both cell lines, but different pathways may be activated after the disruption of microtubules in PC12 and HeLa cells.

164 ESTIMATION OF ALUMINUM BIOAVAILABILITY FROM DEGRADABLE CHAFF COUNTERMEASURES USING A PHYSIOLOGICALLY-BASED EXTRACTION TEST.

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Aluminum is the third most abundant element on earth and comprises roughly 8% of earth's crust. Few adverse human health effects resulting from aluminum exposure have been reported largely because this element is poorly absorbed from the human gastrointestinal tract. Aluminum has been implicated in the etiology of at least one neurodegenerative disease, although data supporting this hypothesis are inconclusive. United States military aircraft use aluminized glass chaff as a passive countermeasure to radar-guided threats. In an effort to assist in human health and ecological risk assessment of the geo-

graphical areas over which chaff is deployed, current studies are evaluating the effects of exposure to aluminized chaff. Results from an *in vitro* physiologically-based extraction test predict that intestinal absorption of aluminum derived from ingested chaff is minimal (5-10% bioaccessible). Validation of this experimental approach *in vivo* has been described elsewhere for both lead and arsenic and current efforts focus on validation of the technique for ingested aluminum. Preliminary results in male Fischer-344 rats dosed with aluminum lactate, aluminum hydroxide, or one of five different forms of chaff for five consecutive days suggest that aluminum from chaff is minimally absorbed via the gastrointestinal tract, as determined by blood and urine total aluminum concentration. No significant differences in body or organ weights were observed between control animals and animals dosed with any form of chaff. Results will be discussed within the context of application of the physiologically-based extraction test in human exposure assessment. Funding sponsor: Naval Air Warfare Center, Aircraft Division, Patuxent River, MD.

165 BRAIN ALUMINUM CLEARANCE IS SLOW.

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Objectives: To estimate the percentage of Al that enters the brain from the primary Al species in plasma (Al•transferrin, Al•Tf) and the duration of Al residence in the brain. **Experimental procedures:** Fischer 344 rats were given 1 ml of a ^{26}Al•Tf complex containing 14.9 ng (0.57 nmol) ^{26}Al bound to 37 nmol Tf. This was given i.v. over 1 h. Controls received identical treatment without ^{26}Al. Subjects received either saline or 0.15 mmol/kg desferrioxamine (DFO) thrice weekly i.p. beginning 1 day after the Al•Tf. Saline-injected subjects were terminated 1, 4, 16 and 32 days, whereas DFO-injected subjects were terminated 4, 16 and 32 days after ^{26}Al•Tf. Brain ^{26}Al was determined by accelerator mass spectrometry. **Results:** Approximately 0.007% of the ^{26}Al dose was in the brain 1 day after ^{26}Al•Tf. In the absence of DFO treatments, brain ^{26}Al concentration decreased by 30% from day 16 to 32. DFO treatments decreased brain ^{26}Al concentration 18 and 12% on days 16 and 32 when compared to that seen following saline treatments. **Discussion:** These results are consistent with previous studies suggesting that a small fraction of Al in blood is able to enter and be retained by the brain. Calculations suggest this Al could have entered the brain by transferrin receptor-mediated endocytosis. Al appears to be stored in a relatively non-mobile compartment in the brain within 1 day of brain entry, from which Al is slowly cleared. The small effect of DFO is consistent with the necessity of prolonged DFO therapy to significantly reduce Al-induced encephalopathy. These studies are being continued to better estimate the half-life of Al in the brain in the absence and presence of DFO treatment. (Supported by US-EPA R 825357.)

166 ALUMINUM BIOAVAILABILITY FROM DRINKING WATER IS NOT INFLUENCED BY STOMACH CONTENTS OR WATER HARDNESS.

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Objectives: To estimate the bioavailability of Al from drinking water and the effects of water hardness and food in the stomach on this measure. **Experimental procedures:** Fischer 344 rats were acclimated to consumption 10 h/day of a 10% protein diet. This resulted in no appreciable stomach contents 14 h after diet and water removal when coprophagy was prevented by a fecal collection cup. All subjects had access to 2 ml of drinking water for 2 h. In a two × two design with n=5/group, subjects then had 2 h access to 0 or 1 g food prior to gastric (i.g.) administration of 50 ng ^{26}Al in soft water ($CaCO_3$ equivalent < 10 mg/l) or hard water ($CaCO_3$ equivalent 300 mg/l). All rats received a continuous i.v. infusion of 447 µg ^{27}Al/kg/hr, as $AlK(SO_4)_2$, from 2 h prior to 120 h after i.g. dosing. Blood was repeatedly obtained up to 120 h after i.g. dosing and analyzed to determine plasma ^{26}Al by accelerator mass spectrometry and ^{27}Al by electrothermal atomic absorption spectroscopy. Oral Al bioavailability was determined from the $AUC_{i.g.\ Al}/AUC_{i.v.\ Al} \times dose_{i.v.\ Al}/dose_{i.g.\ Al}$. The term $dose_{i.v.\ Al}/AUC_{i.v.\ Al}$ was replaced by the equivalent term clearance (Cl_s) which was determined from the ^{27}Al infusion rate/plasma ^{27}Al concentration. Therefore, oral Al bioavailability was determined in a model of drinking water consumption utilizing a physiologically-relevant oral Al dose and concurrent administration of two Al isotopes. **Results:** Overall bioavailability of Al averaged 1.5%. This was not significantly influenced by the presence of food in the stomach or high $CaCO_3$ concentration in the water. **Discussion:** The estimated oral bioavailability of Al from drinking water is higher than most reported estimates. Al bioavailability from drinking water appears to be independent of stomach contents or water hardness. (Supported by US-EPA R 825357.)

167 IRON-INDUCED HEPATOTOXICITY: EFFECTS ON NUCLEAR CALCIUM CONCENTRATION AND DNA FRAGMENTATION.

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Although it is well known that iron overdose can produce hepatotoxicity, the exact mechanism of cell death has yet to be determined. Previous research has suggested that the mechanism of iron toxicity involves an increase in nuclear calcium and an increase in DNA fragmentation. This study was performed to examine the time course of iron-induced DNA fragmentation and nuclear calcium disruption and also to examine methods of protecting against cytotoxicity. The study was performed using hepatocytes isolated from male Sprague-Dawley rats. Incubation of hepatocyte cultures with ferrous sulfate (3 µM or 5 µM) over the course of 20 hours produced concentration-dependent decreases in cell viability, as measured by Lactate Dehydrogenase (LDH) release and concentration-dependent increases in nuclear calcium concentrations, as measured by o-cresolphthalein complexation. DNA fragmentation was also shown to be increased in the hepatocytes as measured by agarose gel electrophoresis and by using an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of 5-Bromo-2-Deoxy-Uridine (Brd-U) labeled end fragments. Nuclear calcium levels and DNA fragmentation became elevated at approximately four hours into the incubation and cell viability became significantly lower at eight hours. Verapamil (25 µM), a calcium channel blocker, and 80 µM Aurinetricarboxylic acid (ATA), an endonuclease inhibitor, offered significant protection against iron-induced loss of viability and lessened DNA fragmentation. Verapamil decreased nuclear calcium accumulation while ATA did not. These results suggest that iron-induced hepatotoxicity may be mediated by increases in nuclear calcium, perhaps through the activation of calcium-dependent endonucleases resulting in DNA fragmentation and ultimately cell death.

168 PCR CLONING AND FUNCTIONAL CHARACTERIZATION OF ZEBRAFISH METALLOTHIONEIN GENE PROMOTER.

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Metallothioneins (MT) are metal binding proteins that are implicated in heavy metal ion detoxification processes and their quantitations are commonly used as biomarkers for heavy metal exposure. Zebrafish is a common tropical fish model for toxicity testings and developmental biology research. We are interested in using zebrafish as a model for our MT gene expression studies. To obtain MT gene promoter from zebrafish, two gene specific reverse primers designed from a published cDNA sequence (accession X97278) were applied for promoter finding using PCR, and a 859bp zebrafish MT promoter with 4 putative metal responsive elements, 3 AP1 sites and 1 Sp1 site was amplified and cloned for nucleotide sequence determination. A zebrafish MT gene with its promoter and coding region in a total of 1773kbp was further amplified by PCR. Deletion mutants of the promoter region lacking MRE sites were linked to luciferase gene and will be subjected to functional characterization in PLHC-1 cell line. For application as biomarker model fish, zebrafish MT promoter constructs will be transfected into various cell lineages and zebrafish embryos. The recombinant cell lines and transgenic zebrafish will thus be used in metal toxicity testings.

169 INHIBITION OF RECOMBINANT HUMAN SQUALENE MONOOXYGENASE (SQUALENE EPOXIDASE) BY TELLURIUM COMPOUNDS.

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Tellurium is an element with a number of industrial applications, including the manufacture of semiconductors, doping of metal alloys, and vulcanization of rubber; however, little is known about its toxicology. Weanling rats fed 1% elemental tellurium develop a transient peripheral demyelination due to the inhibition of squalene monooxygenase, a microsomal enzyme in the pathway

for cholesterol biosynthesis. The active metabolite responsible for this inhibition is not known. To study the potential mechanisms of tellurium toxicity in humans, a truncated human squalene monooxygenase (HSM) was cloned, expressed and purified. We previously reported that tellurite inhibits HSM with an IC50 of 17 μM. Kinetic analysis of the tellurite inhibition shows slope-parabolic and intercept-parabolic inhibition with respect to squalene, implicating more than one binding site for tellurite. To date, a wide variety of related compounds have been tested as inhibitors: dimethyltellurium dichloride is the most potent compound tested, having an IC50 of 1.5 μM. Because tellurite has been shown to bind sulfhydryl groups we hypothesized that the compounds bind to cysteine residues in HSM. Preliminary support for this hypothesis is provided by the observation that the sulfhydryl reagents glutathione, dithiothreitol, and beta-mercaptoethanol are able to protect against as well as reverse the inhibition. The presence of seven cysteine residues in HSM is consistent with the kinetic evidence for multiple tellurite binding sites on HSM. (Supported by a grant from the Ohio Valley Affiliate of the American Heart Association, NIEHS Training Grant ES-07266, and the UK Medical Center Research Fund.)

170 METAL INTERACTION WITH PHYSICAL AND CHEMICAL AGENTS IN THE INDUCTION OF CYTOGENETIC EFFECTS IN HUMAN LYMPHOCYTES.

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Chromium and Nickel are widely spread environmental contaminants and known human carcinogens. Among the most frequently used cytogenetic endpoints to identify exposure to genotoxic agents are the induction of micronuclei (MN) and the induction of sister chromatid exchanges (SCE) in human lymphocytes.Treatment of human lymphocytes with NiSO4 (1-100 mM) or UV-light (200, 1000 ergs/mm2) induced micronuclei (MN) in a dose-dependent fashion. Statistical analysis of the interaction factor (IF), showed that combined treatments of Ni(II) (1-100 mM) with UV-light (200, or 1000 ergs/mm2) interacted antagonistically for the induction of MN. Furthermore Ni(II) [0.5-10 mM] with UV-light (200 or 1000ergs/mm2) or Cr(VI) interacted antagonistically for the induction of sister chromatid exchanges (SCE), in peripheral human lymphocytes. Based on the fact that Ni(II) alters DNA replication, we studied the toxicity of nickel in relation to the cell cycle of human lymphocytes. We also devised an index of cell cycle progression (ICCP) to quantitate the effect of Ni(II) treatments on the cell cycle. There was a delay of 2.1 h for the 5 and 10 mM Ni(II) treatments and a delay of 5.3 h for the 25 mM Ni(II) treatment. These observations has raised a concern that nickel present in complex mixtures may reduce the response, even in the presence of strong MN or SCE inducers, and may lead, therefore, to an underestimate of chemical exposure as assessed by these assays. Since metals affecting certain microsteps in the process of DNA replication or repair (eg. histones, polymerases, ligases) may have similar antagonistic effects, further studies, are recommended.

171 DIFFERENTIAL ABILITY OF TRANSITIONAL METALS TO INDUCE PULMONARY INFLAMMATION.

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Transition metals are components of airborne particles and have been implicated in adverse health effects. Relative toxicity and inflammatory potential of these metals are usually inferred from separate studies since little directly comparable data are available. The objective of this study was to compare the pulmonary effects of intratracheally-instilled, equimolar, soluble forms of six metal sulfates. Rats received either phosphate-buffered saline, 0.1 umol/kg, or 1.0 umol/kg of vanadium, nickel, iron (II), copper, manganese, or zinc. Bronchoalveolar lavage was performed at 0, 4, 16, or 48 hrs post-installation. At the 0.1 umol/kg dose, only Cu induced significant neutrophil influx at 16 and 48 hrs ($p<0.05$). For the 1.0 umol/kg dose at 4 hrs, Cu and Fe(II)-exposed animals had a significant increase in percent neutrophils compared to saline controls, and Cu had a significantly higher percentage than all other metals. After 16 hrs, each metal tested induced significant neutrophilia compared to controls, and Cu and Mn induced significantly higher neutrophilia than the other metals. At 48 hrs, neutrophilia was still increased in all metal exposures except Fe(II). Interestingly, Mn was the only metal to induce a significant increase in eosinophils (16 hr post-instillation, $p<0.05$). Additionally, Cu and Ni-exposed rats had significantly higher levels of lactate dehydrogenase in lavage supernatant compared to the other metals and controls. These results indicate that transition metals differ in their ability to induce pulmonary inflammation and toxicity. Cu appears to be the most pro-inflammatory metal, followed by Mn and Ni, while V, Fe(II), and Zn induced similar levels of neutrophila. We conclude that the extent and cellular nature of metal-induced pulmonary inflammation depends on the individual metal. (Supported by: ES00002, HL54958, HL07118, and HL05947.)

172 CARCINOGENIC NICKEL INDUCES GENES INVOLVED WITH HYPOXIC STRESS.

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Carcinogenic nickel compounds altered the program of gene expression in normal cells and induced a pattern of gene expression similar to that found in nickel-induced cancers. Here we have demonstrated that exposure of mouse normal fibroblasts, or human A549 cells to 1 mM nickel induced hypoxic signaling pathways by inducing hypoxia-inducible transcription factor (HIF-1) which mediated the induction of genes required by cells to survive hypoxia. We also showed that a new gene Cap43 was dependent upon HIF-1 since only HIF-1 proficient mouse cells induced Cap43 gene expression when exposed to either hypoxia (1%O2) or 1 mM nickel. We also showed that GAPDH, a gene induced by hypoxia through HIF-1, was similar to Cap43 in that it required HIF-1 mouse proficient cells to be induced by either 1 mM nickel or hypoxia (1%O2). These data demonstrated that nickel exposure turned on signaling for a hypoxic cascade that may be important in its carcinogenesis.

173 INHIBITION OF HISTONE ACETYLATION BY NICKEL COMPOUNDS IN *S. CEREVISIAE* AND MAMMALIAN CELLS.

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Environmental factors influence carcinogenesis by interfering with a variety of cellular targets. Carcinogenic nickel compounds are inactive in most gene mutation assays, but they induce chromosomal damage in heterochromatic regions and cause gene silencing on reporter genes located near heterochromatin in both yeast and mammalian cells. Here we studied nickel effects on the acetylation in the N-terminal region of histone H4. We demonstrate that *in vivo* nickel compounds decrease histone H4 acetylation levels in both yeast and mammalian cells. The effects of nickel on the lysine residue targets in the N-terminal region of histone H4 are distinct. In both yeast and A549 cells, lysine 12 is the most sensitive residue. While in yeast the acetylation levels of lysine residues at sites 16, 8 and 5 are also affected by nickel, in A549 cells the effect is limited mostly to lysine 12. Unlike in yeast cells, where acetylation levels are reduced by soluble nickel, the acetylation in A549 cells is inhibited only by non-soluble nickel particles (nickel subsulfide). Comparison among the yeast cells treated with a variety of metals shows that CuSO4 causes similar effect on histone acetylation as nickel, while CdCl2 or CoCl2 only slightly inhibits acetylation. In vitro inhibition of H4 acetylation by nickel is also observed using a purified acetyltransferase (GCN5).

174 NICKEL COMPOUND-INDUCED TOXICITY AND MORPHOLOGICAL TRANSFORMATION IN 10T1/2 CELLS.

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Certain insoluble nickel compounds are carcinogenic. We wanted to determine whether short-term *in vitro* assays could predict relative carcinogenic potentials of nickel compounds. We therefore examined abilities of these compounds to be phagocytosed by and induce cytotoxicity, micronuclei, chromosomal aberrations, and morphological transformation in 10T1/2 mouse embryo fibroblasts. The overall ranking for genotoxicity showed that Ni subsulfide and Ni oxides were most genotoxic; elemental nickel particles of <1 μM reported size had intermediate genotoxicity; and elemental nickel particles of 3-7 μM reported size together with soluble nickel sulfate had the lowest overall genotoxicity. Phagocytosis of insoluble nickel compounds and cell transformation endpoints correlated best with existing animal and human carcinogenicity data. Ni subsulfide and green Ni oxide, but not soluble nickel sulfate, were carcinogenic when inhaled by rats. Interestingly, both elemental nickel particles ranked the lowest in cell transformation, together with soluble nickel sulfate. Overall results indicate these *in vitro* assays can be used to prioritize nickel compounds for carcinogenicity in animals. We next induced ten foci in 10T1/2 with black nickel oxide and ten with green nickel oxide, derived transformed cell lines from the foci, and characterized these trans-

formed cell lines. These transformed cell lines all formed type II foci and had a stable focus-forming phenotype.

175 EFFECT OF THE HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A ON REACTIVATION OF A NICKEL-SILENCED GENE.

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We have previously reported that nickel (Ni) silenced the expression of the gpt transgene in the G12 hamster cell line. Silencing of the gpt gene involved increased chromatin condensation and cytosine methylation in the gpt promoter region and was assayed by assessing the cells' ability to grow in selective media containing 10 μg/ml 6-thio-guanine. Recently, our laboratory reported that nickel enhanced telomeric silencing and decreased histone acetylation in yeast cells. To determine if histone deacetylation participates in nickel-induced gpt gene silencing, we treated a Ni-induced 6-thio-guanine resistant (i.e. gpt -) G12 variant with 100 ng/ml trichostatin A (TSA, a histone deacetylase inhibitor) for 48 hr. We then assessed the reversion frequency back to gpt+ by selecting for growth in media containing 100 μM hypoxanthine, 1 μM aminopterin, and 100 μM thymidine (HAT). This treatment increased the gpt reversion frequency only slightly (1.5-3 times higher than the spontaneous reversion frequency) suggesting that by itself, histone acetylation was not sufficient to reactivate the gpt gene. We had previously found that treatment with 5 μM 5-azacytidine (5-azaC), an inhibitor of DNA methylation, resulted in gpt reversion. This effect was abolished when 100 ng/ml TSA was added concurrently with 5 μM 5-aza C probably because TSA slowed the cell cycle resulting in less 5-aza C incorporation into the DNA and hence less inhibition of DNA methylation. Interestingly, treatment of a gpt - clone that was non-responsive to 5 μM 5-aza C by itself with a combination of 5-aza C for 48 hr, followed by recovery for 48 hr, and then 100 ng/ml TSA for 48 hr resulted in a gpt reversion frequency that was 270 times higher than the spontaneous reversion rate. This result suggests that in some instances, perhaps depending upon the degree of chromatin condensation, DNA demethylation and histone acetylation were both involved in reactivation of the Ni-silenced gpt transgene.

176 EFFECT OF ORALLY-DOSED MANGANESE ON THE REPRODUCTIVE EFFICIENCY OF SD-RATS.

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The present study investigated the potential adverse effects of manganese on the reproductive systems in Sprauge-Dawley rats following oral exposure. Moreover, the purpose of this study was concerned with the dose-response of manganese and its toxicologic evaluation. Male and female SD rats (153-175g) were administered manganese acetate for 63 days. Studies were conducted in two phases. Phase I, used a single dose of 306 mgMn/kg. Significant decreases in ovary/uterus (55%) weight and insignificant increases in testicular (2.5%) weights were observed prior to mating. Following a mating period where Mn was discontinued, ovarian/uterus and testis weight decreased 22 and 9%, respectively. No significant changes in treated above control groups with respect to ovarian and uterine changes. Histopathology revealed focal mild prostatitis in treated males while controls remained unchanged. In phase II rats were dosed with 306, 612, 1225 and 1838 mgMn/kg. Prior to mating, decreases of 25, 29, 45, and 14%, respectively, of ovarian/uterine tissue weights were observed. Testicular weights for all treatment groups showed consistent decreases while histologically confirmed testicular degeneration was demonstrated in doses of 612 (33%), 1225 (50%) and 1838 (33%) mgMn/kg. Following mating when Mn administration was discontinued, decreases were observed in ovarian/uterine weights. Testicular weight were decreased in all groups. Affected animals had segmented degeneration of germinal epithelium within seminiferous tubules. This suggests that the male reproductive system is more sensitive than females to the effects of orally administered manganese. (Supported by ATSDR #U50/ATU398948-08.)

177 ACCUMULATION OF ZINC IN F_1 OFFSPRING OF ZINC CHLORIDE TREATED RATS.

M. Brownlow, A. T. Khan, A. Atkinson, T. C. Graham, M. Green, S. Ali and S. Thompson. *Tuskegee University, College of Veterinary Medicine, Nursing and Allied Health, Tuskegee, AL.* Sponsor: R. R. Dalvi.

The accumulation of zinc in the F_1 offspring of Zinc chloride (Zn) treated rats were analyzed to assist in determining the potential for Zn accumulation to influence reproductive performance and other systems in the second generation. Both male and female Fo rats were administered 0.0, 7.50, 15.0 and 30.0 mg/kg/day of Zn (oral gavage) during premating, mating, gestation and lactation periods. After weaning, one male and one female F_1 offspring were selected for Zn analysis The brain, thymus, testis, ovary, and stomach content were analyzed for Zn accumulation. In most instances, Zn accumulation in a dose dependent manner in the tissues that were analyzed. Zn accumulated in the ovary at the mid and high dose was significantly increase and almost twice the level noted their control. Also, Zn accumulation in the thymus of the mid and high dose rats of both sexes were significantly increased when compared to their controls. The pattern of Zn accumulation in the F_1 generation suggests the potential for effects upon immune system function in both sexes and upon reproduction in females. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU398948-07.)

178 EFFECT OF SUBCHRONIC EXPOSURE OF RATS TO ZINC CHLORIDE DURING A BREEDING TRIAL ON HEMATOLOGY, BLOOD CHEMISTRY, ORGAN WEIGHTS, AND HISTOPATHOLOGY.

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The effects of oral Zinc chloride (Zn) on reproductive performance are unknown. Therefore, the effects of 13 weeks of oral administration of Zn on hematology, blood chemistry, organ weights, and histopathology of rats were studied to determine their impact on breeding. Both male and female rats (45-50 days old at study initiation) were administered 0.0, 7.5, 15.0 and 30.0 mg/kg/day of Zn via oral gavage during premating, mating, gestation and lactation periods. Males were sacrificed after mating (13 weeks) and females were sacrificed after weaning (19 weeks). Clinical pathology assessment consisted of evaluations of the hemogram, leukogram, and selected chemistries. Organs from the nervous, urinary, endocrine, digestive, hematopoietic/lymphoreticular, and reproductive systems of both sexes were weighed and subsequently evaluated histopathologically. There were no significant Zn related changes observed in the clinical pathology parameters. Analysis of organ weight data showed Zn treatment related decreases in testicular and ovarian weights and an increase in the brain weights of males. Evaluation of histopathology revealed Zn treatment associated thymic atrophy, depletion of lymphoid tissue in lymph nodes, splenic hemosiderosis, and prostatic acinar atrophy. All effects related to treatment with the test compound were mild. However, those that were noted in reproductive organs could have had adverse effects upon reproduction. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU398948-0.)

179 NITRIC OXIDE INDUCES METALLOTHIONEIN GENE EXPRESSION APPARENTLY BY DISPLACING ZINC BOUND TO MT.

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The metal binding protein, metallothionein (MT), is probably involved in zinc homeostasis since it typically binds large amounts of zinc coordinated to its numerous cysteine groups. Zinc can control MT gene expression by interacting with metal-sensitive transcription factors and free zinc induces the accumulation of large amounts of zinc-containing MT protein. However, the precise factors governing intracellular release of metal ions from MT remain unknown. Aerobic nitric oxide (NO) can nitrosate thiol groups in proteins, and MT overexpression induces resistance to NO toxicity in certain cell lines, while MT-bound Cd is released by NO exposure. Based on these observations, we hypothesized that NO may also be effective at displacing zinc from MT, and that this could be an important physiological control mechanism in zinc homeostasis and utilization. Thus we studied the release of zinc from MT after NO exposure (using the diethylenetriamine nitric oxide adduct, DETA/NO, as the NO source) to help define this as a possible mechanism of

controlled zinc release from MT. In this study, we used TRL1215 cells (which are derived from fetal rat liver) and HUVECs (human umblical vein endothelial cells) as *in vitro* model systems. In these cell lines, MT protein levels and MT mRNA were induced by NO exposure following zinc treatment. Additionally, NO displaced MT-bound zinc, as assessed by gel-filtration chromatography. The released zinc probably then stimulated MT gene expression as assessed by MT mRNA and MT protein. These results suggest that NO may play an important role in regulation of zinc homeostasis in the cells by providing for a controlled release mechanism from metal stored in association with MT. The strong possibility exists that other genes could be activated by NO-induced release of MT-bound zinc.

180 DISTRIBUTION OF ZINC IN TARGET ORGANS OF ZINC CHLORIDE TREATED MICE.

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Male and female (CD) mice were administered either 0.0, 12.5, 25.0, 50.0, 100.0 or 200.0 mg/kg/day of zinc chloride via oral gavage to determine levels of zinc accumulation in various organs. Zinc accumulation in the brain, liver, kidney, pancreas, spleen, thymus, ovary, uterus, epididymis, seminal vesicle and testis were analyzed. Males showed 15.0, 13.0, and 16.0% reductions in body weight while females had 7.0, 9.0 and 8.0 % reductions in body weight gain at the 12.5, 25.0 and 50.0 mg/kg/day dose groups, respectively when compared to controls. The body weights of males in the 12.5, 25.0 and 50.0 mg/kg/day dose groups were significantly lower than their control group, but females had no significant differences in final body weight gain when compared to their control group. In males, Zinc accumulations in the kidney, pancreas, spleen, thymus, prostate and seminal vesicle, and in females, the pancreas, thymus, uterus, and ovary were significantly higher at 50.0 mg/kg/day when compared to control groups. Additionally, Zinc accumulation data showed a dose-dependent increase in all organs of both sexes. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU398948-07.)

181 A PRELIMINARY STUDY ON THE TOXICITY OF ZINC ON RAT SPERMATOGENESIS AS DETERMINED BY FLOW CYTOMETRY.

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The effects of zinc chloride (Zn) on the maturation of rat testicular germ cells are unknown. Therefore, Cell Flow Cytometry (CFC) was used to investigate the effects of Zn on rat testicular cell development. Male rats were administered either 0.0, 7.5, 15.0 or 30.0 mg/kg/day of Zn for 98 days. After the final exposure, rats were scarified and their testes were removed for CFC evaluation. The percentage of diploid, tetraploid and haploid cells were evaluated. All Zn treated groups appear to have a small dose dependent decrease in the percentage of haploid cells and consequently a small dose dependent increase in the percentage of diploid cells. Also, the mean percentage of round, elongating and elongated spermatids were evaluated. Exposure of rats to Zn resulted in a decrease in the percentage of elongated spermatids and an increase in round spermatids In summary, the pattern of all the changes noted may have been due to an arrest of maturation imposed upon an earlier cell stage that prevented the appearance of the next cell stage. (Supported by MHPF/ATSDR Cooperative Agreement #U50/ATU398948-07.)

182 NUCLEAR MAGNETIC RESONANCE (NMR) ANALYSIS OF THE INTERACTION OF EXOGENOUS DIVALENT METALS WITH ZINC FINGER MOTIFS IN THE SYNTHETIC ZINC FINGER MODEL.

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The Cys_2-His_2 family zinc finger motif is regarded as one of the major structural motifs in cellular proteins and is involved in eukaryotic protein-nucleic acid interactions. Zinc ions coordinate this finger-like structure through bonds created with cysteine and histidine amino acids. To determine whether these domains are potential targets for xenobiotic (Cd, Hg, and Pb) metals, we examined the interactions between several metals and a synthetic Cys_2-His_2 finger peptide (26-mer) corresponding to a single domain of the transcription factor Sp1. Recent studies conducted in our laboratory, have supported the ability of Pb(II), Hg(II), and Cd(II) to bind to the zinc finger region of this motif, but did not provide unequivocal interpretations in terms of the sites (residue) involved. Therefore, the metal-binding and structural properties of the synthetic peptide, in the presence or absence of zinc and a variety of other metals (Pb, Cd, Ca, and Hg) was investigated by one and two-Dimensional Nuclear Magnetic Resonance. Transition metals Zn(II), Cd(II) and Hg(II) and the non-transition metal Pb(II) demonstrated distinct signal changes in the aliphatic region, which could allow some structural inferences such as metal-cysteine binding to be drawn. Ca(II), on the other hand, did not exhibit any significant chemical shift of proton resonances in this region. All of the above metals, however, indicated major chemical shifts in the aromatic region of the spectra, which was indicative of metal-histidine binding. In addition, chemical shift assignment and sequential connectivity of the peptide were determined by 2D TOCSY and NOESY experiments. The interaction of the peptide with divalent metals, Zn(II), Pb(II) and Ca(II) were monitored by the cross peak in TOCSY and NOESY spectra. Cysteine and histidine residues show a distinct change in the amide and beta resonances region in the presence of Zn(II) and Pb(II) indicating the metal-ligand binding site are near these residues. However, Ca(II) was not actively involved in the binding site.

183 A ROLE FOR ZINC IN ANTI-APOPTOTIC ACTION.

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Cadmium, an environmental pollutant caused nephroptosis which was inhibitable by zinc. The mechanism of anti-apoptotic action of zinc is poorly understood. In this study, we found the stimulation of DNA synthesis, as assessed by BrdU incorporation during prevention by zinc of apoptosis, suggesting the proliferactive nature of zinc contributes to its inhibition of apoptosis. This finding was consistent with the result that the cells driven by dialyzed fetal bovine serum were resistant to apoptotic stimuli of cadmium. Furthermore, zinc activated the expression of endogenous Bcl-2 proteins. Overexpression of Bcl-2 proteins by transfection, however, did not facilitate zinc-mediated DNA synthesis. Thus, one possible role of zinc in prevention of apoptosis is to promote DNA synthesis , independently of activation of anti-apoptotic proteins Bcl-2.

184 MODULATION OF METALLOTHIONEIN MRNA BY ESTRADIOL IN PERIPHERAL BLOOD LEUKOCYTES FROM GREY SEALS.

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Metallothioneins (MTs) are metal-binding proteins whose synthesis can be induced by heavy metals. MTs are synthesized in immune cells and can modulate several immune functions. Previous studies have reported gender differences in the sensitivity of seal macrophages to metals. The objective of this study was to determine which MT transcripts are present in peripheral blood leukocytes of grey seals and whether or not estradiol can modulate these. Using Northern blot analyses MTs (I, II, III) were undetectable in seal blood mononuclear cells. However, exposure of cells to $ZnCl_2$ (10^{-4} M, 10^{-5} M) increased MTI and II mRNA levels. MTIII mRNA was not detectable even in Zn-stimulated cells. To assess the time response of MT induction, cells were exposed to $ZnCl_2$ (10^{-4} M and 10^{-5} M) from 3 to 24 hours. MTI and MTII mRNA levels increased rapidly reaching maximum levels after 3 hrs of exposure. A dose-response relationship between MT mRNA levels and $ZnCl_2$ concentrations were observed. Exposure of cells to 10^{-4} M $ZnCl_2$ caused a 10-fold higher induction of both MT mRNA levels than 10^{-5} M $ZnCl_2$. To determine if estradiol could alter the response of the mononuclear cells to $ZnCl_2$, cells were exposed to physiological concentrations of $ZnCl_2$ and estradiol (0.05, 0.1, 0.5 nM). Our results show that estradiol can significantly increase MT I and II mRNA levels in the presence of $ZnCl_2$. Estradiol by itself had no effect. Together these results indicate that PBMNs can be stimulated by heavy metals to synthesize transcripts for both MT I and II and that estradiol can increase the sensitivity of these cells to heavy metals. These observations may have important implications in immunotoxicological risk assessment of heavy metal exposures due to male-female differences.

185 ROLE OF METALLOTHIONEIN AND GLUTATHIONE IN AGE-RELATED CHANGE IN CADMIUM-INDUCED LIVER INJURY IN MALE FISCHER 344 RATS.

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The influence of aging on the sensitivity of the liver to the acute toxicity of cadmium (Cd) has not been studied previously in adult rats. In this study hepatotoxicity caused by a single sc injection of CdCl2 was compared in 5-, 18-, and 28-month-old male Fisher 344 rats. Doses of Cd were adjusted on the basis of the mean lean body mass for each age group of rats, and liver injury was evaluated 24 hr after treatment. Cd treatment produced substantial increases in serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities in 5- and 18-month-old rats, whereas no significant increases were observed in 28-month-old rats. Histological examination of r3qepresentative livers from each age group confirmed the findings for serum enzyme activity; hepatocellular necrosis was observed only in livers from 5- and 18-month-old rats. The attenuation of Cd hepatotoxicity in senescent rats did not appear to be related to pre-treatment levels of metallothionein or glutathione. Likewise, resistance to Cd could not be explained on the basis of metallothionein induction, which decreased as a function of aging. Thus, the mechanisms that account for the post-maturational decline in sensitivity to Cd do not appear to be associated with alterations in levels of the major factors that protect against Cd-induced hepatotoxicity.

186 ROLE OF KUPFFER CELLS AND INFLAMMATORY CYTOKINES IN THE ATTENUATION OF CADMIUM-INDUCED LIVER INJURY IN SENESCENT MALE FISCHER 344 RATS.

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Age of rats is one of the critical determinants of the degree of chemical-induced acute hepatotoxicity. In male Fischer 344 rats, subcutaneous cadmium (Cd) treatment (3 mg/kg) produced severe hepatotoxicity in young-adult rats (5 months old) but not in old rats (25 to 28 months old) 24 hr after treatment. We investigated this effect of aging from the aspect of the role of Kupffer cells and inflammatory cytokines. The phagocytic activity of Kupffer cells, determined as the rate of carbon clearance from blood, was stimulated by the administration of a hepatotoxic dose of Cd (3 mg/kg, sc) only in young-adult rats. Increases in hepatic concentrations of interleukin (IL)-1beta and cytokine-induced neutrophil chemoattractant (CINC) produced by Cd treatment were greater in young-adult rats compared to old rats. Pretreatment of young-adult rats with gadolinium chloride or cyclosporin A inhibited the elevations in hepatic cytokines and prevented Cd-induced liver damage. The results suggest that: (1) Kupffer cell activation is essential for inflammatory liver damage from Cd, (2) IL-1beta and CINC are important mediators of the inflammatory response induced by Cd, and (3) the attenuation of Cd-induced liver injury in senescent rats is caused by an impairment in Kupffer cell activation, leading to a lower production of CINC and less inflammatory liver injury.

187 THE INFLUENCE OF ADVANCED AGE ON THE HEPATOTOXICITY OF CHLOROFORM (CHCl3) AND BROMODICHLOROMETHANE (BDCM) IN MALE F-344 RATS.

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Disinfection of water containing organic matter by chlorination or by ozonation followed by either chlorine or chloramine results in the production of a variety of halogenated byproducts, including trihalomethanes (THMs). CHC13 and BDCM are the two most prevalent THMs. Exposure to CHC13 and BDCM is virtually ubiquitous across all segments of the U.S. population, including the aged. Aged (22-23 month old) male F-344 rats are markedly more susceptible to carbon tetrachloride-induced hepatotoxicity than their younger counterparts (either 3- or 5- months old) (Schoeffner et al., 1999). Thus, the present objective was to investigate the influence of advanced age on susceptibility to the hepatotoxic effects of BDCM and CHC13. Each THM was evaluated in a separate experiment in which young (~90 day old) and aged male F-344 rats (22-24 months old) were administered 0, 0.5, 1.0 or 2.0 mmol THM/kg by oral gavage in an aqueous vehicle (10% Alkamuls) at a gavage volume of 5ml/kg at ~3 p. m (n=4/group). Hepatotoxicity was assessed 24 hr later by serum activities of sorbitol dehydrogenase (SDH) and alanine (ALT) and aspartate (AST) aminotransferases. In both young and old rats, the hepatotoxicity of CHC13 and BDCM was dose-dependent. The influence of age on THM hepatotoxicity was evaluated, in separate analyses for each THM, by two-way analysis of variance that included a THM*age interaction term as well as the main factors of THM and age. The THM*age interaction terms were significant for SDH, ALT and AST for both CHC13 and BDCM, indicating that the hepatotoxic effects of CHC13 and BDCM were dependent on rat age. For both CHC13 and BDCM, the differences between young and old rats were most apparent at the highest dosage. At 2.0 mmol CHC13/kg, relative to the young rats, mean ALT, AST and SDH values were between 4-and 7-fold greater in the old rats. At 2.0 mmol BDCM/kg, mean Alt, AST and SDH values were ~10-fold greater in old rats relative to young rats. In conclusion, advanced age increased the hepatotoxic response to both CHC13 and BDCM, indicating that the aged may be a susceptible subpopulation for THMs. (Abstract may not reflect EPA policy.)

188 DOSE RELATED INCREASE IN LIVER INJURY OF CHLOROFORM IS TEMPERED BY TISSUE REPAIR.

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Dose response assessment is a fundamental basis of quantitative relationships between exposure to an agent and the incidence of an adverse outcome versus the opposing compensatory response. Present studies were designed to investigate the dynamics of injury and tissue repair during chloroform toxicity. Male S-D rats were injected with ten-fold dose range of chloroform (0.05, 0.125, 0.25 and 0.5 ml/kg or 74, 184, 368 and 736 mg/kg ip in corn oil) and hepatotoxicity and tissue repair were assessed during a time course of 0-96 h. Blood levels of chloroform were estimated by gas chromatography after chloroform administration. Liver injury, as assessed by plasma alanine aminotransferase (ALT), peaked at 24 h and declined by 96 h for the lower three doses (74,184 and 368 mg/kg). A significant and progressive increase in plasma ALT was observed in the highest dose (736 mg/kg) treated group after 24 h. Tissue regeneration response as measured by 3H-thymidine incorporation into hepatonuclear DNA peaked by 36 h in rats treated with lower three doses of chloroform (74,184 and 368 mg/kg). This correlated with prompt recovery from liver injury without any mortality. However, with the highest dose of chloroform (736 mg/kg), tissue repair was delayed and attenuated allowing unrestrained progression of liver injury and 90% animal death. Histopathology of the liver samples correlated well with the biochemical parameters of liver injury. Chloroform concentrations in blood increased with dose reaching maximum at 1 h before declining to undetectable levels by 6 h after chloroform administration regardless of the dose. These results support the concept that quantifying tissue repair response along with injury inflicted by chloroform should yield a greater insight into the mechanism of toxicity on one hand and increase the usefulness of dose-response relationships on the other. (Supported by ATSDR # U61/ATD 681482.)

189 GAVAGE VEHICLE AND VOLUME MODULATION OF CHLOROFORM-INDUCED HEPATOTOXICITY IN MICE.

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Digestible oils are frequently used as vehicles for oral administration of volatile lipophilic compounds. Previous research from our laboratory in the male F-344 rat has shown that chloroform (CHCl3) toxicity is modulated by both gavage vehicle and gavage volume. CHCl3 is a disinfection byproduct ubiquitously present in drinking water disinfected either by chlorination or by ozonation processes that involve post-treatment with chlorine or chloramine. In the rat, greater hepatic and renal toxicity is seen with oil administration, with increased toxicity at higher oil volumes. As less attention has been focused on the effects of gavage vehicle and volume in the mouse, the objective of the present study was to examine the influence of these factors on CHCl3-induced hepatotoxicity in this species. Female B6C3F1 mice (~70 days old) received a single oral gavage of 0, 238 or 477 mg CHCl3/kg (the NCI bioassay dosages). CHCl3 was administered in either 2.5, 5.0 or 10 ml corn oil/kg or 10 ml/kg of an aqueous-based vehicle (10% Alkamuls®). Toxicity was assessed at 24, 48 and 72 hr by serum activities of sorbitol dehydrogenase (SDH) and alanine (ALT) and aspartate (AST) aminotransferases. CHCl3 toxicity was dose- and time-dependent following delivery in either

vehicle. Under all conditions, toxicity was most severe at 24 hr. Vehicle differences were most apparent at 477 mg CHCl3/kg, with oil gavage resulting in greater toxicity than aqueous delivery. Enhancement of liver injury by oil delivery is consistent with previous findings in the male F-344 rat. At the high dose (477 mg/kg), the volume of oil had little or no apparent effect on CHCl3 hepatotoxicity. In contrast, at the low dose (238 mg/kg), CHCl3 toxicity was dependent on oil volume. Relative to the 10 ml/kg gavage volume, greater toxicity was observed at the 2.5 ml/kg gavage volume. This is in contrast to our findings in the rat, where compared to an oil gavage volume of 2.5 ml/kg, much greater toxicity was seen at 10 ml/kg. These data indicate that gavage vehicle and volume are significant modulators of CHCl3 hepatotoxicity in the mouse. In summary, while the effects of gavage vehicle appear to be consistent across species, the effects of gavage volume do not. Therefore, careful attention must be given to the experimental parameters of gavage vehicle and gavage volume when making toxic potency comparisons among chemicals and across species. (This abstract does not reflect EPA policy.)

190 DIFFERENCE IN RESPONSE OF LIVER AND KIDNEY TO HEPATIC DAMAGE.

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We have examined the effects of preceding hepatic damage on response of liver and extrahepatic sites to a subsequent dose of xenobiotics. Adult male ICR mice were pretreated with a hepatotoxic dose of carbon tetrachloride (CCl_4; 0.05 ml/kg, ip) 24 hr prior to a challenging dose of either CCl_4 (0.02 ml/kg, ip), acetaminophen (APAP; 350 mg/kg, ip) or chloroform ($CHCl_3$; 0.025 or 1.0 ml/kg, ip). The CCl_4 pretreatment reduced the hepatotoxicity of challenging toxicants, but elevated the renal toxicity significantly as determined by changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), and blood urea nitrogen (BUN) and creatinine levels in serum. Glutathione (GSH) level in the liver or in the renal cortex was not altered by the pretreatment. In mice pretreated with a 24 hr prior dose of CCl_4 the hepatic microsomal *p*-nitrophenol hydroxylase, *p*-nitroanisole demethylase, and aminopyrine demethylase activities were decreased, however, the renal microsomal drug metabolizing activities were all induced. In partially hepatectomized male mice there was a significant increase in the renal microsomal metabolizing activities, and the renal toxicity resulting from administration of CCl_4 or $CHCl_3$ was potentiated correspondingly. In female or castrated male mice, however, induction of the renal microsomal metabolizing activities by CCl_4 was diminished significantly. Also partial hepatectomy did not produce an increase in the renal metabolizing activities in female mice. But in female mice conditioned with testosterone a dose of CCl_4 induced the renal metabolizing activities to a level comparable to those of male mice. It is strongly suggested that testosterone play a critical role in the compensatory induction of the renal drug metabolizing enzyme activities in the male mice with preceding liver injury. The results obtained from this study emphasize the complexity of *in vivo* response problematical to predict in a limited test system.

191 EARLY ALCOHOL-INDUCED LIVER INJURY: SEX DIFFERENCES AND PYRUVATE INTERVENTION.

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While chronic alcohol abusers of both sexes frequently experience disruption of sex hormone homeostasis, men and women differ in susceptibility to alcohol-induced liver injury, suggesting influence by sex hormones. This study determined if the sexes differ in early alcoholic liver injury and hepatic estrogen (E) metabolism, and if intervention with pyruvate, reputed to be hepatoprotective, might attenuate injury. Male and cycling female rats were fed standard alcohol-containing (36% alcohol, AF) or isocaloric (0% alcohol, IC) diets for 6 weeks. One-half of each group received pyruvate supplement equivalent to 10% of diet carbohydrate (AFP and ICP), the other half no addition. Liver injury was scored blindly using a 0 (none) to 4 (significant) scale in categories of fat drops, inflammation, and fibrosis. Compared to females, male rats had greater overall alcohol-induced injury ($p=.04$) regardless of intervention. In males, pyruvate treatment reduced inflammation ($p=.01$) and fibrosis ($p=.02$), and reduced overall injury ($p=.002$). In females, pyruvate reduced fat drops, inflammation and fibrosis slightly but not significantly, and reduced overall injury ($p=.01$). Alcohol-induced effects on E metabolism differed with sex. In males, E 2-hydroxylase (E2-OHase) activity was reduced 37% in AF ($p=.04$) with a 2.1-fold increased serum E ($p<.05$). Pyruvate normalized E2-OHase activity and serum E. In contrast, in females, E2-OHase activity was increased 14% in AF with 30% reduction in serum E; pyruvate treatment further enhanced E metabolism and reduced serum E levels, with E2-OHase activity increased 67% and serum E level reduced 33% in AFP. These data suggest that AF males sustain more early alcohol-induced injury than AF females; pyruvate intervention attenuates liver injury; and alcohol-induced effects of E metabolism differs between the sexes.

192 TNF-α AND TGF-α STIMULATE THE ENHANCED COMPENSATORY TISSUE REPAIR RESPONSE IN DIET RESTRICTED RATS AFTER THIOACETAMIDE-INDUCED LIVER INJURY.

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Studies have shown that 35% diet restriction (DR) protects rats from a lethal dose of thioacetamide (TA) due to enhanced compensatory tissue repair in DR rats. The objective was to investigate the role of TNF-α and TGF-α in hepatocyte proliferation after low (50 mg/kg) and a high (600 mg/kg) doses of TA in DR and *ad libitum* (AL) fed rats. TNF-α was estimated using rat-specific ELISA. DR itself did not change TNF-α expression. After low dose, TNF-α increased within 12 h in both AL and DR rats. Expression of TNF-α remained elevated until 96 h in DR rats while in AL rats it declined to control by 24 h. Similar results were obtained after high dose of TA. Regiospecific TGF-α expression was evaluated immunohistochemically in paraffin embedded rat liver sections during a time course after TA administrations. After high dose TGF-α expression in DR rats was sustained and 2-fold higher. The intensity of staining was much greater after high dose than low dose in DR rats. After low dose DR rat livers had higher TGF-α than AL rats. Staining pattern after low dose in DR rats paralleled the pattern in AL rats during 24-96 h. In both low dose and high dose studies, expression of TNF-α and TGF-α coincided with cell proliferation measured by ^{3}H-thymidine incorporation. TNF-α elevated during early time points while TGF-α increased during later time points indicating that TNF-α expression stimulates production of TGF-α, which in turn stimulates cell division. These results indicate that higher expression of TNF-α and TGF-α are responsible for the higher tissue repair in DR rats after TA-induced liver injury.

193 REGULATION OF INFLAMMATORY MEDIATOR PRODUCTION IN THE LIVER BY TUMOR NECROSIS FACTOR-α (TNF-α) IN CARBON TETRACHLORIDE (CCL_4)-INDUCED HEPATOTOXICITY.

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Macrophages (MP) are known to release a number of cytotoxic mediators which have been implicated in the pathogenesis of liver injury. In the present studies we analyzed the role of MP-derived TNF-α in carbon tetrachloride (CCl_4)-induced hepatotoxicity. Treatment of mice with CCl_4 (0.3 ml/kg, i.p.) resulted in a time-dependent induction of centrilobular hepatic necrosis. This was associated with increases in hepatic lipid peroxidation and serum transaminase levels. CCl_4-induced toxicity was also correlated with increases in serum and hepatic TNF-α, as well as expression of several gene products thought to mediate liver inflammation, including inducible nitric oxide synthase (NOS II), cyclo-oxygenase-2 (COX-2), and heme oxygenase-1 (HO-1). To study the role of TNF-α in hepatotoxicity, we used TNF-α knockout mice. CCl_4 was less effective in inducing hepatotoxicity in these mice, suggesting that TNF-α contributes to hepatotoxicity in this model. Western blot analysis revealed that TNF-α knockouts treated with CCl_4 expressed greater quantities of hepatic COX-2 and HO-1 than wild-type mice, but less NOS II, demonstrating that TNF-α regulates production of these mediators. Many genes regulating production of inflammatory mediators contain binding sites for the transcription factor nuclear factor-kappa B (NF-κB). CCl_4 treatment of wild-type mice resulted in a time-dependent increase in NF-κB binding activity. Using knockout mice lacking the p50 subunit of NF-κB we analyzed the role of NF-κB in CCl_4-induced liver injury. CCl_4 was less effective in inducing injury in these mice. This was correlated with decreased TNF-α but increased hepatic COX-2 and HO-1. These data suggest that the hepatotoxic effects of TNF-α in CCl_4-induced liver injury may be mediated by altering expression of pro- and anti-inflammatory mediators and antioxidants. (NIH GM34310, ES06897; Burroughs Wellcome Fund).

194 INFLAMMATION AND APOPTOSIS IN RAT LIVER INDUCED BY MONOCROTALINE.

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Monocrotaline (MCT) is a pyrrolizidine alkaloid that is hepatoxic, mutagenic and carcinogenic. Human exposure to MCT occurs from consumption of contaminated grains and herbal teas and medicines. MCT given intraperitoneally (i.p.) produced dose-dependent hepatocellular injury in rats that was significant at 200 mg/kg. Injection of 300 mg/kg MCT produced time-dependent hepatotoxicity beginning at 12 hours. Histologic examination of liver sections revealed widespread, panlobular hepatocellular necrosis and congestion, with injury being most pronounced in centrilobular regions. There was also an inflammatory infiltrate consisting of neutrophils (PMNs) and morphological evidence of apoptosis. This suggested that there might be both inflammatory and apoptotic components to MCT-induced liver injury. To quantify these, rats were treated with 300 mg/kg MCT (i.p.) for 4, 8, 12 and 18 hours, and liver sections were analyzed for PMN accumulation using immunohistochemistry. A time-dependent increase in the number of PMNs in areas of hepatocellular injury appeared as a trend by 4 hours and reached statistical significance by 18 hours. These same liver sections were analyzed using TUNEL staining (which identifies apoptotic cells by labeling DNA strand breaks, a hallmark of apoptosis). Only TUNEL-stained cells with apoptotic morphology were enumerated. MCT caused a statistically significant increase in TUNEL staining in the liver beginning at 8 hours. TUNEL staining was panlobular, with more extensive staining occurring in areas of hepatocellular necrosis. These studies revealed that MCT induces both inflammation and apoptosis in the rat liver. (Supported by NIH grant ES 04139.)

195 IMPORTANCE OF THE CXC CHEMOKINES MIP-2 AND KC FOR NEUTROPHIL-INDUCED LIVER INJURY DURING ENDOTOXEMIA.

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Neutrophils (PMNs) can cause parenchymal cell injury in the liver. Prerequisite for the attack on hepatocytes is the migration out of sinusoids, induced by a chemotactic signal. Thus, our objective was to evaluate the two murine chemokines MIP-2 and KC in a murine model of PMN-induced liver injury [700mg/kg galactosamine, 100μg/kg endotoxin (Gal/ET)]. Serum levels of MIP-2 and KC increased 950-fold and 380-fold, resp., within 90 min. Although the conc. declined 3 h after Gal/ET treatment, levels were significantly elevated during the entire observation period. RNase protection assays showed transcriptional activation of both chemokines in the liver. Gal/ET injection into ET-resistant strain C3H/HeJ did not result in MIP-2 or KC formation. However, i.v. injection of murine TNF-α, IL-1α, or IL-1β (2μg/kg) induced MIP-2 and KC formation. At 7 h after Gal/ET, large numbers of PMNs (262±9 PMN/50 high power fields) accumulated in sinusoids and partially extravasated into the parenchyma. High plasma ALT activities (2300±830 U/L) and histological evidence of necrosis (54±4% of hepatocytes) indicated severe liver injury. Administration of an antiserum against MIP-2 2 h before Gal/ET significantly reduced ALT activities and necrosis (20±4%). The number of hepatic PMNs was not affected, but less cells appeared to be in the parenchyma. Control serum or anti-KC antiserum were ineffective. **Conclusion:** Our data showed a cytokine-dependent transcriptional activation of CXC chemokine genes in the liver *in vivo*. MIP-2 appears to be the critical chemotactic signal for neutrophil-induced liver injury during endotoxemia. (Supported in part by 2 R01 NIEHS 06091.)

196 ROLE OF PEROXYNITRITE (PN) IN ACETAMINOPHEN (AA)-INDUCED HEPATOTOXICITY.

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Recent studies have shown that the toxicity of AA is mediated, in part, by inflammatory mediators produced by liver cells. In the present studies, we analyzed the role of reactive nitrogen intermediates in the pathogenic process. Treatment of mice with AA (100-300 mg/kg, ip) results in a dose and time dependent induction of centrilobular hepatic necrosis and increases in serum ALT which are evident within 6 hr. This was correlated with the appearance of inducible nitric oxide synthase (NOS II) in the liver. Immunohistochemistry also revealed PN-mediated injury which was identified by nitrotyrosine staining. This was observed in sinusoidal cells within 1 hr and was followed by hepatocyte staining by 6 hr. To study the role of nitric oxide and PN in AA-induced hepatotoxicity, we used aminoguanidine (AG, 100 mg/kg, 2x/day, ip), a selective NOS II inhibitor, and aminoethylselenourea (AEseU, 25 mg/kg, 2x/day, ip), a NOS II inhibitor and a PN scavenger. Both compounds were found to block AA-induced hepatotoxicity. AEseU was a more effective inhibitor of toxicity than AG. These findings suggest that reactive nitrogen intermediates, in particular PN, play a role in AA-induced liver injury. These data are supported by our observations that NOS II knockout mice are significantly less sensitive to AA-induced hepatotoxicity than control mice. These mice also do not exhibit evidence of PN-mediated tissue injury. To exclude the possibility that the reduced toxicity in NOS II knockouts was due to alterations in AA metabolism, we examined AA covalent binding to liver microsomal proteins by Western blotting. We found that the extent of covalent binding in microsomal proteins from wild type and NOS II knockout mice was similar. Moreover, although baseline hepatic glutathione (GSH) levels were 30% greater in NOS II knockouts, AA administration resulted in a 90% reduction in GSH in both genotypes. These data demonstrate that AA-induced toxicity involves both direct and indirect mechanisms. (Supported by NIH grants GM34310, ES06897and ES07163 and the Burroughs Wellcome Fund.)

197 RETINOL'S POTENTIATION OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY DOES NOT INVOLVE THE DIRECT FORMATION OR CONJUGATION OF NAPQI.

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We have previously shown that retinol potentiates acetaminophen-induced hepatotoxicity in the BALB/C mouse. 4 days of retinol (75 mg/kg/d) was required to potentiate acetaminophen (400 mg/kg) hepatotoxicity (ALT activity; 3260±583 IU/L retinol + acetaminophen vs.1056±225 IU/L vehicle + acetaminophen, $p<0.05$). Retinol, alone and in combination with acetaminophen, decreased the catalytic activity and polypeptide levels of CYP3A as compared to the vehicle and untreated groups (catalytic activity; 0.72±0.08 nmol/mg/min retinol + acetaminophen vs. 1.21±0.07 nmol/mg/min untreated, $p<0.05$). However, no statistical difference has been demonstrated between any groups for the catalytic activity or polypeptide levels of CYP1A2 or CYP2E1. Furthermore, GSH concentrations in the liver were unaltered by the administration of retinol alone. However, our results demonstrate a significant decrease in the GSH content of retinol + acetaminophen treated animals, when compared to acetaminophen treated alone (0.36±0.11 vs. 0.87±0.19 respectively, $p<0.05$), which is consistent with an increase in the formation of the toxic metabolite, NAPQI. We can conclude that retinol's potentiation of acetaminophen-induced hepatotoxicity is not due to an induction of CYP450 isoforms or a reduction in available glutathione at the time of NAPQI formation. (Supported by a Laurenson Award from the Otago Medical Research Foundation and by a University of Otago Research Grant.)

198 MULTIDRUG RESISTANCE PROTEIN 2 (MRP2) MESSAGE RNA EXPRESSION IN MOUSE LIVER FOLLOWING CHEMICAL INDUCTION OF COMPENSATORY HEPATOCELLULAR PROLIFERATION *IN VIVO*.

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Chemically induced compensatory hepatocellular proliferation can result in increased protection against hepatotoxicants. Enhanced expression of liver plasma membrane ATP-dependent transport proteins may contribute to this protection and, indeed, Mrp2 protein expression is elevated following acetaminophen treatment. Thus, the present studies were designed to investigate the relative abundance of Mrp2 mRNA. **Methods:** Male CD-1 mice received 350 mg acetaminophen/kg i.p. or 15 ul carbon tetrachloride/kg i.p. Control mice received vehicle only. Liver poly A+RNA was isolated at 12 and 24 hr after treatment, followed by reverse transcription-polymerase chain reaction (RT-PCR) using mouse Mrp2 specific primers, and agarose gel electrophoresis. Mrp2 cDNA bands were normalized to the housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (HPRT), cDNA bands. **Results:** No change in Mrp2 mRNA abundance was detected by image analysis at either

time point examined. **Conclusions:** The increase in Mrp2 protein induced by acetaminophen treatment cannot be accounted for by a concurrent change in mRNA levels. (Supported by grants from AstraZeneca and PhRMA Foundation.)

199 HIGH SENSITIVITY OF NRF2 KNOCKOUT MICE TO ACETAMINOPHEN HEPATOTOXICITY.

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Nrf2 is a transcription factor that activates Antioxidant Response Element (ARE)-regulated genes including some members of Phase II drug metabolizing enzymes and those involved in oxidative stress defense mechanisms. In order to examine the effect of Nrf2 on xenobiotic metabolism *in vivo*, acetaminophen (APAP) hepatotoxicity was evaluated in Nrf2 knockout mice. When APAP was administered to 2 to 4-month-old male mice at dosage of 0, 150, 300 or 600 mg/kg (p.o., 4 mice/genotype/dose) after overnight fasting, 1 at 300 mg/kg and 3 at 600 mg/kg of homozygous knockout mice died within 24 hours, and those that survived for 24 hours showed higher plasma alanine aminotransferase activities, lower hepatic glutathione levels, and increased severity of centrilobular hepatocellular necrosis when compared to heterozygous knockout or wild-type mice. Time-course changes in blood biochemistry and histopathology at 0, 2, 8 and 24 hours after dosing of APAP at 300 mg/kg (4 mice/genotype/time point) indicated earlier development of hepatotoxicity in homozygous knockout mice than those of other genotypes. Hepatic glutathione levels were decreased markedly at 2 hours in all mice; at 8 hours, they remained lower than the pre-dose values in homozygous knockout mice while those in other mice recovered. These results clearly show Nrf2 affects sensitivity to APAP hepatotoxicity and suggest it plays an important role in xenobiotic detoxification *in vivo*.

200 INDUCTION OF HEME OXYGENASE (HSP 32) IN HEPATIC MACROPHAGES DURING ACETAMINOPHEN HEPATOTOXICITY.

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Heme oxygenase (HO) is the rate-limiting enzyme for the physiological degradation of heme into biliverdin, iron, and carbon monoxide. Two isoforms have been identified, a constitutive form (HO-2) and an inducible form (HO-1). Both isozymes have been shown to be important in protecting against oxidant-induced injury. We have previously demonstrated that acetaminophen-induced hepatotoxicity is mediated in part by oxidants, in particular, nitric oxide, which is released by parenchymal and nonparenchymal liver cells. Macrophages are known to upregulate mediators which aid in antioxidant defense. In the present studies, we analyzed expression of HO-1 and HO-2 in hepatic macrophages during acetaminophen-induced toxicity. Treatment of male Long Evans Hooded rats with acetaminophen (1 g/kg, i.p.) caused a time-dependent induction of liver injury which was measured by increases in serum transaminase levels. This was observed within 3 hr of acetaminophen administration and reached maximal levels by 12 hr and was directly correlated with expression of inducible nitric oxide synthase (NOS II) protein in the liver. Hepatocytes, as well as macrophages isolated from acetaminophen treated rats also produced more nitric oxide in response to inflammatory mediators. We also found that acetaminophen treatment of rats resulted in a time-dependent induction of hepatic HO-1 expression which was maximum after 24 hr. Immunohistochemical analysis revealed that HO-1 expression was predominantly localized in hepatic macrophages, with weaker staining of hepatocytes. In contrast, no changes were observed in HO-2 expression. These data suggest that HO-1 may play a protective role in this model of hepatotoxicity. (Supported by GM34310 and the Burroughs Wellcome Fund.)

201 OVEREXPRESSION OF HEAT SHOCK PROTEINS IN HEPG2 CELLS USING ADENOVIRAL GENE DELIVERY.

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Increased expression of the heat shock proteins (hsps) is a highly conserved response to cellular stress in both eukaryotic and prokaryotic cells. In cultured cells, increased levels of hsps induced by mild hyperthermic treatment can result in tolerance to both severe hyperthermia and chemical stressors (e.g. tButylhydroperoxide, Cd, diethylnitrosamine, cyclophosphamide). Elucidation of the mechanism of cytoprotection afforded by hsps has been hampered by the induction of multiple hsps or concomitant physiological responses brought about by the inducing agents. In an effort to address these issues, replication deficient adenoviral vectors containing hsp25 (Ad25), hsp27 (Ad27), hsp70i (Ad70i), or a constitutively active heat shock factor (Ad-hsfB) were developed. HepG2 cells (ATCC - human liver cell line) were inoculated with adenovirus at levels of 1-200 multiplicity-of-infection (MOI) for 6-24 hours. Sub-lethal heat stress controls were incubated at 43°C for 60 min. Significant elevation of hsp protein levels was observed for an MOI of 10 or more. By 24 hours post-inoculation an MOI of 100 produced results most comparable to sub-lethal heat shock for the hsp70i and hsp27 adenovirus. Strong elevation of hsp25 was observed after Ad25 at an MOI of 10 or above. As anticipated, inoculation of HepG2 cells with Ad-hsfB at an MOI of 10 or more elevated the levels of all hsps tested. Elevated levels of hsps were specific to the inoculating strain of adenovirus (i.e. only the targeted hsp protein levels were elevated). The use of specific hsps delivered by adenoviral vectors will enable hypothesis testing of fundamental mechanisms for the role of hsps in hepatotoxicity. (Supported by ES-07213.)

202 GROWTH HORMONE DEPENDENT REGULATION OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α.

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Peroxisome proliferators (PP) are a structurally diverse group of rodent hepatocarcinogens. It is known that the peroxisome proliferator-activated receptor α (PPARα) plays an essential role in mediating the effects of PP. A recent study using Snell dwarf mice treated with the PP methylclofenapate showed increases in liver peroxisome proliferation but not hepatocellular proliferation (Styles *et al.*, 1990. *Carcinogenesis* **11**:387-91). Dwarf mice have very low levels of pituitary hormones, of which growth hormone (GH) is known to negatively regulate PPARα post transcriptionally. Based on these findings, we are using dwarf mice as a model to determine the role of peroxisome proliferation in carcinogenesis. PP-dependent gene and protein expression was examined in Snell (dw) and Ames (df) dwarf mice using our PP cDNA arrays and immunoblot analysis. These mice have mutations in the *pit*-1 and *prop*-1 genes, respectively, resulting in low levels of GH. To determine the effect of PP, dw mice were administered a single dose of the PP Wy-14,643 (50 mg/kg) and sacrificed 12 hr later. A number of PP-dependent genes (acyl-CoA oxidase, Cyp4a, fibrinogen) were constitutively altered in the absence of PP treatment in dw and df mice. However, some PP-dependent gene/protein expression was independent of dwarf status but altered by Wy-14,643 exposure (multi-functional protein-1, acyl-CoA thioesterase, Cu/Zn superoxide dismutase). Our results also indicate that PPARα is constitutively elevated in dw mice compared to their heterozygous counterparts. Because dwarf mice live 1.5-2.0 times as long as wild type mice, the markers of PP exposure that are constitutively activated in dwarf mice and thought to be important in hepatocarcinogenesis need to be reassessed as to their relevance for predicting liver cancer.

203 PPARα KNOCKOUT MICE ARE NOT PROTECTED AGAINST ACETAMINOPHEN (APAP) HEPATOTOXICITY BY CLOFIBRATE (CFB) PRETREATMENT.

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The purpose of this study was to investigate whether the activation of the nuclear receptor PPARα mediates the protection against APAP hepatotoxicity by peroxisome proliferator pretreatment (T.A.P. 129:252, 1994). **Methods:** Female PPARα +/+ and PPARα -/- mice were given corn oil or 500 mg CFB/kg, i.p., daily for 10 days. They were then fasted overnight and either killed without challenge or at 4 or 24 hr after challenge with 600 mg APAP/kg, p.o. Controls were given 50% propylene glycol vehicle only. **Results:** At 24 hr, hepatotoxicity was evidenced by elevated plasma SDH and histopathology in corn oil pretreated PPARα +/+ and PPARα -/- mice receiving APAP. As expected, CFB pretreated PPAR +/+ mice were protected against APAP hepatotoxicity, while liver damage was present in CFB pretreated PPARα -/- mice. However, lesion severity in this group of mice was less than that of either group of corn oil pretreated mice receiving APAP. At 4 hr, hepatic glutathione depletion and selective arylation of 44 and 58 kDa cytosolic proteins by APAP were both greatly diminished in CFB pretreated PPARα +/+ mice, but unchanged in CFB pretreated PPARα -/- mice. **Conclusions:** These findings suggest that the protection against APAP hepa-

totoxicity by peroxisome proliferator pretreatment is in part mediated by the activation of PPARα receptor, which may lead to diminished net availability of reactive intermediate of APAP. (Supported by a grant from PhRMA Foundation.)

204 CHANGES IN THE EXPRESSION OF MOUSE LIVER PLASMA MEMBRANE PROTEINS INDUCED BY CLOFIBRATE.

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Dosing with the peroxisome proliferator clofibrate (CFB) protects mice against acetaminophen (APAP) hepatotoxicity. Previous studies showed that clearance of APAP from liver is significantly faster in mice receiving CFB. This rapid clearance occurs in the absence of noticeable changes in bioactivating or detoxifying pathways for APAP, suggesting a possible role for plasma membrane transport proteins in the hepatoprotection. In this study, changes in the expression of mouse liver plasma membrane proteins were analyzed by two-dimensional gel electrophoresis (2-D gel). **Methods:** Male CD-1 mice received 500 mg CFB/kg i.p., daily for 5 or 10 days. Controls were given corn oil vehicle only. Liver plasma membrane (mLPM), canalicular (cLPM) and basolateral (bLPM) membranes were prepared by sucrose density gradients, and then subjected to 2-D gel analysis. **Results:** The most prominent change observed in mLPM was an increased expression of a cluster of acidic proteins with an apparent Mr of 47,000. Further separation of cLPM and bLMP revealed that this cluster of proteins is native to the basolateral domain of the plasma membrane. No prominent changes in expression of canalicular proteins were detected. **Conclusion:** This studies suggests that pretreatment with CFB induces primarily bLPM proteins, with no noticeable changes in the profile of cLPM proteins (Supported by grants from PhRMA Foundation and AstraZeneca.)

205 THIAZOLIDINEDIONES PRODUCE SEVERE HEPATIC STEATOSIS IN MICE EXHIBITING INCREASED HEPATIC EXPRESSION OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ.

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Background: Thiazolidinediones (TZDs), used to treat non-insulin-dependent diabetes mellitus (NIDDM), are selective ligands for the peroxisome proliferator-activated receptor γ (PPARγ>). This receptor, which is involved in the regulation of lipid homeostasis, is normally not abundantly expressed in the liver. **Aim:** We explored the hypothesis that PPARγ expression in the liver of obese mice exhibiting NIDDM is upregulated and that, therefore, treatment with TZDs produces increased hepatic lipid deposition. **Methods:** Basal hepatic PPARγ gene expression was analyzed by quantitative PCR both in genetically obese KKA^y mice, an animal model for NIDDM, and lean C57BL/6 control mice. KKA^y and lean controls were treated orally with vehicle or the TZDs, troglitazone (100 mg/kg/day) or rosiglitazone (2.5 mg/kg/day) for 4 weeks. At the end of the study, the extent of hepatocellular lipid accumulation and its distribution was evaluated histopathologically in liver sections. **Results:** Untreated KKA^y mice exhibited upregulated hepatic expression of both PPARγ1 and PPARγ2 (6-fold and 2-fold, respectively) mRNA, as compared to transcript levels in lean C57BL/6 mice. While, in lean mice, prolonged treatment with the TZDs had no apparent effect on hepatic lipid homeostasis, treatment of obese KKA^y mice with troglitazone or rosiglitazone resulted in severe hepatocellular steatosis. **Conclusions:** TZD treatment of diabetic mice exhibiting high expression levels of hepatic PPARγ results in increased lipid deposition in the liver. These augmented hepatocellular fat stores may not only be a reservoir for the accumulating lipophilic drug but also predispose for progressive liver injury.

206 EFFECT OF TROGLITAZONE ON THE LIVER OF THE ZDF RAT MODEL OF DIABETES MELLITUS.

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Troglitazone was developed as a curative medicine for type II diabetes mellitus. As troglitazone was clinically used, serious case of liver dysfunction, the cause of which was suggested to be the dosing of the agent, were reported. Based on analysis of the clinical data, these rare adverse effects were classified as idiosyncratic hepatocellular injury, however, it was not clear what kind of factor was involved in the idiosyncracy. In this study, we administered troglitazone to male ZDF rats, an animal model of diabetes mellitus, at dose levels of 0, 100 or 400 mg/kg for up to 3 months to investigate the possibility that liver dysfunction was induced by long term treatment of troglitazone under diabetic condition. The results indicated that no abnormalities were observed in general conditions or blood biochemical parameters, i.e., AST, ALT, ALP and total bilirubin, which are good indicators of liver dysfunction. Moreover, in liver histopathological examinations, although fatty change of hepatocyte was observed in one rat given 400 mg/kg, hepatocellular degeneration or necrosis was not observed in any rats given troglitazone. In summary, it was concluded that 3-month treatment of troglitazone did not induce liver dysfunction in ZDF rats. Thus, the liver dysfunction in humans was not reproducible in the ZDF model, suggesting that diabetic condition itself may not directly related to idiosyncracy reported in diabetes mellitus patients treated with troglitazone.

207 COMPARATIVE TOXICITY OF THIAZOLIDINEDIONES IN ISOLATED RAT HEPATOCYTES.

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Thiazolidinediones, including troglitazone and rosiglitazone, are agents for the treatment of adult-onset insulin resistant diabetes. Thiazolidinediones have caused sporadic hepatic dysfunction, that led to hepatic failure in a small number of cases. The potential to induce toxicity was compared in isolated rat hepatocytes with troglitazone (TRO), its quinone (TRO-Q) and sulfate metabolite, the dione and tocopherol segments of TRO, and rosiglitazone (ROSI). Hepatocytes were exposed for 2 hours and evaluated for LDH, ALT and AST leakage (membrane integrity) by solid-phase assay, MTT activity changes (reductive metabolism) by spectrophotometry and for mitochondrial transmembrane potential alterations, $\Delta\psi_m$ (cell energetics) by flow cytometry. Increased LDH, ALT or AST leakage ranged from 2.0 and 6.5-fold with TRO at ≥300 μM concentrations. MTT activity decreased 47% to 90% with TRO at ≥300 μM. TRO-Q exposures caused 52% and 79% decreased MTT activity at 500 and 600 μM, respectively. Reductive metabolism decreased 46% with 600-μM ROSI. TRO, TRO-Q and ROSI caused significant $\Delta\psi_m$ decreases. The IC_{50} for effects on cell energetics was 277, 524 and 595 μM for TRO, TRO-Q and ROSI, respectively. No effects were seen with the sulfate metabolite or the dione or tocopherol segments of TRO. The results indicate a relative order of potency for hepatic effects where TRO > TRO-Q > ROSI. Based on the mechanism of liver cell effects, hepatotoxicity may be a class effect with thiazolidinediones.

208 KINETIC DIFFERENCES IN γ-GLUTAMYLTRANSFERASE (GGT) FROM LIVER OF DIABETIC RATS MAY BE DUE TO CHANGES IN CARBOHYDRATE STRUCTURE.

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Recent studies have shown that GGT activity is increased in the liver of insulin-dependent diabetic rats; however, this increased activity was not due to increased expression of the enzyme. In our current work, GGT was purified from the livers of male and female Sprague-Dawley rats after induction of diabetes with streptozotocin. Michaelis-Menten kinetic analysis of GGT from diabetic male rats versus controls showed an average increase of 360% in maximal GGT velocity (V_{max}), an average decrease of 8% in the Michaelis constant (K_m), and an average increase of 803% in the ratio V_{max}/K_m, using L-glutamic acid-p-nitroanilide as the γ-glutamyl donor and 6 different acceptors (glycyl-glycine, glycyl-alanine, methionine, glutamine, glutamate and alanine). Under the same conditions, female rats showed a 60% increase in V_{max}, an 18% increase in K_m and a 50% increase in the V_{max}/K_m ratio. Because GGT is glycosylated, these kinetic results indicate probable conformational changes due to differences in the carbohydrate structures attached to the enzyme. The activity of several glycosyltransferases from the livers of the diabetic rats were compared to controls. β1-4 Galactosyltransferase was increased 257% in diabetics versus controls (0.157 nmol NADH depleted/μg protein/minute vs. 0.044 nmol NADH depleted/μg protein/minute, $p<0.05$); however, no significant changes were found in the activities of α1-2 fucosyltransferase, α1-3 fucosyltransferase, N-acetylglucosaminyltransferase II or α2-3 sialyltransferase. These data indicate that glycosylation is altered in diabetic rat liver, which could alter GGT carbohydrate structure in such a way that the enzyme is more catalytically active.

209 DIABETES PROTECTS MICE FROM LETHAL EFFECT OF THIOACETAMIDE HEPATOTOXICITY.

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The diabetic state has been shown to potentate hepatotoxicity of classical hepatotoxicants like thioacetamide (TA), CCl4 and chloroform in male Sprague-Dawley rats. Results from our laboratory have shown that TA liver injury in streptozotocin (STZ)-induced diabetic rats is highly exaggerated (~6-fold higher) and is also accompanied by decreased compensatory tissue repair. Higher liver injury further progressed owing to compromised tissue repair, leading to 90% mortality from an ordinarily non-lethal dose of TA. The objective of the present studies was to characterize the modulation of TA hepatotoxicity due to diabetes in male C57BL6 mice. Diabetes was induced by a single dose of STZ (200 mg/kg, i.p. in citrate buffer). Diabetes was confirmed by plasma glucose concentration (>350 mg/dl) prior to administration of TA or saline vehicle. Paradoxically, a dose of TA (1000 mg/kg i.p. in 1 ml saline/ kg) which caused 90% mortality in non-diabetic mice, caused only 10% mortality in diabetic mice. The diabetic state perplexingly protected from TA-induced liver injury and consequent lethality. A time-course of liver injury revealed ~5-fold higher liver injury in the non-diabetic mice as measured by alanine aminotransferase (ALT) activity. Liver injury was also confirmed by histopathology of liver sections by H & E staining. Tissue repair response as measured by 3H-thymidine incorporation into hepatonuclear DNA, was ~3-fold higher in the diabetic mice (at 48 hr) compared to non-diabetic mice. The present data indicate a striking species-difference in the way diabetes modulates the hepatotoxicity of TA. Our data also suggests that the tissue repair response in the normal mice is sluggish and appears at a later time (Supported by Board of Regents Support Fund.)

210 POTENTIATION OF THIOACETAMIDE-INDUCED LIVER INJURY IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Earlier studies revealed that enhanced liver injury accompanied by compromised liver tissue repair that leads to 90% lethality from a non-lethal dose of thioacetamide (TA, 300 mg/kg, ip) in streptozotocin-induced diabetic rats. The object of this study was to investigate underlying mechanisms for the increased liver injury and decreased liver tissue repair. We tested two hypotheses: (1) the increased TA liver injury is due to induction of CYP2E1 and FMO1 in diabetic condition; (2) the inhibition of tissue repair in diabetes is a function of diabetic condition rather than high liver injury of TA. Male Sprague-Dawley rats were pretreated with isoniazid (INH), a CYP2E1 inducer, or diallyl sulfide (DAS), a CYP2E1 inhibitor. Plasma ALT and SDH showed that TA liver injury was significantly increased in the INH-pretreated rats, whereas it was decreased 60% in DAS-pretreated rats. DAS pretreatment to the diabetic rats decreased a maximum of 75% TA liver injury. FMO1 is induced 1.8-fold in the diabetic rats. Paradoxically, after FMO1 was inhibited by dietary administration of indole-3-carbinol (0.25%, 10 days), TA liver injury was highly increased. Further, we compared tissue repair by employing equi-toxic doses of TA (30 mg/kg in the diabetic rats yields near equal liver injury as observed with 300 mg/kg in the non-diabetic rats). [^{3}H]-thymidine pulse labeling revealed DNA synthesis is delayed in the diabetic rats in spite of equal liver injury in diabetic and non-diabetic rats. We conclude that (1) CYP2E1 might primarily mediate bioactivation-based TA liver injury. (2) Compensatory tissue repair is delayed at low dose of TA, and delayed and decreased at higher dose of TA in the diabetic rats (Louisiana Board of Regents Support Fund).

211 MOLECULAR MECHANISMS OF CYTOPROTECTION AGAINST HYDROGEN SULFIDE TOXICITY.

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Hydrogen sulfide is a well known industrial toxin, yet it is produced in the body and has been postulated to have a physiological role. Therefore, metabolism may contribute to its biological effects. Glycine and alanine which block chloride ion channels in hepatocytes decreased NaHS cytotoxicty in freshly isolated hepatocytes suggesting that NaHS enters the cells via chloride ion channels. Hepatocyte GSH was depleted by NaHS although GSH did not react with NaHS in a cell free cystem. This suggests that an NaHS metabolite reacts with GSH. Furthermore GSH-depleted hepatocytes were more susceptible to NaHS than control cells, suggesting that GSH detoxifies this NaHS metabolite. Cytochrome P-450 inhibitors e.g. cimetidine, metyrapone and phenylimidazole were protective against NaHS toxicity, suggesting that cytochrome P-450 is involved in the metabolic activation of NaHS. Methionine was protective against NaHS and its protective effect was overcome by adenosine, an S-adenosyl-L-homocysteine hydrolase inhibitor. This suggests that intracellular methylation by S-adenosylmethionine detoxifies NaHS.

212 BETAINE - ITS ROLE IN METABOLISM OF GLUTATHIONE AND *S*-CONTAINING AMINO ACIDS.

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We have examined the role of betaine in metabolism of glutathione (GSH) and *S*-containing amino acids to elucidate the mechanism of its dual action against chloroform hepatotoxicity previously observed in this laboratory (Kim *et al., Fd Chem Toxicol* 36: 655, 1998). Betaine (1000 mg/kg, ip) rapidly decreased the hepatic GSH level, but not the plasma GSH, in rats and mice. However, both hepatic and plasma GSH levels were elevated significantly 24 hr following the treatment. Hepatic efflux of GSH into plasma or the ratio of GSSG/GSH was not altered by betaine. Decreases in hepatic cysteine, glutamate and glycine were evident from t = 0.5 to 4 hr. Cysteine in plasma was increased from t = 0.5 hr. In liver, taurine and several amino acids, such as aspartate, tyrosine, isoleucine and leucine were declined whereas methionine was significantly increased. Hepatic *S*-adenosylhomocysteine (SAH) and *S*-adenosylmethionine (SAM) levels were also elevated, but cystathionine was decreased by a 4 hr prior dose of betaine. The activities of hepatic cystathionine β-synthase (CβS), cystathionine γ-lyase (CγL) and γ-glutamylcysteine synthetase (GCS) were all increased 24 hr after the betaine treatment. Hepatic uptake of cysteine was also decreased at t = 2 hr as determined by arterial-venous difference for cysteine in liver. In mice pretreated with propargylglycine (200 μmole/kg, ip), an irreversible inhibitor of CγL, betaine decreased the hepatic GSH and cysteine levels at t = 4 hr, however, betaine-induced increase of GSH in liver, kidney and plasma was diminished at 24 hr following the treatment. The results suggest that reduction of cysteine uptake from blood in conjunction with inhibition of the transsulfuration pathway be responsible for the rapid decrease in hepatic GSH, and that activation of the transsulfuration pathway and related enzymes play an important role in the elevation of this endogenous tripeptide in later time periods in animals treated with betaine.

213 IDENTIFICATION OF RAT HEPATIC PROTEINS ADDUCTED BY MALONDIALDEHYDE AND 4-HYDROXYNONENAL.

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Lipid peroxidation results in the production of α, β–unsaturated aldehydes, the most abundant products being malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). These aldehydes initiate cellular effects including dysregulation of Ca^{2+} homeostasis and signal transduction. In addition, their chemical reactivity facilitates adduction of cellular proteins possibly mediating cytotoxic events resulting from exposure to chemicals with prooxidative potential. The aim of this study is to identify hepatic aldehyde adducted proteins in rats following acute or chronic prooxidatvie challenge. Acute prooxidative challenge was by intragastric administration of CCl_4 (1ml/kg) after which the rats were sacrificed at 2.5 to 72 hrs following treatment. Chronic prooxidative challenge was by administration of an ethanol-containing diet for up to 120 days. Hepatic subcellular fractions were prepared and subjected to immunoprecipitation -immunoblotting using polyclonal antibodies specific for 4-HNE or MDA-protein epitopes. Immunoisolated proteins were subjected to tryptic digestion and HPLC separation of peptides for amino acid sequence analysis. MDA and 4-HNE adducted proteins with molecular weights of 205 and 170 kDa were isolated from either CCl_4 or alcohol-treated rats and identified as non-muscle myosin heavy chain A and carbamoyl phosphate synthetase I, respectively. 70 kDa and 35 kDa proteins adducted with wither 4-HNE or MDA were identified as urate oxidase. These results indicate that 4-HNE and MDA adduct common target proteins that are relatively abundant and have toxicologic significance with respect to disruption

of cytoskeletal function, urea cycle inhibition and uric acid oxidation. (Supported by NIAAA/NIH AA-09300.)

214 PROTEIN ADDUCT FORMATION BY NORCOCAINE NITROXIDE, AN N-OXIDATIVE METABOLITE OF COCAINE.

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Several lines of evidence indicate that N-oxidative metabolism of cocaine is required for its hepatotoxicity. It was demonstrated over 20 years ago that cocaine N-oxidative metabolism leads to formation of a reactive metabolite that binds irreversibly to proteins, and this binding correlates closely with cocaine-induced liver injury. The identity of the reactive metabolite has not been established with certainty. Early reports suggested that the stable nitroxide metabolite of cocaine (norcocaine nitroxide; NCNTX) was not sufficiently reactive to be responsible for protein binding. However, to test this more directly, 14C-labelled NCNTX was synthesized and incubated with mouse hepatic microsomes. Rapid, irreversible binding of radiolabel to protein was observed. The addition of NADPH to the incubation produced only a modest increase in radiolabel binding, indicating that oxidation of NCNTX is not required for protein-adduct formation. In subsequent experiments, incubation of unlabelled NCNTX with mouse whole liver homogenate was found to result in selective adduction of specific proteins as determined by Western blot analysis. Many of these proteins appear to have similar molecular masses as proteins adducted during cocaine hepatotoxicity *in vivo*. Two of the protein targets for cocaine binding *in vivo* have been identified recently as hsp60 and transferrin. Incubation of NCNTX with these proteins *in vitro* resulted in protein adduct formation. Incubation with cocaine under these conditions, in comparison, produced little or no protein adduct formation. These observations suggest that NCNTX formed from N-oxidative metabolism of cocaine is spontaneously reactive with proteins and that it could be responsible for much, if not all, of the protein adduct formation associated with cocaine hepatotoxicity. (Supported by DA-06601.)

215 COVALENT BINDING OF KETOCONAZOLE IN RAT HEPATIC TISSUE.

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It has been speculated that ketoconazole's (KT) hepatotoxicity may be a result of the parent compound and/or reactive metabolite(s) that covalently binds with hepatic macromolecules, thereby producing hepatic damage. Presently, mechanism(s) of toxicity or bioactivation of KT has not been investigated *in vivo*. The results of this study provide evidence that covalent binding to hepatic tissue is dose- and time-dependent. To determine whether covalent binding occurs, male Sprague-Dawley rats were injected with radiolabeled ^{3}H-KT (1.5 μCi/mg) at doses of 40 mg KT/kg and 90 mg KT/kg *i.p.* The liver was harvested at 0, 0.5, 1, 2, 4, 8, 12 and 24 hr after *i.p.* administration. Acute studies were performed because it is expected that formation of reactive metabolites will occur soon after KT administration. Liver tissues were homogenized and the protein was precipitated with 0.9 M TCA. The protein pellet was exhaustively washed with 80% methanol. Radioactivity was measured. The radioactivity was normalized to dpm/mg protein. Significant covalent binding occurred as early as 0.5 hr for both doses evaluated. The 40 mg KT/kg dose peaked at 2-hr (0.026 nmole KT/mg protein ± 0.005 nmole KT/mg protein) with a steady decline by 24 hr (0.018 nmole KT/mg protein ± 0.008 nmole KT/mg protein). The 90 mg KT/kg dose peaked at 8 hr (0.052 nmole KT/mg protein ± 0.001 nmole KT/mg protein) with a steady decline by 24 hr (0.030 nmole KT/mg protein ± 0.007 nmole KT/mg protein). The results represent three separate experiments. The binding of KT to hepatic tissue may be responsible for the hepatic injury and hepatic necrosis seen in the clinical literature. Moreover, the significant increase in covalent binding seen with the higher dose may warrant caution for those patients treated with higher doses of KT for prostate cancer. (Supported by Pharmaceutical Research and Manufactures of America Foundation.)

216 EFFECT OF DIMETHYLNITROSAMINE EXPOSURE IN MICE ON SELECTED MRNA TRANSCRIPTS FROM PERITONEAL, BONE MARROW, AND LIVER CELLS.

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Dimethylnitrosamine (DMN) is a hepatotoxicant and a suspected carcinogen found in foods and in some industrial settings. We have studied the influence of murine macrophage heterogeneity and activation state by monitoring selected transcript expression following daily dimethylnitrosamine exposure. The purpose of this study was to determine whether expression of selected genes is altered during DMN exposure in the liver and peripheral tissue. Cells from the peritoneal cavity responded to 7 days of DMN exposure by increasing transcripts for the chemokine C10 and phosphoglycerate kinase in the latter part of the experimental period. Thioglycollate-elicited cells from the peritoneal cavity, however, responded to DMN exposure with an early increase in the relative amount of transcripts for the chemokine C10 and phosphoglycerate kinase and then a decrease in the latter days of the experiment. Cells recovered from the bone marrow showed little change in expression for these transcripts due to DMN exposure, whereas, these same cells from mice pretreated with thioglycollate responded with substantial increases in transcripts for the chemokine C10 and for mouse myeloid secondary granule protein. In the liver, the transcript for murine calcium binding protein pp52 increased coincidentally with an increase in pathology due to exposure to DMN. Thus, DMN exposure *in vivo* has both tissue-specific effects and can influence peripheral macrophage populations. (Supported by NIH grants 5F32 ES05745-03 and ES04348.)

217 HEPATIC ISCHEMIA/REPERFUSION INJURY REDUCED BY DIMETHYL SULFOXIDE.

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Many potential transplant recipients die waiting for livers. Use of organs from non-heart-beating donors (NHBD) might reduce the current organ shortage. However, warm ischemia in NHBD produces damage that can lead to graft failure in recipients post-transplantation. The hepatic microvascular system is the target of ischemia/reperfusion (I/R) injury. Dimethyl sulfoxide (DMSO) has several well-known biological activities that may prevent or reduce such damage. Male Fischer 344 rats were pretreated with DMSO (2 ml/kg, ip) or saline (control) 30 minutes prior to the induction of ischemia. The rats were then subjected to 60 minutes of left lateral lobar ischemia and 60 minutes of reperfusion. Sham-operations were performed in an identical manner without vascular occlusion. The hepatic microvasculature of the left lateral lobe was observed using established high resolution *in vivo* microscopic methods. The numbers of leukocytes adhering to the sinusoidal wall, swollen endothelial cells (EC) and sinusoids containing blood flow (SCF) were counted. The phagocytic activity of Kupffer cells was assessed by counting the number of Kupffer cells that phagocytosed injected fluorescent latex particles in relation to the number of SCF. Results demonstrated that I/R caused significant increases in the numbers of adhering leukocytes, swollen EC, and phagocytic Kupffer cells as well as Kupffer cell phagocytic activity when compared to sham-operated animals. Pre-treatment with DMSO prevented these adverse events from occurring in the ischemic lobe. The number of SCF was significantly decreased by I/R, however, pre-treatment with DMSO restored SCF to sham-controls levels. Results indicate that pre-ischemic treatment of rats with DMSO reduced damage caused by warm ischemia. This suggests that pre-treatment with DMSO should prevent tissue damage in NHBD livers, thus providing additional organs suitable for transplantation. (Supported by the ADCRC [JU, PN], the AHA, AZ Affiliate [JU], the Richard Siegel Foundation and NIEHS Center Grant P30-ES-06694 [JU].)

218 EFFECT OF CRUCIFEROUS VEGETABLES (CV) ON CAFFEINE METABOLISM IN HUMAN VOLUNTEERS.

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In this study we have examined the effect of CV on the metabolism of caffeine (which reflects the activity of hepatic CYP1A2) in a group of 20 male, non-smoking, Caucasian volunteers. The study was conducted in three phas-

es, each of 12 days with 2 days between each phase. In phases 1 and 3 (washout periods) the subjects abstained from consumption of CV, whereas in phase 2 the subjects consumed 500 g per day of CV (250 g each of cooked Brussels sprouts and broccoli, eaten at breakfast and dinner). For the last 48 hr of each phase, the subjects refrained from consumption of caffeine and cooked and smoked meat and fish products. The morning after the end of each phase the subjects ingested a 2 mg/kg oral dose of caffeine. Saliva samples were collected at 0, 1, 2, 4, 6, 9, 12 and 22 hr and assayed by LC-MS-MS for caffeine content and at 6 hr for the paraxanthine/caffeine (17X/137X) ratio. Urine samples were collected from 2 hr before until 8 hr after the caffeine dose and were assayed by LC-MS-MS for caffeine and selected caffeine metabolites. Compared to phase 1, caffeine metabolism was significantly induced by CV consumption. Clearance values were 3356 and 3580 ml/hr, t½ values were 4.83 and 4.32 hr and $AUC_{0-\infty}$ were 58.7 and 50.0 μg-hr/ml for phases 1 and 2, respectively. No statistically significant differences were noted between phases 1 and 3. In contrast, CV treatment had no significant effect on various caffeine urinary metabolite ratios for CYP1A2, N-acetyltransferase 2 or xanthine oxidase activities, or on the 17X/137X saliva ratio after 6 hr. These results demonstrate that CV can induce CYP1A2-dependent caffeine metabolism in humans and that effects on caffeine metabolism are more reliably assessed by saliva kinetics than by the use of metabolite ratios. (Supported by UK MAFF.)

219 BENOXAPROFEN FORMS PROTEIN ADDUCTS IN THE BILE CANALICULI OF FEMALE CD1 MICE.

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The NSAID benoxaprofen (Oraflex; B) was withdrawn from use after a number of cases of fatal cholestatic jaundice in elderly patients receiving the drug. The formation of protein adducts of various NSAIDs has been associated with their hepatotoxicity in both animal and human studies (Pumford et al (1997) Drug Metab Rev 29, 39). We now report on an investigation of the mechanisms of the hepatotoxicity of benoxaprofen. Female CD1 mice were given single doses of benoxaprofen (5-200 mg/kg) or dose vehicle. The mice were sacrificed after 6 h, the livers excised and subcellular fractions prepared by differential centrifugation. The various fractions were subjected to SDS-PAGE and Western blotting with a rabbit anti-B-KLH antibody. There was a single major 110 kDa adduct concentrated in the 600 x g_{av} fraction. The amounts of this adduct increased in a dose-dependent manner. Adduct recognition was inhibited by B and B-RSA but not RSA. Immunohistochemical analysis showed these adducts to be localised in the bile canalicular plasma membrane. The molecular mass and intrahepatic location of these adducts, currently being characterised further, raises the possibility that they may be involved in the hepatotoxicity of B. We have shown with other hepatotoxic NSAIDs (diclofenac, sulindac) that the formation of 110 kDa adducts in the bile canalicular plasma membrane is associated with impairment of the active secretion of bile salts suggesting that the hepatotoxicity of these drugs is mediated by the intrahepatocytic accumulation of bile salts. (Supported by EC Biomed 2 Contract BMH4-CT96-0658 and Universiti Putra Malaysia.)

220 EFFECTS OF BILIRUBIN AND ARSENITE ON GLUTATHIONE S-TRANSFERASE AND NAD(P)H: QUINONE OXIDOREDUCTASE ACTIVITIES IN MOUSE HEPATOMA HEPA 1C1C7 CELLS.

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The current study evolved from recent results in our laboratory showing that bilirubin induces Cyp1a1 gene transcription through direct interaction with the aryl hydrocarbon receptor (AHR).The aims of this study were to; 1) determine if the increased expression of Cyp1a1 also occurs with other AHR battery genes such as glutathione s-transferase (GST) and NAD(P)H: quinone oxidoreductase (NAD(P)H:QOR), and 2) characterize the effects of arsenite, a potent inducer of heme oxygenase, on GST and NAD (P)H:QOR in the mouse hepatoma Hepa 1c1c7 wild-type (WT), AHR deficient (C12) and AHR nuclear translocator (ARNT) deficient (C4) cell lines. Bilirubin (1-250 μM) caused a concentration-dependent inhibition of GST and NAD(P)H:QOR in all cells with the largest inhibition in WT cells. Arsenite (5-20 μM) treatment resulted in a concentration-dependent decrease in GST and NAD(P)H:QOR activities in WT cells and to a lesser extent in C4 cells, whereas in C12 cells arsenite had no effect on GST but increased NAD(P)H:QOR activity. Co-administration of beta-naphthoflavone (10 μM) and arsenite (5-20 μM) further potentiated the inhibition of GST and NAD(P)H:QOR in WT cells with no changes in C4 cells while it further potentiated the increase of NAD(P)H:QOR activity in C12 cells. We conclude that the AHR mediated at least part of the inhibitory effect of bilirubin and arsenite on GST and NAD(P)H:QOR. Furthermore, ARNT appears to be required for the increase of NAD(P)H:QOR by arsenite and the AHR may prevent ARNT from fully participating in other ARNT-dependent pathways.

221 Mrp2 IS CRITICAL FOR CHOLESTASIS MEDIATED BY ESTRADIOL-17 β(β-D-GLUCURONIDE) ($E_2$17G).

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$E_2$17G is an endogenous metabolite of estradiol that decreases bile flow in the rat while estradiol-3-β-D-glucuronide ($E_2$3G) increases bile flow under the same experimental conditions. The present studies were designed to test the hypothesis that the accumulation of $E_2$17G in bile is essential for its cholestasis and whether direct inhibition of taurocholate (TC) transport is responsible for $E_2$17G cholestasis. Membrane vesicles from Sf9 cells expressing the bile salt export pump (Bsep) and rat canalicular membrane vesicles were used for transport studies. The single pass isolated perfused liver from male TR⁻ (mrp2-deficient Wistar rats) and control Wistar rats was used to monitor $E_2$17G cholestasis. In Sf9 membrane vesicles, Bsep-mediated ATP-dependent transport of TC (1 μM) was 110 ± 12.5 and 108 ± 17.3 % of control in the presence of 10 and 50 μM $E_2$17G, respectively. In rat canalicular membrane vesicles, 50 μM $E_2$17G and $E_2$3G inhibited ATP-dependent transport of 1 μM TC by 67 and 64% respectively. Infusion of 24 μmol ^{3}H-$E_2$17G was not able to induce cholestasis in TR⁻ rats, while 2 μmol ^{3}H-$E_2$17G inhibited bile flow by 51% in control rats. The peak biliary concentration of $E_2$17G, determined by HPLC, was 3.4 and 2.5 mM in male control and TR⁻ rats, respectively, whereas 2.5 mM $E_2$17G in bile is associated with cholestasis in control rats. Cumulative biliary excretion of $E_2$17G equivalents over 70 min was 1205 ± 136.5 and 908 ±15.8 nmol in control and TR⁻ rats, respectively. These data demonstrate that the accumulation of $E_2$17G in bile alone is not sufficient to induce cholestasis. Furthermore, direct inhibition of Bsep-mediated TC transport is not the mechanism by which $E_2$17G induces cholestasis. These data indicate that mrp2-mediated transport of $E_2$17G is critical to its ability to induce cholestasis. (GM55343)

222 BILE ACIDS AFFECT LIVER MITOCHONDRIAL BIOENERGETICS: POSSIBLE RELEVANCE FOR CHOLESTASIS THERAPY.

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Liver disease ranks as the third leading disease-related cause of death in the world. Two important but poorly understood manifestations of liver disease are cholestasis and steatosis, that can progress to primary billiary cirrhosis (a form of cholestatic liver disease). For years, several mechanisms are pointed out for this type of liver disease but they still poorly understood, like the exact therapy that should be prescribed to these patients, claiming our attention to this fact. The use of bile acid therapy, usually in the form of ursodeoxycholic acid (UDCA) and its conjugates (TUDC and GUDC) as increased, but the mode of action remains poorly understood. Several observations support the concept that bile acids may be cytotoxic by causing mitochondrial dysfunction. In this work we evaluate the toxicity of the bile acids cholic, ursodeoxycholic, deoxycholic, lithocholic and chenodeoxycholic on liver mitochondrial bioenergetic functions. Both cholic and ursodeoxycholic acids don't affect mitochondrial membrane potential nor the phosphorylative system in concentrations ranging from 10 to 200 μM. Opposingly, lithocholic and chenodeoxycholic acids decrease mitochondrial membrane potential for concentrations of 25 and 50 μM, respectively. Deoxycholic acid for concentrations ranging from 10 to 100 μM decrease mitochondrial membrane potential and additionally, at 100 μM the phosphorylative system is strongly affected, suggesting a strong effect at the ATP-synthase level. Furthermore, the hepatoprotective effects of UDCA and its conjugates were evaluated in terms of mitochondrial permeability transition pore (MPT) induction. UDCA, TUDC and GUDC decreases the susceptibillity of induction of MPT promoted by the toxic bile salts. These results claim attention for the fact that the impairment of mitochondrial function by bile acids can be clinically relevant for patients with cholestasis.

223 CYTOTOXIC EFFECTS OF METHYLENE DIANILINE TO PRIMARY CULTURED RAT BILIARY EPITHELIAL CELLS.

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Methylene dianiline (4,4'-diaminodiphenylmethane, DAPM), a starting ingredient in the production of polyurethanes and epoxy resins, causes cholangitis and cholestasis in rats and humans by an unknown mechanism. DAPM initially targets biliary epithelial cells (BEC) in rats; early *in vivo* effects include increases in biliary levels of glucose and inorganic phosphate. *In vitro* studies using a primary BEC culture system could provide information regarding potential impairments in BEC structure/ function induced by DAPM. Our objectives were to 1) isolate, culture, and characterize primary rat BEC, and 2) perform dose-response and time-course studies of DAPM toxicity using these primary BEC cultures. BEC were isolated using a collagenase liver perfusion method (*Gastroenterology 104:840, 1993*) and cultured on collagen coated plates. Purity was assessed by specific markers for BEC: cytokeratin 19, OC.2 (a BEC-specific surface antigen), γ-glutamyl transpeptidase (GGT) activity and by ultrastructure. BEC cytotoxicity to xenobiotics was assessed by release of GGT, reduction in transepithelial resistance (TER), and formation of a formazan dye from the tetrazolium salt, XTT. The sensitivity of these assays was XTT > TER > GGT. The cytotoxicity of DAPM and α-naphthylisothiocyanate (ANIT) was assessed using BEC and HepG2 cells. BEC were more sensitive to ANIT (0.1 mM) and DAPM (2 mM) than HepG2 cells (0.2 mM and >10mM, respectively). BEC cytotoxicity was demonstrated at 2 mM DAPM, but this concentration is ~3X that found in bile. Thus, the toxic effects of DAPM *in vivo* may be due to 1) proximate metabolite(s) of DAPM, 2) synergistic effects of DAPM with biliary constituents, or 3) unknown phenotypic changes of cultured BEC. (Supported by NIEHS 06348; NIEHS T32-ES 07254; F31-ES 05831.)

224 NITRIC OXIDE ABOLISHES POST-NICOTINE DETRIMENTAL IMPACT ON STRESS-INDUCED GASTRIC ULCERS.

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Chronic nicotine treatment or nitric oxide (NO) reduction intensifies stress-induced gastric ulcers. We tested whether a NO donor could prevent cold-restraint stress-induced gastric ulcers in chronic nicotine-treated rats. Nicotine 50μg/ml in drinking water was given ad lib for 20 days to male SD rats (160-180g, 8 rats/group). NO donor (Isosorbide dinitrite, 10mg/kg, PO) was given 1h and 10min before stress. Stress (2h) induced gastric ulcers, and nicotine treatment significantly intensified the ulcers. However, NO donor treatment failed to reduce this potentiated action. Ulcer index (mm): Water: 6.0±0.7; Nicotine: 19.0±1.7*; Nicotine+NO donor: 17.8±1.7*. Gastric mucus was measured (alcian blue). Mucus content (mg/g): Water: 200±17; Nicotine: 122±14*; Nicotine+NO donor: 143±15*. In another series of experiments, rats drank nicotine solution for 20 days, then the solution was replaced by water. Some ex-nicotine rats were treated a NO donor (10mg/kg, PO, tid). After 10 days, all animals were stressed. Ten-day nicotine cessation still remained a markedly intensifying action, and ten-day NO donor treatment completely abolished this detrimental impact. Ulcer index: Water: 6.3±0.7; Ex-nicotine: 11.0±0.9*; Ex-nicotine+NO donor: 5.8±0.7. Mucus content: Water: 190±18; Ex-nicotine: 142±16* Ex-nicotine+NO donor: 210±19. Conclusion: nicotine treatment reduced gastric mucus and intensified stress-induced gastric ulcers. Neither nicotine cessation alone nor acute NO donor treatment reversed nicotine worsening action on either mucus content or ulcers. However, nicotine cessation plus chronic NO donor treatment completely abolished the impact of nicotine on mucus reduction and ulcerogenic action. *$p<0.05$ vs water group.

225 DECREASE IN SERUM ACTIVITY OF INTESTINE-DERIVED ALKALINE PHOSPHATASE AS A POTENTIAL NON-INVASIVE INDICATOR OF DICLOFENAC-INDUCED ENTEROPATHY.

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Endoscopy of patients and volunteers receiving therapeutic doses of the non-steroidal anti-inflammatory drug diclofenac (Voltaren®) has revealed bleeding and ulcers in the proximal small intestine. In addition, post-mortem studies have linked NSAID use to ulcer formation in the distal small intestine [SI], a region difficult to evaluate by endoscopy. Our laboratory has observed decreases in serum activity of intestine-derived alkaline phosphatase [iALP] in rats given a single, large enteropathy-inducing dose of diclofenac (50 mg/kg). Determine if repeated treatment with diclofenac at the lower recommended therapeutic dosage (10 mg/kg for rodents) leads to decreases in serum iALP activity. Fed adult male Sprague-Dawley rats were treated by oral gavage for 5 days with 10 mg/kg diclofenac given once per 24 hr or in a divided dose of 5 mg every 12 hrs. Control animals were gavaged with the vehicle every 12 hr. Rats were sacrificed 12 hrs after the final dose, the gastrointestinal tract was examined for ulcer numbers and locations, and serum was analyzed for activities of ALP isozymes. Both the single and divided dosage regimens for diclofenac treatment resulted in a similar and appreciable number of ulcers in both the distal SI (15.6±10.2 and 17.8±12.3) and the cecum (10.8±7.9 and 5.0±6.5) without appreciable stomach lesions. Serum activities of iALP in the single and divided diclofenac dosage regimens were diminished by 32% and 51%, respectively. This observed decrease in serum activity of intestine-derived ALP after repeated treatment with a therapeutic dosage of diclofenac suggests that iALP might be used as a cost-effective non-invasive test for detecting NSAID-induced enteropathy in place of upper endoscopy. (Supported by NIH DK 34806, NIH T32ES07254, and Brown Family Foundation.)

226 ENZYME POLYMORPHISM IN COLON CANCER CASES.

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Colon cancer is associated with genetic, occupational, and environmental factors. The polymorphic enzymes N-acetyltransferase 2 (NAT2), glutathione transferase M1 (GSTM1), and glutathione transferase T1 (GSTT1) are predisposing factors for several human cancers. Therefore, the study was carried out in an area of former coal, iron, and steel industries with a known elevated colon cancer mortality. The distribution of the genotypes of NAT2, GSTM1, and GSTT1 was investigated in 100 colon cancer cases and 150 controls (suffering from non-malignant diseases) from the same department. They were genotyped from leukocyte DNA by RFLP and PCR. Additionally, possible occupational and non-occupational risk factors were investigated using a questionnaire. In cancer cases 65% were "slow" acetylators, 53% were GSTM1 negative and 15% were GSTT1 negative. In controls 61% were "slow" acetylators, 51% were GSTM1 negative, and 14% were GSTT1 negative. In contrast to most studies on colon cancer cases, an overrepresentation of the "rapid" acetylator status was not observed in our study group from an area with known elevated colon cancer mortality. The impact of the "rapid" acetylator genotype as a risk factor for colon cancer may vary in different areas possible due to occupational and/or environmental factors. (Work was supported by Deutsche Forschungsgemeinschaft.)

227 EXPRESSION OF FLAVIN-CONTAINING MONOOXYGENASES IN HUMAN INTESTINE.

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Drug metabolism in the small intestine can be considered an initial source of bioconversion of orally administered drugs resulting in decreased drug absorption. Thus, presystemic intestinal metabolism by cytochrome P-450 (CYP) and/or flavin-containing monooxygenases (FMO) may have significant effect on oral drug bioavailability. Exhaustive literature exists on intesti-

nal-CYP effect of drug absorption, while there is minimal research in the area of intestinal-FMO. The results of this study provide evidence that FMO isozymes are present in the GI tract. Western blotting techniques were used to determine the presence of FMO isozymes from microsomes prepared from human jejunal tissue and the human intestinal cell line, Caco-2. The results indicate that FMO1 was present in both the jejunal tissue as well as the Caco-2 cell line. FMO3 was present only in the jejunal tissue while FMO5 was present in the Caco-2 cells. These results are the first to demonstrate that a human intestinal cell line expresses various isozymes of FMO (FMO1 and FMO5). Moreover, the presence of FMO1 in the jejunal tissues and the Caco-2 cells correlate with published reports of FMO1 in rat and rabbit intestine. Lastly, these results demonstrate that the human jejunum expresses at least two forms of FMO (FMO1 and FMO3) which can result in presystemic metabolism of orally administered pharmaceuticals such as cimetidine thereby resulting in low bioavailability.

228 PEROXYNITRITE IN ACETAMINOPHEN (APAP)-INDUCED LIVER NECROSIS: DETOXIFICATION BY APAP.

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Metabolism of APAP by CYP450 to N-acetyl-*p*-benzoquinone imine is an initial step leading to toxicity. Recent data indicate that a second step may be important. We reported that in APAP intoxicated livers nitrotyrosine- and APAP-protein adducts co-localized in the hepatic centrilobular cells, the site of necrosis. Nitration of tyrosine is by peroxynitrite (PN), formed from NO with O_2^- by activated Kupffer cells. Nitration was directly proportional to toxicity. Since both tyrosine and APAP are phenols, we investigated the reaction of APAP with PN. GC-MS analysis of incubations of APAP with PN indicated formation of an APAP dimer, a product formed via the semiquinone imine, a free radical. The potential role of APAP in detoxification of PN was studied in mice. A time course/dose response for APAP toxicity was determined in mice. At 6 hrs following APAP serum ALT levels were inversely proportional to dose: 10,493 ± 981 IU/L for 300 mg/kg, 3,138 ± 91 IU/L for 450 mg/kg, and 1,961 ± 323 IU/L for 600 mg/kg; however, by 12 hrs ALT levels were directly proportional to dose. Tyrosine nitration was inversely proportional to dose at early times and directly proportional to dose at late times. APAP covalent binding was directly proportional to dose at all times. The APAP $t_{1/2}$ was 2 hrs and hepatic APAP levels were calculated to be 1 μmole/gm at 2.5, 7.0 and 9.8 hrs in the low, middle and high dose groups, respectively. These data support the hypothesis that PN is the toxic species, it may be detoxified by APAP, and APAP clearance is necessary for toxicity.

229 NITRATION OF TYROSINE IN THE 85 KDA REGULATORY SUBUNIT OF PHOSPHATIDYLINOSITOL 3-KINASE DURING ACETAMINOPHEN-INDUCED LIVER NECROSIS IN MICE.

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Metabolism of acetaminophen (APAP) by CYP450 to N-acetyl-*p*-benzoquinone imine and covalent binding to proteins is a correlate of toxicity. We recently showed that a second step may be important. We reported that following a toxic dose of APAP, nitrotyrosine protein adducts and APAP-adducts co-localized in the hepatic centrilobular cells, the site of necrosis. Nitration is by peroxynitrite (PN), formed by reaction of NO with O_2^- from Kupffer cells. In this study we performed immunoblot analyses using an anti-nitrotyrosine antiserum. Immunoblot studies indicated three significant nitrated proteins in liver homogenates. These adducts were at low levels in hepatic homogenates from saline- and increased in livers of APAP-treated mice. Subsequently, we performed studies to determine if the major 85 kDa nitrated protein was the regulatory subunit of PI-3 kinase (P-85), a protein reported to be nitrated by PN *in vitro*. Proteins of the same molecular weights stained using anti-P-85 and anti-nitrotyrosine. A time course using anti-nitrotyrosine revealed that 4 hrs following APAP (300 mg/kg) to mice the nitrated 85 kDa hepatic protein increased 14X over saline controls. Similar analyses using anti-P-85 indicated a 19X increase. Serum ALT levels peaked at 8 hrs. Immunohistochemical analyses of liver sections indicated that P-85 was uniformly distributed in the lobule of saline-treated mice; however, in the livers from the APAP-treated mice P-85 was concentrated in the centrilobular areas, the site of necrosis. These data indicate that alterations of a signal transduction protein occur in APAP-induced hepatotoxicity.

230 PRODUCTION OF A RECOMBINANT 56-KDA SELENIUM BINDING PROTEIN ASSOCIATED WITH ACETAMINOPHEN (APAP) TOXICITY AND COVALENT BINDING.

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APAP toxicity has been highly correlated with covalent binding of APAP's reactive metabolite to a 58-kDa protein (58ABP) in murine liver. Sequence analysis of purified 58-ABP from liver cytosol revealed that this protein was homologous to a 56-kDa selenium binding protein (56SBP). Additional studies demonstrated that the 2 proteins were not products of the same gene and that 58ABP was expressed solely in the liver whereas the 56SBP was expressed in both liver and kidney. Interestingly, it appears that both cytosolic proteins are major targets for APAP binding. Therefore, to study additional aspects of APAP binding and toxicity a 56SBP recombinant protein was produced using the pET28b vector containing sequence for histidine and T7 tags (Novagen). 56SBP cDNA (from P. Harrison) was ligated into pET-28b (+) at the *HinD*III site and used to transform *E. coli* DH5α cells. Digestion with *HinD*III and *Apa*I confirmed the 56SBP insert was in frame with the T7 promoter (pSDC8). BL21(DE3)pLysS cells were transformed with pSDC8, a 60 kDa protein was expressed after IPTG induction. Western blot analysis showed that this protein reacted with 58-ABP antisera, T7 tag antisera, and a histidine conjugate confirming the protein was the recombinant 56SBP. The use of recombinant 56SBP will significantly enhance the ability to explore the mechanisms of covalent binding in APAP toxicity. (Supported by U. Conn. Res. Foundation, NIH ES07163, Boehringer Ingelheim Pharmaceutical, Inc.)

231 FORMAMIDINE, A NOVEL INHIBITOR OF NITRIC OXIDE PRODUCTION.

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The free radical gas nitric oxide is generated in biological systems by a family of oxidoreductases known as the nitric oxide synthases using arginine as the substrate. Elevated levels of nitric oxide have been observed in several inflammatory disorders. Our laboratory has been using substrate analogs to characterize mechanisms regulating production of nitric oxide in cell lines expressing either NOS 2, the calcium-independent cytokine inducible nitric oxide synthase (CX-1 and RAW 264.7 cells), or NOS 1, the calcium-dependent constitutively expressed isoform (GH3 cells). In both CX-1 and RAW 264.7 cells, nitric oxide production is induced by gamma-interferon. In GH3 cells, nitric oxide release is stimulated by potassium ionophores or high levels of KCl (25 mM), a process resulting in membrane depolarization and the opening of calcium channels. We found that in each cell type, formamidine, a compound structurally related to arginine, readily inhibits nitric oxide production. The effects were time- and dose-dependent (IC50 = 1-5 mM). In all cell types, the effects of formamidine were apparently not due to cytotoxicity, as determined by trypan blue dye exclusion. Using RAW 264.7 cells, we found that formamidine had no effect on expression of NOS 2 suggesting that the compound acts at the level of enzyme activity. Using a polarographic microsensor to measure real-time flux of nitric oxide release from RAW 264.7 cells, we found that formamidine required 2-4 hours to inhibit enzyme activity. This may be due to slow uptake of the drug. Taken together, our data suggests that formamidine acts to inhibit nitric oxide production by inhibiting the activity of both the NOS 1 and NOS 2 isoforms of nitric oxide synthase. (Supported by NIH grants ES 03647 and ES 06897.)

232 IDENTIFICATION OF OXIDATION PRODUCTS PRODUCED BY MAMMALIAN ALCOHOL DEHYDROGENASE FROM THIODIGLYCOL *IN VITRO*.

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Sulfur mustard (2,2'-bis chloroethyl sulfide) is a chemical warfare agent causing delayed injury to epithelial tissue ranging from erythema to massive fluid-filled blisters and necrosis in a dose dependent manner. In aqueous media, sulfur mustard spontaneously rearranges to 2,2'-bis hydroxyethyl sulfide (thiodiglycol, TDG). Because TDG inhibited protein phosphatase activity in tissue cytosol but not in purified enzyme preparations, we hypothesized that

metabolic activation may be necessary for phosphatase inhibition. Using horse liver ADH, we isolated and instrumentally characterized two products arising from the enzymatic oxidation of TDG. One, with an Rf of 0.65- 0.70 by thin-layer chromatography (TLC)(silica gel, hexane:ethyl acetate 1:3, vol), gave a color consistent with an aldehyde when sprayed with 0.4% 2,4-dinitrophenyl hydrazine (DNPH) in 2.0 M HCl. The second, with an Rf of 0.74-0.78 in the same TLC system, did not react with DNPH. Electron impact (EI) GC-MS analysis of material isolated by preparative TLC indicated a small molecular ion peak at m/e 120 for the lower Rf compound with a base peak at m/e 46 and additional peaks at m/e 61 and 74. Methane chemical ionization GC-MS, which characteristically gives an unambiguous M+H+ peak, instead gave a peak at m/e 103 with this compound indicating the loss of H2O (120 + H+ - 18), behavior characteristic of an aldehyde. The higher Rf compound gave an EI mass spectrum with a base peak at m/e 46, additional peaks at m/e 59 and 74 and a molecular ion at m/e 118. This is consistent with published mass spectral data for the 1,4-oxathian-2-one. Methane chemical ionization produced an M+H+ at m/e 119. We conclude that the compound with Rf of 0.65-0.70, DNPH (+) and with a molecular ion at m/e 120 is the monoaldehyde of TDG. The compound with the higher Rf, DNPH (-), and a molecular ion at m/e 118 is the 1,4-oxathian-2-one.

233 MECHANISM OF INHIBITION OF LUNG CYP2E1 BY DIALLYL SULFONE.

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We have tested the hypothesis that lung CYP2E1 is inactivated by diallyl sulfone ($DASO_2$) through formation of the metabolite diallyl monoepoxide ($DASO_3$). $DASO_3$ was chemically synthesized and reacted with [^{3}H]glutathione (GSH) to produce the $DASO_3$-GSH standard. HPLC analysis of lung microsomal incubations with $DASO_2$ in the presence of [^{3}H]GSH and NADPH showed the formation of the $DASO_3$-GSH conjugate. This conjugate was not produced in incubations performed in the absence of $DASO_2$ or NADPH. The formation of the $DASO_3$-GSH conjugate in the incubations was time-dependent, peaking at 2 h. Conjugate formation was concentration-dependent; saturation was detected at 0.8 mM of $DASO_2$. Generation of $DASO_3$ in the microsomal incubations, as estimated by formation of a 4-nitrobenzyl pyridine derivative, peaked at 35 min. Levels of $DASO_3$ formed in the incubations were concentration-dependent, and were maximal at 1.0 mM of $DASO_2$. The $DASO_3$-GSH conjugate was also generated in incubations containing CYP2E1-expressed human lymphoblastoid microsomes. Furthermore, the formation of this conjugate was significantly inhibited in lung microsomes preincubated with an inhibitory CYP2E1 antibody. CYP2E1-mediated *p*-nitrophenol hydroxylase activity was significantly inhibited by $DASO_2$ in the microsomal incubations. This inhibitory effect was also observed in microsomes isolated from the lungs of mice treated with $DASO_2$. The loss in catalytic activity coincided with diminution of immunodetectable CYP2E1 protein in both the *in vitro* and *in vivo* systems, as assessed by protein immunoblotting. These results supported the conclusion that $DASO_3$ is responsible for the inactivation of lung CYP2E1. (Supported by NCI Grant RO1 CA73220-01.)

234 EFFECTS OF QUINONE METHIDES ON MOUSE LUNG EPITHELIAL CELL LINES.

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There is substantial evidence that butylated hydroxytoluene (BHT)-mediated mouse lung injury and tumor promotion are consequences of metabolic activation. BHT is oxidized by pulmonary cytochrome P450 forming two quinone methides, BHT-QM and BHTOH-QM. Both of these electrophiles readily form covalent adducts with cellular thiols but the latter is several-fold more reactive. In order to probe the targets of these metabolites possibly related to tumor promotion, cell lines derived from normal alveolar type II pneumocytes (C10 and E10) and the corresponding spontaneous transformants (A5 and E9) were incubated with quinone methides. Compared to the transformed cells, C10 and E10 cells had lower levels of glutathione S-transferase (GST) activity and greater sensitivity to quinone methide-induced toxicity. In all four cell lines, BHTOH-QM was substantially more toxic than BHT-QM in agreement with previous results using isolated bronchiolar Clara cells. The quinone methides irreversibly inhibited GST activity and both toxicity and GST inhibition were enhanced by depleting glutathione (GSH) levels. These results demonstrate that both GSH and GST are intracellular targets of BHT-derived quinone methides and suggest a possible mechanism for tumor promotion involving selective destruction of normal versus transformed cells. (Supported by NIH Grant CA41248.)

235 SUCCINYLACETONE ELICITS AN OXIDATIVE STRESS RESPONSE IN MOUSE HEPATOMA HEPA-1C1C7 CELLS.

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Succinylacetone is a tyrosine catabolite that accumulates in the absence of fumarylacetoacetate hydrolase (FAH), the final enzyme in the tyrosine catabolism pathway. Succinylacetone is a reactive oxygenated metabolite (yet a weak Michael reaction acceptor) found in the tissues, plasma, and urine of *Fah*(-/-) knockout and *14CoS/14CoS* mice, both of which lack a functional FAH enzyme. The presence of urinary succinylacetone is also used to screen patients for hereditary tyrosinemia type 1 (HT-1), an inherited deficiency of FAH. An oxidative stress response, characterized by the induction of stress-inducible genes and changes in glutathione status, has been observed in *Fah(-/-)* and *14CoS/14CoS* mice. We have tested the hypothesis that succinylacetone might cause an oxidative stress response. Northern blot analysis of succinylacetone-treated mouse hepatoma Hepa-1 cells demonstrates significant elevations in mRNA levels of the oxidative stress-responsive genes glutamate-cysteine ligase (catalytic and regulatory subunits, *Gcls* and *Gclr*), NAD(P)H: quinone reductase-1 (*Nqo1*) and heme oxygenase-1 (*Ho1*). Transient transfection of succinylacetone-treated cells with an electrophile response element (EPRE)-driven luciferase (*LUC*) construct generated a 3-fold increase in luciferase activity. Succinylacetone is a potent inhibitor of 5-aminolevulinic acid (ALA) dehydratase, the second enzyme in the heme synthesis pathway. Northern blot analysis and transient transfection of EPRE-*LUC* in ALA-treated cells demonstrate that an oxidative stress response is caused directly by ALA. We hypothesize that FAH deficiency leads to accumulation of succinylacetone, thus inhibiting ALA dehydratase, leading to build-up of ALA and resulting in the oxidative stress response. These findings may have clinical relevance for HT-1 patients as well as porphyria patients, and may partially explain the oxidative stress response observed in untreated *Fah(-/-)* and *14CoS/14CoS* mice. (Supported in part by NIH Grant R01 AG09235.)

236 ANTIOXIDANT BALANCE AND FREE RADICAL GENERATION IN VITAMIN E DEFICIENT MICE AFTER DERMAL EXPOSURE TO CUMENE HYDROPEROXIDE.

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Organic peroxides are widely used in the chemical industry as initiators of oxidation for the production of polymers and fiber-reinforced plastics, in the manufacture of polyester resin coatings, and as antimicrobial agents in pharmaceuticals. Free radical production is considered to be one of the key factors contributing to skin tumor promotion by organic peroxides. *In vitro* experiments have demonstrated metal-catalyzed formation of alkoxyl, alkyl and aryl radicals in keratinocytes incubated with cumene hydroperoxide. The present study investigated *in vivo* free radical generation in lipid extracts of mouse skin exposed to cumene hydroperoxide. The electron spin resonance (ESR) spin-trapping technique was used to detect the formation of *alfa*-phenyl-*N*-*tert*-butylnitrone (PBN) radical adducts, following intradermal injection of 180 mg/kg PBN. It was found that 30 min after topical exposure, cumene hydroperoxide (12mmole/kg) induced free radical generation in the skin of female Balb/C mice (13-14 weeks old) kept for 10 weeks on vitamin E deficient diets. In contrast, no radical adducts were detected when cumene hydroperoxide was applied to the skin of mice fed a vitamin E sufficient diet. Importantly, levels of GSH and vitamin E in the skin of vitamin E deficient mice decreased 30% and 80%, respectively, compared to vitamin E sufficient controls. PBN adducts detected by ESR in vitamin E deficient mice provide direct evidence for *in vivo* free radical generation in the skin after exposure to cumene hydroperoxide.

236A THE ARYL HYDROCARBON RECEPTOR LIGANDS MODULATE MCF-10F BREAST EPITHELIAL CELL GROWTH.

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor implicated in the growth regulation of many cell types. Previous data have demonstrated that AhR mRNA levels correlated with the malignant state in human breast cancer cell lines. In addition, AhR protein expression correlated with the rate of cell growth. Analysis of AhR protein levels in MCF-10F breast epithelial cells determined that AhR protein expression decreased to undetectable levels when cells were grown in serum free media. However, when cells were rescued with media containing 5% serum, AhR protein expression increased in 6 hours. AhR ligands have been implicated in suppression of cell cycle. In order to determine if AhR can modulate cell growth, three AhR ligands (galangin, alpha-naphthoflavone, and indol-3-carbinol) were used. These compounds have been shown to be both AhR agonists and antagonists depending on concentration used. MCF-10F breast epithelial cells grown in media containing these compounds showed a decrease in both thymidine incorporation and cell number in a dose dependent manner. To determine if this decrease was due to cell cycle arrest, G0/G1 synchronized cells were grown in media containing serum and/or 10-5 to 10-6 M of AhR ligands for 24 hours. PI staining of MCF-10F cells grown with these inhibitors demonstrated that 60-80% of cells were arrested in G0/G1 ($p<0.05$) and that these cells did not progress into S-phase. These data demonstrate that the AhR can modulate malignant cell growth by suppressing cell cycle progression.

236B IDENTIFICATION OF A MEDIATOR OF THE CYTOSTATIC EFFECTS OF 12-0-TETRADECANOYLPHORBOL-13-ACETATE IN HUMAN BREAST MYOEPITHELIAL CELLS.

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The effects of 12-0-tetradecanoylphorbol-13-acetate (TPA) on cell cycle progression were examined in the human breast myoepithelial cell line MCF10A-Neo. Exposure of cultures to TPA induced a rapid but transient G_1 arrest (IC_{50}~0.5 nM). Cultures produced a conditioned media within one hr of TPA treatment that could totally account for the cytostatic effects of TPA. Conditioned media lost its cytostatic activity within 16-20 hr of TPA treatment. However, a cytostatic conditioned media was quickly generated by refeeding previously treated TPA cultures with new media. Removal of latent TGFβ from culture media, or supplementation of culture media with soluble $TGF\beta_{II}$ receptor, or preabsorption of conditioned media with an antibody to TGFβ, all reduced the cytostatic effects of TPA by ~50%. Cotreatment of cultures with the serine protease inhibitors aprotinin or plasminogen activator inhibitor 1 also suppressed (~50%) the cytostatic activity of TPA. Pretreatment with two cell permeable protein kinase C inhibitors (Ro-32-0432 and bisindolymaleimide) completely suppressed the cytostatic activity of TPA. In contrast, inhibitors of MEK (PD98059) and PI3 kinase (LY294002 and Wortmannin), two signaling pathways commonly activated by TPA, were without effect. These studies suggest that in MCF10A-Neo cells TPA activates protein kinase C-dependent processes which lead to the proteolytic activation of latent TGFβ being supplied by the serum in the culture media. However, TPA also stimulates the production of an additional cytostatic factor(s) which signals through a mechanism not involving the $TGF\beta_{II}$ receptor. (Supported by NIH Grant CA34469.)

236C EFFECTS OF DIETARY GENISTEIN ON UTERINE CELL CYCLE IN RATS: ANALYSIS BY FLOW CYTOMETRY.

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The phytoestrogen genistein is a naturally occurring isoflavone consumed by humans mainly through the use of soy products. Genistein has been identified as an endocrine disrupting chemical affecting the growth and development of the reproductive tract and mammary glands in rats. This study is to evaluate the effects of dietary genistein on uterine tissue by flow cytometric cell cycle analysis. Genistein was fed in a soy-free casein-containing diet at 0, 25, 250, or 1250 ppm to F_0 dams beginning on gestation day (GD) 7 and continued to the weaning of F_1 pups. Exposure of F_0 dams to genistein continued until sacrifice at PND 120 or 154. Exposure of F_1 pups to genistein continued via lactation and after weaning by feed until sacrifice at PND 21, 25, 63, or 77. Uterine tissue was collected, preserved in citrate-sucrose-DMSO buffer, and frozen at -80°C until assayed using flow cytometry. The percentage (%) of cells in each cell cycle phase of the cell cycle was measured and analyzed by ModFit program. The results showed that 1250 ppm genistein did not cause cell cycle changes in uterus of the F_0 dams sacrificed at either ages. In the genistein-treated F_1 pups, no alterations of uterine cell cycle were observed at PND 77. However, in the PND 21 F_1 pups, significant decreases in the % of S- and G_0-cells were detected in the 25 and 1250 ppm groups, respectively. A significant increase in the S-cell was found in the 1250 ppm group of the PND 21 pups. In the PND 25 pups, exposure of dietary genistein at 1250 ppm caused an increase in the % of cells in both S- and G_2M-phases. At the same dose, the % of G_0-cells was significantly decreased. At PND 63, 1250 ppm genistein lowered the % of S-cells. However, a significant increase in the G_2M-cell was detected at all dosages evaluated. The results indicated that dietary exposure of genistein caused alterations in cell cycle in uterine tissue of the developing rats. Immature animals were found to be more sensitive to induction of changes in % S-cells inferring that the uterotrophic effect of genistein is more sensitive and detectable in early developmental age. The G_2M-arrest may occur after 3 weeks of age and appears obviously at PND 63. The effects may be age-dependent; younger animals may be more susceptible to the damage exerted by the compound.

236D INITIAL DEVELOPMENT OF A MULTISTAGE CANCER MODEL BASED ON SYRIAN HAMSTER EMBRYO (SHE) CELL TRANSFORMATION STUDIES.

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To better incorporate biologic information into quantitative cancer modeling, the two-stage MVK model has been modified for use with SHE cell neoplastic progression. Five phenotypic stages are included in this model: normal cells could either become senescent or mutate into immortal cells followed by anchorage-independent growth and tumorigenic stages. Cells in each stage have distinct division, death and mutation rates, and mutation is assumed to occur during cell division. Model development and related experiments are focused on studying the abilities of lead, arsenic, chromium and a mixture of these three metals to induce progression of SHE cells from one phenotype to the next. Cell division and death rates have been assessed using flow cytometric analysis for inclusion in the model. Cell division rates were measured using bromodeoxyuridine (BrdU) incorporation with propidium iodide staining, which allows for the calculation of potential doubling time, a measure of cell cycle time that takes growth fraction, but not cell loss, into account. Potential doubling times of normal SHE cells ranged from 12 to 59 hours, depending on the degree of confluence of cell cultures. Cell death was measured by a flow cytometry method based on propidium iodide staining specifically related to membrane damage. The individual metals and their mixture did not induce immortalization or further mutations of SHE cells in our laboratory. However, the growth of SHE cells were inhibited by 5.4 μM of arsenic and became senescent after only 16 population doublings, whereas normal cells and cells exposed to lower arsenic concentrations lasted for at least 30 population doublings. The model developed in our laboratory was proven to predict the growth of normal cells successfully. (This work was supported by ATSDR [Cooperative Agreement U61/ATU 881475] and NIEHS Superfund Basic Research Program [P42 ES05949].)

236E TRICHOSTATIN AFFECTS CELL PROLIFERATION AND DETOXIFICATION ENZYME ACTIVITY COMPARABLE TO THE DIFFERENTIATING AGENT BUTYRATE IN HUMAN HT29 COLON CARCINOMA CELLS.

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Chemoprevention of carcinogenic processes involve a variety of mechanisms. Previously, we have shown that compounds such as benzyl isothiocyanate (BIT), found in cruciferous vegetables and butyric acid, released from dietary fiber fermentation act to increase detoxification enzyme activity and decrease cell proliferation. Differentiation of the human colon adenocarcinoma cell line HT29 with sodium butyrate (NaB) results in increased activity of glutathione S-transferase (GST) and NAD(P)H:quinone reductase (N:QR), with a further increase in enzyme activities in response to 25 μM BIT. One proposed mechanism for the differentiating effect of NaB is inhibition of histone

deacetylase. We compared the effect of trichostatin, a histone deacetylase inhibitor, to that of NaB. HT29 cell proliferation, measured by [^{3}H]-thymidine incorporation, was dose-dependently decreased by trichostatin (TC) (0.25 - 2.0 μmol/l) and sodium butyrate (NaB) (1 - 5 mmol/l). Cell proliferation decreased by at least 90% with a 24 h exposure to 0.25 μM TC or 5 mM NaB. Both NaB and TC increased GST and NQR activities in response to BIT. NaB and TC, due to their affect on histone acetylation, may increase the susceptibility to enzyme inducers acting on promoter regions exposed by decreased contact between histone proteins and DNA. (Support: GM-08248, ES-09047.)

236F HEPATOMA CELL LINES FROM HUMAN, MOUSE AND RAT SHOW SIMILAR CELL CYCLE RESPONSES WHEN INCUBATED WITH CRUCIFEROUS VEGETABLE-DERIVED CRAMBENE.

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Cruciferous vegetables have cancer preventive effects in both animals and humans. These effects are believed to be due mainly to glucosinolates in crucifers. The glucosinolate breakdown product, crambene, is naturally produced when endogenous myrosinase hydrolyses the glucosinolate progoitrin. We earlier showed that crambene (1-cyano-2-hydroxy-3-butene) induces liver detoxification enzymes. Here we evaluated the effect of crambene (5mM) on the cell cycle in hepatoma cell lines from three different species: mouse (Hepa 1c1c7 cells), rat (H4IIEC3 cells) and human (HepG2 cells). By 24 hours, crambene caused cell cycle arrest in the G2-phase in all three cell lines. Cell viability was unaffected (>95%). The G2-population increased by 2-fold (HepG2), 2-4-fold (Hepa 1c1c7) or 1.5-2-fold (H4IIEC3) compared with control (0.1% DMSO). These findings indicate that cruciferous vegetables may protect after initiation of cancer by slowing the cell cycle, in addition to preventing initiation of carcinogenesis. (Supported by The Illinois council on Food & Agricultural Research.)

237 CELL-CYCLING EFFECTS FROM *IN VITRO* EXPOSURE TO SODIUM ARSENITE ON DEVELOPING RAT MIDBRAIN CELLS.

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Arsenic and its metabolites distribute to all organs in the body, and can pass through the placental barrier as well as the blood brain barrier, which is not fully developed in the fetus. High-dose exposure to arsenic during development has resulted in neural tube defects, exencephaly, and other defects, while molecular mechanisms of lower-dose effects are undefined. In this study, gestation day 12 primary rat midbrain neuroepithelial cells were exposed to sodium arsenite (0, 2, 2.5, 4, and 5 μM, 0-48 hr) *in vitro*. Cell-cycling effects were determined by continuous BrdU labeling and bivariate flow cytometric Hoechst-ethidium bromide analysis. We observed a time- and concentration-dependent inhibition of cell proliferation in both G1 and G2/M as early as 12 hours after sodium arsenite exposure at the lowest concentration tested (2 μM), with no associated cytotoxicity at 24 hours as shown by the neutral red assay. Because of the observed G1- and G2/M-phase effects, fewer cells entered DNA synthesis or completing one round of cell division, respectively. The magnitude of this effect increased with increasing arsenic concentration. Although cell proliferation was inhibited, cell cycle progression was observed to occur under all exposure conditions. (Supported by Child Health Center Grant jointly funded by EPA and NIEHS.)

238 INVOLVEMENT OF P21$^{WAF1-CIP1}$ IN METHYLMERCURY-INDUCED CELL CYCLE INHIBITION.

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Methylmercury (MeHg) has been recognized to exert toxicity particularly to developing central nervous system (CNS). To evaluate the role of p21, a cell cycle protein involved in the G1 and G2 phase checkpoints, in the cell cycle inhibition induced by MeHg we have utilized primary mouse embryonic fibroblasts (MEFs) of different p21 genotypes (wild type, heterozygous and null). MEFs were isolated at day 14 of gestation and treated at passage 4-6 with 2, 4 and 6 μM MeHg for 24 hr. Changes in cell cycle distribution after continuous MeHg treatment were analyzed by DNA content-based flow cytometry using DAPI. MeHg induced an increase in proportion of cells in G2/M at 2 and 4 μM MeHg irrespective of p21 genotype (p*0.05). Effects of MeHg on cell cycle progression were also evaluated using BrdU- Hoechst flow cytometric analysis. Inhibition of cell cycling was observed in all p21 genotypes after continuous exposure to MeHg for 24 and 48 hrs. p21 null (-/-) cells reached the second-round G1 at a higher fraction compared to the wild type (+/+) and heterozygous (+/-) cells (p> *0.05). The present study confirms previous observations that MeHg inhibits cell cycle progression through delayed G2-M transition. Furthermore, the G2/M accumulation induced by MeHg is independent of p21. Further studies on other cell cycle regulation pathways will provide further understanding of the effects of MeHg on cell proliferation. (This study was supported by EPA grant numbers R825358, R826886, R825358, and NIEHS grant number 5 P30 ES07033.)

239 ELEVATIONS IN MRNA LEVELS FOR CYCLINS FOLLOWING TRIMETHYLTIN-INDUCED HIPPOCAMPAL NEURODEGENERATION.

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The interactions of glia and neurons during injury and subsequent neurodegeneration is a subject of interest in both disease and chemical-induced brain injury. One such model is the prototypical hippocampal toxicant trimethyltin (TMT). An acute injection of TMT (2.0 mg/kg, ip.) to postnatal day 20 CD-1 male mice produced neuronal necrosis and loss of dentate granule cells, astrocyte hypertrophy, and microglia activation in the hippocampus within 24 hours. While microglia show an early response to injury, their morphological progression to a phagocytic phenotype did not occur until 72 hours. The purpose of this study was to examine the role of microglia in the onset and progression of neurodegeneration and their differentiation to a phagocytic state. Based upon previous reports in the literature on the response of macrophages to injury, we examined both elevations in mRNA levels for pro-inflammatory cytokines and cell cycle control genes. TNFα, IL-1α, and IL-1β mRNA levels were significantly elevated in the hippocampus by 12 hrs. By 72 hrs, mRNA levels for cyclin A2 and cyclin B1 were elevated in the hippocampus by approximately 10-fold, decreasing to 2-fold at 10 days post-dosing. Immunohistochemistry showed a cellular localization of cyclins A2 and B1 to microglia in the region of neuronal necrosis. mRNA levels for cyclin C, cyclin D1, and cyclin D2 were elevated 2-fold by 72 hrs and remained elevated for 10 days. No changes were seen in cortex or cerebellar tissue from dosed animals. Immunohistochemistry for PCNA showed no evidence of cell proliferation, suggesting that the upregulation of these cell cycle genes is associated with cellular processes other than proliferation and may contribute to the differentiation of microglia to a phagocytic phenotype.

240 CELL CYCLE DEPENDENT NUCLEAR LOCALIZATION OF METALLOTHIONEIN IN 3T3-L1 FIBROBLAST.

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Previous studies on human tumor cells have shown that metallothionein (MT) is expressed mainly in proliferating cells, and its nuclear localization occurs during the S-phase of cell cycle. The role of nuclear MT in cell proliferation was studied in 3T3-L1 fibroblasts using inhibitors of cell cycle and phosphorylation. The changes in localization of MT were followed by immunofluorescence using a polyclonal MT-antibody and anti rabbit IgG labelled with FITC. In these cells, MT was localized in the nucleus at late G1/early S-phase after blocking of the cell growth with Aphidicolin (0.25μg/ml), an inhibitor of DNA polymerase-α cell cycle with Aphidicolin (0.25μg/ml) treatment leads to relocation of MT to the cytoplasm at the end of M-phase. Flow cytometry analysis confirmed that the cell cycle was arrested at S-phase for most of the cells after treatment with Aphidicolin (4μg/ml), and MT was retained primarily in the nucleus. Rapamycin, an immunosuppressant drug can block progression of cell cycle at the G1 phase by inhibiting activation of serine/threonine p70S6 kinase. Addition of Rapamycin, PD98059 (an inhibitor of MAP kinase) or LY294092 (an inhibitor of PI3 kinase) blocked the translocation of MT in the nucleus of 3T3-L1 fibroblast, suggesting that the nuclear translocation of MT is dependant on MAP-kinase and PI3-kinase activity. The results show that the localization of MT in the nucleus occurs during G1/S-phase of the cell cycle, and it is transferred to the cytoplasm during M-phase. (Supported Medical Research Council of Canada.)

241 C-MYC ANTISENSE PHOSPHORODIAMIDATE MORPHOLINO OLIGOMERS LIMIT RAT LIVER REGENERATION AND CYTOCHROME P450 3A ACTIVITY.

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Expression of c-myc protein is associated with cell proliferation. The present studies utilize antisense oligomers to inhibit c-myc expression in the regenerating rat liver following 70% partial hepatectomy (PH). Antisense phosphorodiamidate morpholino oligomers (PMO, novel DNA analogs) were administered intraperitoneally immediately after surgery to block expression of c-myc within the first 24 hours after PH. A 20-mer PMO complimentary to the c-myc mRNA at the translation start site was an effective sequence (AVI-4126: 5'-ACGTTGAGGGGCATCGTCGC-3'). A single intraperitoneal dose of 0.5 mg/kg AVI-4126 caused reduction of the regenerating liver c-myc protein in a sequence-specific and dose-dependent manner. Inhibition of c-myc expression resulted in reduction of proliferating cell nuclear antigen (PCNA) and arrested cells in the G0/G1 phase of the cell cycle. The ratio of G2:G0 cell populations in the regenerating liver twenty four hours after PH dropped from 29.1 in saline vehicle treated rats to 18.0 in rats treated with 2.5 mg/kg AVI-4126. Further, the expression of cell cycle checkpoint protein, p53 was inhibited with increasing doses of AVI-4126, but expression of p21(waf-1) was unaffected. The activity of cytochrome P-450 3A2 (CYP3A2) was evaluated by immunoblot analysis and erythromycin N-demethylation. AVI-4126 did not alter CYP3A activity in non hepatectamized animals but showed a dose dependent decrease in PH rats. We conclude that AVI-4126, antisense oligomer to c-myc, can reduce cell proliferation in the regenerating rat liver. Further, inhibition of c-myc may indirectly influence the expression of CYP3A.

242 EARLY INHIBITION OF HEPATOCYTE PROLIFERATION BY DICHLOROACETIC ACID (DCA) IN THE MALE B6C3F1 MOUSE AND F344 RAT.

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DCA occurs in finished drinking water as a by-product of the chlorine disinfection process and was found to induce hepatocellular cancer in rats and mice. Mice were exposed to 0, 0.5, 1, 2, and 3.5 g/l and rats were exposed to 0, 0.05, 0.5, and 1.5 g/l DCA. Animals were euthanized at 5, 10 (rats), 15 and 30 days. Hepatocyte proliferation was measured by BRDU (mice) or PCNA (rats) immunohistochemical methods. DCA at 2 and 3.5 g/l depressed the hepatocyte labeling index (LI) in mice at 5 and 15 days; 0.5 and 1 g/l DCA depressed the Li at 15 days ($p<0.05$). The inhibitory effect was lost by 30 days. The inhibitory effect was not as marked in rats, as in mice. Treatment with 0.5 and 1.5 g/l DCA depressed the LI at 10 days ($p<0.05$); 1.5 g/l depressed the 30 day LI ($p<0.05$). At no time did DCA stimulate hepatocyte proliferation in mice or rats. To further investigate the effect of DCA on DNA synthesis, primary hepatocytes isolated from 28 day old male B6C3F1 mice and F-344 rats were exposed *in vitro* to 10^{-3} to 10^{-11} M DCA. DCA inhibited [3H]-thymidine incorporation into the DNA of mice, but not rats. The differential ability of DCA in mice and rats to inhibit hepatocyte proliferation in cell culture may be important in establishing the relationship between inhibition of cell proliferation and hepatocarcinogenesis. The early dose-dependent inhibition of hepatocyte proliferation *in vivo* correlated with DCA induction of hepatocellular carcinogenesis in male mice and rats. *This is an abstract of a proposed presentation and does not reflect EPA policy.*

243 26-WEEK DERMAL AND DOSED-FEED STUDIES OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP) AND WY-14643 IN HOMOZYGOUS TG.AC TRANSGENIC MICE.

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DEHP and WY-14643 were used to study mechanisms of carcinogenesis in genetically altered mice. DEHP and WY-14643 were administered topically in acetone (DEHP-0, 100, 200, and 400 mg/kg; WY-14643-0, 2, 10, and 20 mg/kg) or in feed (DEHP-0, 1500, 3000, and 6000 ppm; WY-14643-0, 10, 50, and 100 ppm) to male and female homozygous Tg.AC mice for up to 28 weeks. The toxicity produced by topical and dosed-feed exposure to WY-14643 and dosed-feed exposure to DEHP (hepatomegaly, hepatocellular hypertrophy) were consistent with effects of peroxisome proliferating chemicals. Peroxisomal enzyme activity (β-oxidation and acyl CoA oxidase) and cell cycle biomarkers [proliferating cell nuclear antigen (PCNA) and cyclin-dependent kinase (CDK)] were included in these studies to assess oxidative effects and hyperplasia, respectively. Both administration routes of WY-14643 produced increases in β-oxidation and acyl CoA oxidase at all three dose levels and PCNA and CDK at the upper two dose levels. DEHP dosed-feed administration led to increases in peroxisomal enzyme activity (all three dose groups) and cell cycle biomarkers (high dose only). Topical administration of DEHP led to increases in β-oxidation and acyl CoA oxidase at the two highest dose groups but did not produce hepatocellular hyperplasia or any change in PCNA or CDK. Changes seen with DEHP were generally smaller in magnitude than those observed with WY-14643. Despite these increases, changes in enzymatic activity were not sensitive predictors of carcinogenic activity in this model as topical and dosed-feed exposure to DEHP and WY-14643 produced minimal to no treatment related proliferative lesions. In agreement with previous evaluations, the Tg.AC strain may not be responsive to non-genotoxic mouse liver carcinogens. (Supported by NIEHS Contract No. N01-ES-65406.)

244 DEXAMETHASONE SELECTIVELY INHIBITS WY-14,643 INDUCED CELL PROLIFERATION AND NOT PEROXISOME PROLIFERATION IN MICE.

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It has been proposed that NFκB activation may be involved in the hepatocellular proliferation induced by peroxisome proliferators. We compared the induction of peroxisome proliferation and cell proliferation in C57Bl/6 mice treated with 100 mg/kg/day WY14,643 in the presence or absence of increasing doses of dexamethasone (DEX), an inhibitor of NFκB activation. Biochemical markers of peroxisome proliferation, including fatty acyl-CoA oxidase activity, CYP4A content, and liver to body weight ratios were markedly increased in the WY14,643 treated mice. DEX co-administration, up to the maximum dose of 50 mg/kg/day, did not prevent the induction of these parameters. Acyl-CoA oxidase mRNA levels increased 5-fold with WY14,643 treatment and 15-fold with DEX co-administration at 5 mg/kg/day. ApoCIII mRNA levels were decreased by 50% in WY14,643 treated mice. DEX alone at 5 mg/kg/day increased the ApoCIII mRNA 4-fold, but WY14,643 co adminstartion also inhibited this induction by greater than 50%. In addition, immunohistochemical detection of peroxisomes with anti-PMP-70 antibodies demonstrated marked proliferation of hepatocellular peroxisomes in WY14,643 treated mice regardless of DEX treatment. In contrast, co-administration of DEX at 2 mg/kg/day partially inhibited the cell proliferation response (measured by BrdU incorporation or Ki-67 immunohistochemical detection). Moreover, DEX at doses of 5 mg/kg/day or higher completely inhibited the induction of cell proliferation and, at the higher doses, reduced the cell proliferation rate to levels below the vehicle treated control mice. Our studies clearly demonstrate that the hepatocellular proliferation induced by a peroxisome proliferator can be modulated independently of the other pleiotropic effects usually induced by these agents, suggesting an indirect mechanism of hyperplasia.

245 PEROXISOME PROLIFERATOR-INDUCED CELL PROLIFERATION USING A PRECISION-CUT RAT LIVER SLICE MODEL.

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Precision-cut rat liver slices were evaluated for the ability to detect *in vitro* cell proliferation induced by the peroxisome proliferator WY14,643. No induction of S-phase DNA synthesis as measured by [^{3}H]-thymidine incorporation into DNA was observed after WY14,643 treatment when the media contained as little as 5 nM dexamethasone. However, when dexamethasone was omitted from the culture media, WY14,643 induced S-phase DNA synthesis. The presence of dexamethasone did not prevent the induction of DNA synthesis in slices treated with EGF or TNFα. The absence of dexamethasone in the culture media did not affect measures of viability such as protein synthesis, ATP, LDH leakage, or glutathione levels. In contrast to hepatocyte monolayer cultures, marked induction of peroxisomal β-oxidation was achieved in the absence of dexamethasone. The induction of [^{3}H]-thymidine incorporation by WY14,643 was inhibited by hydroxyurea suggesting the induction was due to replicative DNA synthesis and not DNA repair. WY14,643 induced cell proliferation and peroxisomal β-oxidation in a dose-

dependent manner with maximal effect at 3 μM at 48 hours of treatment. Bromodeoxyuridine labeling and immunohistochemistry demonstrated that cell proliferation was observed mainly in the bile duct epithelial cells in untreated liver slices, suggesting that the baseline labeling in this model can be further reduced if bile duct epithelial cell proliferation can be specifically suppressed. These data suggest that after careful consideration and optimization of culture conditions precision-cut rat liver slices are an effective *in vitro* model to measure hepatocellular proliferation in response to peroxisome proliferators.

246 THE EFFECT OF A DOMINANT-NEGATIVE CONNEXIN43 ON GAP JUNCTIONAL COMMUNICATION AND DIFFERENTIATION OF SEVERAL MAMMALIAL PROGENITOR CELL LINES.

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Gap junctional intercellular communication (GJIC) has been associated with the regulation of cell proliferation, cell differentiation and synchronized electrotonic and metabolic functions of cells. To test the hypothesis that modulation of GJIC could affect differentiation of mouse osteoblast (MC3T3-E1), rat liver epithelial (WBF344), and human neural-glial (SVG) cell lines, we transfected these cells, which express normal Cx43 proteins, with either a plasmid containing the neo gene and the mutant Cx43 (Cx43delta, 7 residues deleted from the internal loop at positions 130-136). The wild type cells of each cell line, as well as those clones transfected with the control plasmid and the clones of each cell line transfected with Cx43delta were shown to have equivalent ability to transfer Lucifer yellow dye (MW=457), using the scrape loading/dye transfer technique to measure GJIC. The D/N Cx43 cell clones transferred Lucifer yellow iodoacetamide (MW=649) much slower (~50% less) than the control cells. The induction of differentiation in each cell type containing D/N Cx43 resulted in a large decrease in differentiated cells as compared to the wild-type controls. Phorbol ester blocked GJIC in all clones equally well. However, recovery of GJIC in the D/N Cx43 clones was significantly inhibited compared to the control cells. Results suggest a significant role of GJIC in the differentiation of these three progenitor cells. (Research supported by a grant from NCI [CA21104].)

247 PHENOLIC COMPONENTS OF CIGARETTE TAR INHIBIT DNA SYNTHESIS BY QUENCHING THE TYROSYL RADICAL IN RIBONUCLEOTIDE REDUCTASE.

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Cigarette smoke is known to induce profound suppression of T cell responses in the lungs. We have found that the two major phenolic components of cigarette tar, hydroquinone (HQ) and catechol, block DNA synthesis in human T lymphocytes. The purpose of these experiments was to measure the effects of cigarette tar and its phenolic components on the tyrosyl radical in the M2 subunit of ribonucleotide reductase, a major rate-limiting enzyme in DNA synthesis. This radical is essential for enzyme activity and is the only stable tyrosyl radical in sufficient concentration to be detectable by electron paramagnetic resonance (EPR) in lymphocytes. The human Jurkat T cell line was cultured at 10^6 cells/ml in RPMI1640 + 10% FBS and treated for 5 min with cigarette tar extract, or 40μM HQ, catechol or phenol. Cigarette tar extracts were prepared by 'smoking' a single cigarette into 10 ml of medium via a vacuum pump at 125 ml/min. The cells were then centrifuged into quartz EPR tubes and frozen in liquid nitrogen. EPR spectra were obtained on a Varian E9 spectrometer with a TE_{102} resonator at 93K, 150mW and a frequency of 9.10 GHz (X-band). To improve spectra resolution, some samples also were analyzed on a Bruker E580 at 50K, 128mW. The tyrosyl radical was detected in Jurkat cells with an absolute signal intensity of 0.2 μM. Extracts from a low-tar cigarette (*Carlton*) quenched the M2 tyrosyl radical by <40%, while treatment with high-tar extracts (unfiltered *Camel*) resulted in >95% quenching. HQ and catechol quenched the tyrosyl radical by >99%, but phenol quenched only 25% of the radical. Nicotine (1mM) had no effect. The results indicate that the benzene derivatives, HQ and catechol, block DNA synthesis by quenching the M2 tyrosyl radical and thereby inhibiting ribonucleotide reductase. (Supported by NIH grants HL60538 and ES05673.)

248 EVALUATION OF THE UTILITY OF ACCELERATED INFUSIONS FOR THE DETERMINATION OF PHARMACOKINETIC LINEARITY.

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Accelerated infusions are useful in the investigation of pharmacokinetic linearity. Little information, however, exists to validate this technique or to demonstrate its limitations. This investigation was performed (1) to determine whether accelerated infusion regimens reliably estimate the range of pharmacokinetic linearity for molecules of varying pharmacokinetic properties, (2) to evaluate the ability of accelerated infusions to identify pharmacokinetic nonlinearity, and (3) to compare results from the accelerated infusion technique to experimental values obtained independently with specific compounds. Simulations using accelerated infusions as the input function resulted in the anticipated profiles that contained an initial lag phase before reaching a linear slope. This lag phase increased with increasing half-life, and in some instances was sufficiently great to obscure the linear portion of the profile. These simulations also revealed that clearance estimated from the apparently linear portion of the concentration-time profile can be substantially overestimated (up to 2000% for the simulation conditions employed here) for compounds with long half-lives. Simulations of structured nonlinearity produced the predicted profiles for compounds with short to moderate half-lives, while demonstrating that compounds with longer half-lives produce acceleration profiles that do not allow distinction between linear and nonlinear pharmacokinetics. Finally, experiments employing accelerated infusions with 3 test compounds produced clearance estimates within 30% of those determined by independent means under linear conditions, and demonstrated the clear identification of nonlinearity for one compound. The data presented in this investigation indicate that accelerated infusions may be used to determine pharmacokinetic linearity for compounds within certain pharmacokinetic boundaries (i.e., for which half-life is sufficiently small), but that caution is required in the interpretation of such studies.

249 COMPARATIVE TOXICOKINETIC PROFILE OF HIGH AND LOW DOSE PROPANIL USING ACCELERATED MASS SPECTROSCOPY.

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Toxicokinetic measurements using radiolabeled compounds are frequently hampered by lack of sensitive methods to measure low (environmentally relevant) levels of xenobiotics. This limitation can be overcome by using accelerator mass spectrometry (AMS). AMS can routinely measure low abundance ^{14}C accurately and reproducibly at 1 part in 10e12 to 10e15. This project was designed to measure the distribution and elimination of the aniline herbicide, propanil. Propanil was custom synthesized substituting ^{14}C for ^{12}C in the ring structure (Sp. Activity 12.3 mCi/mmol [Moravek Biochemicals]). C57Bl/6 mice were injected intraperitoneally with either a low dose of 0.88 ng/g ^{14}C-propanil or a high dose of 2 mg/g "cold" propanil-spiked with 0.88 ng/g 14 C-propanil dissolved in peanut oil. Starting 1 minute after injection the blood, spleens, and livers were removed from 3 animals per time point and flash frozen for shipment to the Center for AMS at LLNL. Additional samples were taken at 10, 20, 40, 80, 120, 240, 360, and 720 minutes after injection. The data profiles from the two dosing schemes differed substantially. Blood and tissue samples from animals receiving the low dose showed a sharp peak in activity at approximately 10 minutes after injection and activity dropped to a very low level by 40-80 minutes after injection. In contrast, samples from animals given the high concentration of propanil did not peak in activity until approximately 40 minutes and showed a substantial plateau of activity throughout the time course of the experiment. These differences may reflect saturation of the tissues by the high dose. Development of PBTK models of these data is continuing. (Supported by NIH grant, ES07512.)

250 THE DISTRIBUTION OF PULEGONE IN B6C3F1 MICE AND F-344 RATS.

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Pulegone is a monoterpene ketone found in the essential oils from many mint species. It is the major constituent of pennyroyal oil (*Mentha pulegium*, *Hedeoma pulegoides*, and others). It is used as a flavoring and fragrance and as an herbal to induce menstruation and abortion. Ingestion of pennyroyal oil

has caused human poisonings resulting in CNS toxicity, gastritis, hepatic and renal failure, pulmonary toxicity, and death. It has been nominated to the National Toxicology Program for toxicity and carcinogenicity studies. A distribution study of 14C-pulegone in B6C3F1 mice and F344 rats conducted to aid in the design of the NTP study has determined that pulegone is rapidly absorbed and excreted in urine and feces in both species. At doses from 0.8 to 80 mg/kg, male mice excrete about 73 to 85% in urine and 15 to 24% in feces in 24 hr. Rats take longer to excrete an oral dose, eliminating 44% in urine and 15 to 23% in feces in 24 hr. Tissue to blood ratios in male mice are highest in the liver and kidney (less than 9 at 2 hours) and in the gastrointestinal tract (up to 21 at 2 hr depending on tissue). These ratios drop at 24 hr to below 3 in the liver and kidney and to less than 2 in the G.I. tract. At 24 hr 0.6 to 0.9% of the radioactivity remains in the liver, a target tissue for pulegone toxicity. This data suggests that even though the majority of the pulegone is rapidly excreted, there may be bioaccumulation of pulegone-derived material in liver following multiple doses.

251 PRELIMINARY TOXICOKINETIC STUDIES OF METHYLEUGENOL (MEG) IN FEMALE FISCHER 344/N RATS AND B6C3F1 MICE.

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The National Toxicology Program is conducting toxicokinetic studies of methyleugenol (MEG) to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. The primary purpose of this study was to determine plasma concentrations of MEG and to estimate toxicokinetic parameters to assist in the design of a definitive study. MEG disposition was assessed in female rats and mice dosed intravenously (37 mg/kg in ethanol:emulphor:water, 1:1:8) or by gavage (37 or 150 mg/kg in corn oil). Plasma concentration-versus-time profiles after IV dosing revealed that elimination of MEG was bi-phasic. Estimates of the terminal half-life, Cltot and AUC were 1.9 hr, 3100 mL/hr-kg and 12 µg-hr/mL for elimination of MEG from rat plasma. Estimates of the terminal half-life, Cltot and AUC were 1.3 hr, 13000 mL/hr-kg and 2.8 µg-hr/mL for elimination of MEG from mouse plasma. Vss for MEG in rat and mouse plasma were 1500 and 6100 mL/kg, respectively. Plasma concentration-versus-time profiles for animals dosed by gavage were characterized by an early absorption phase (~15-30 min post-dosing) followed by at least one secondary peak. These secondary peaks in the profiles were attributed to the use of corn oil as an administration vehicle and occurred at earlier time points in the higher dose groups. Bioavailability was 2.6% for rats and 2.4% for mice. Saturation of MEG metabolism occurred in gavaged animals as revealed by non-linear (dose-dependent) toxicokinetic behavior. Additionally, based on these results a GC/MS method ranging from 0.005 to 32 µg MEG/mL plasma was validated for use in the definitive MEG toxicokinetic study. (This work was supported by the NTP Chemistry Support Services Contract, N01-ES-55395.)

252 PRELIMINARY TOXICOKINETIC STUDIES OF ISOEUGENOL (IEG) IN FEMALE FISCHER 344/N RATS AND B6C3F1 MICE.

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The National Toxicology Program is conducting toxicokinetic studies of isoeugenol (IEG) to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. The primary purpose of this study was to determine plasma concentrations of IEG and to estimate toxicokinetic parameters to assist in the design of a definitive study. IEG disposition was assessed in female rats and mice dosed intravenously (37 mg/kg in ethanol:emulphor:water, 1:1:8) or by gavage (37 or 150 mg/kg in corn oil). Plasma concentration-versus-time profiles after IV dosing revealed that elimination of IEG was bi-phasic. Estimates of the terminal half-life, Cltot and AUC were 2.4 hr, 4900 mL/hr-kg and 7.6 µg-hr/mL for elimination of IEG from rat plasma. Estimates of the terminal half-life, Cltot and AUC were 3.3 hr, 4000 mL/hr-kg and 9.2 µg-hr/mL for elimination of IEG from mouse plasma. Vss for IEG in rat and mouse plasma were 3910 and 2000 mL/kg, respectively. Plasma concentration-versus-time profiles for animals dosed by gavage were characterized by an early absorption phase occurring at ~15-20 min post-dosing followed by at least one secondary peak. These secondary peaks in the profiles were attributed to the use of corn oil as an administration vehicle and occurred at earlier time points in the higher dose groups. Bioavailability was ~20% for rats and mice. There was no evidence for saturation of IEG metabolism in gavaged animals. Additionally, based on these results a GC/MS method ranging from 0.015 to 32 µg IEG/mL plasma was validated for use in the definitive IEG toxicokinetic study. (This work was supported by the NTP Chemistry Support Services Contract, N01-ES-55395.)

253 PHARMACOKINETIC DATA FROM INTRAVENOUS HIGH DOSE IBUPROFEN IN ANESTHETIZED WEANLING SWINE.

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A weanling swine model has been developed to assess the efficacy of treatments for phosgene (CG) intoxication. CG is a choking agent used during World War I and an industrial hazard today. CG produces pulmonary edema (PE) after an asymptomatic period of up to 24 hr. Pharmacokinetic data on ibuprofen (IBU) were collected using eleven pigs (sus scrofus, ~20 kg) that were initially anesthetized with isoflurane and catheters placed into a femoral artery and vein. Once catheterized, the anesthetized state was maintained by slow infusion (~3 mL/hr) of pentobarbital sodium (65 mg/mL) and ~45 mL/hr of Lactated Ringer's solution for 72 hr. Five ventilated, anesthetized test animals were exposed for 10 min to air, and 30 min later treated iv with a loading dose of 45 mg IBU/kg over 30 min. This was followed by a 22.5 mg IBU/kg maintenance dose over 30 min every two hours for the initial 24 hr. The remaining six anesthetized test animals were exposed for 10 min to a target concentration of 450 mg CG/m3 and received the same IBU treatment regimen. Heparinized blood samples were drawn from each animal every 30 min over the initial 26 hr and every 4 hr for 72 hr. Whole blood IBU concentration was determined using High Performance Liquid Chromatography (HPLC). Selected blood gases and electrolytes were analyzed using I-Stat System. The resultant data indicate that the elimination curve for IBU in anesthetized swine is rapid and similar to that reported for man. Similar IBU elimination curves were observed for air and CG-exposed animals, suggesting that CG exposure did not significantly affect IBU metabolism. IBU appears toxic at a total dose of ~6,300 mg when administered as described above. The anesthesia regimen used may alter liver metabolism of swine, increasing energy requirements and possibly IBU metabolism. When a high iv dose of IBU is used as treatment, electrolytes and glucose levels should be monitored. (Supported by DAMD 17-89-C-9050.)

254 BROMODICHLOROMETHANE AND DICHLOROACETIC ACID PLASMA PROFILES FOLLOWING DOSED-WATER EXPOSURE USING FVB/N MICE: PRELIMINARY TIME POINT COLLECTION STUDIES.

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Preliminary studies for bromodichloromethane (BDCM) and dichloroacetic acid (DCA) were performed to ascertain the feasibility of obtaining useful blood concentration data for toxicology studies being conducted by the National Toxicology Program (NTP) to evaluate water disinfectant by-products. Male and female FVB/N mice, 10 to 11 weeks old, were exposed to BDCM or DCA in drinking water (tap water vehicle) at concentrations of 175 or 700 mg/L (BDCM) and 500 or 2000 mg/L (DCA) for up to 9 days. Serial blood samples were collected over a 24-hr period on Days 3 (BDCM and DCA) and 8 (BDCM only). The plasma samples were analyzed for BDCM or DCA using gas chromatography methods, which utilized electron capture detection. The limit of quantitation (LOQ) values were 2.5 ng/mL for BDCM and 0.1 µg/mL for DCA. BDCM plasma concentrations for the 175 mg/L group (Days 3 and 8) or the 700 mg/L group (Day 3) were generally below the LOQ. On Day 8, the profile for the male 700 mg/L group had a C_{max} value of 4.4 ng/mL (T_{max} of 12:00 AM), whereas the female group's highest plasma concentration (2.3 ng/mL at 12:00 AM) was just below the LOQ. For DCA, plasma concentrations for the 500 mg/L group (Day 3) were generally below the LOQ. For the 2000 mg/L group, the C_{max} values were 0.96 µg/mL (males) and 2.1 µg/mL (females), which occurred at 1:00 AM (T_{max}). The males exhibited a second peak at 6:00 AM of 0.94 µg/mL. It was recommended that no blood samples be collected for the BDCM study based on these results. For the DCA study, times of 12 AM or 6 AM will provide an estimate of the peak plasma concentration after at least three days of exposure. (This work was supported by the NTP Chemistry Support Services Contract N01-ES-55395.)

255 DOSE-DEPENDENT TOXICOKINETICS OF MONOCHLOROACETIC ACID IN ADULT MALE SPRAGUE-DAWLEY RATS AFTER ORAL ADMINISTRATION.

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Male rats were administered a nontoxic 10 mg/kg and a toxic 225 mg/kg dose (~$1.D_{20}$) of ^{14}C-MCA (2.5-3.0 µCi/kg) in saline by gavage at 2 ml/kg. They were housed individually in metabolism cages. Urine and feces were collected separately. Animals were euthanized 15, 45 min, 2, 4, 8, 16, and 32 h after dosing and tissues collected by gross dissection. Radioactivity in tissue aliquots and excreta was determined by scintillation counting after digestion. At the nontoxic dose, the administered dose rapidly disappeared from the stomach (absorption + gastric emptying) leaving only 34 and 10% of dose in stomach at 15 and 45 min after administration, respectively. Within 45 min after gavage, 45% of the dose appeared in the small intestine and was rapidly absorbed leaving only about 3% of dose in the small intestine at 4 h. Concentration of MCA in plasma peaked at 0.1% of dose by 2 h and declined thereafter. Most tissue profiles of MCA paralleled that of plasma with notable exceptions (liver, heart, brain, thymus). At the toxic dose much of the dose remained in the stomach due to edema which lasted about 8 h and led to extremely slow release of MCA into the small intestine (5% of dose at 2 h being the highest value). Most of dose was absorbed through the stomach as indicated by >54 and 17% of dose present in stomach 4 and 16 h after gavaging, respectively. Tissue concentrations remained below those seen after the nontoxic dose. Peak plasma concentration was reached within 15 min without an apparent subsequent uptake phase. About 70-80% of MCA recovered from the small intestine was parent compound at both doses. Fecal excretion was negligible (<1.5%) after either dose. The nontoxic dose was rapidly excreted in urine; 43% within 4 h and >56% within 8 h. However, at the toxic dose, urinary excretion was slow, especially at early time points (9% at 4 h and 18% at 8 h). Urinary excretion accelerated after the 8 h time point. A total of 72 and 66% of the dose was recovered in urine within 32 h after the nontoxic and toxic dose, respectively. Oral administration of MCA did not cause any overt toxicity up to 200 mg/kg. Doses ≥450 mg/kg caused 100% mortality.

256 TISSUE DISTRIBUTION AND EXCRETION STUDIES OF EMIVIRINE IN MONKEYS AND RATS.

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Emivirine (EMV; Coactinon®; MKC-442) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) for treatment of HIV-1 infection that is currently in Phase III clinical development. Tissue distribution and excretion of [^{14}C]EMV-derived radioactivity were studied in cynomolgus monkeys (CM), and in Sprague Dawley (SD; non-pigmented) and Long-Evans (LE; pigmented) rats. CM were orally dosed with 180 mg/kg/day (90 mg/kg x 2) EMV for 13 days, then given a single oral dose of 90 mg/kg containing 63 µCi/kg [^{14}C]EMV. Blood, urine and feces were collected at several intervals up to 96 hr post-dose. Dosing with EMV continued for 6 days to steady state, after which a second [^{14}C]EMV dose was given. Tissues were collected at 1 hr post-dose. Rats were given a single oral dose of 50 mg/kg containing 88 µCi/kg [^{14}C]EMV and blood and tissues were collected at 5 (LE rats) or 6 (SD rats) post-dose intervals. Blood and tissue samples were analyzed for [^{14}C]EMV-derived radioactivity ([^{14}C]EMV-RA) by liquid scintillation counting. In CM, [^{14}C]EMV-RA peaked at 2 hr post-dose in plasma, with detectable levels remaining at 96 hr. Highest [^{14}C]EMV-RA levels were found in liver, bile, kidneys, and plasma. Mean total dose recovery was 70% in urine and 19% in feces, with 81% of the dose recovered by 48 hr. [^{14}C]EMV-RA distributed well into aqueous humor, brain, CSF, and testes. In SD rats, distribution of [^{14}C]EMV-RA to tissues was rapid, with maximum levels occurring at 0.5 hr post-dose. Levels then declined rapidly, but remained detectable in all 23 tissues studied for >24 hours post-dose. The highest levels were found in (decreasing order) stomach, small intestine, large intestine, liver, adrenal gland, and kidneys. Terminal elimination half-lives ranged from 4.2 hr (abdominal fat) to 27.8 hr (liver). Distribution of [^{14}C]EMV-RA was similar in SD and LE rat tissues, indicating that there was no association of EMV with melanin. In both species, EMV was rapidly absorbed following oral administration and EMV and its metabolites were widely distributed to all tissues examined.

257 COMPARATIVE DISTRIBUTION BETWEEN URINE AND BIOLOGICAL TISSUES IN MICE EXPOSED TO ARSENITE. DOSE-DEPENDENT EFFECT.

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The concentration of arsenicals in urine has been generally accepted as a good biological indicator of arsenic (As) exposure, because excretion via kidney is the major route for elimination of most As species. The efficiency of As methylation is often evaluated by the relative distribution of As species in the urine. However, urinary methylated As metabolites are excreted more quickly than inorganic arsenic (iAs), in consequence, relative amounts of As species in the urine might not reflect the methylation efficiency in the body. Comparative As distribution patterns between urine and biological tissues were made in adult female B6C3F1 mice exposed to a single oral dose of 0, 1, 10, 30 or 100 µmol/kg arsenite (AsIII). Concentrations of iAs, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were determined in urine collected during 6h postdosing and in blood, liver, lung, and kidney from mice sacrificed 6 h after AsIII exposure. Tissue distribution and concentration of arsenicals increased in a dose-dependent manner. The concentration of iAs and metabolites (%dose/g tissue) was greatest in the urine, followed by in descending order: liver, kidney, lung, and blood. The relative proportions of iAs and MMA were significantly higher in internal organs than in urine while the urinary relative proportion of DMA was higher than in biological tissues. At doses higher than 10µmol/kg AsIII, an increased percentage of iAs and decreased percentage of DMA were observed in biological tissues (kidney was the exception), which indicates that the metabolism of As was affected by the dose. With increased dose, urinary excretion of iAs and MMA increased and DMA decreased, suggesting inhibition or saturation of methylation of As. The urinary As metabolite profile was a sensitive indicator of the dose-effect alteration on As metabolism. (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.) (Supported in part by CR824915.)

258 TISSUE DISTRIBUTION OF ARSENITE (AsIII) AND ITS METHYLATED METABOLITES IN MICE.

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The relationship of exposure dose and tissue concentration of parent chemical and metabolites is a critical issue in cases where toxicity may be mediated by a metabolite or parent chemical and metabolite acting together. This has emerged as an issue for inorganic arsenic (IAs) because its principle methylated metabolite, dimethylarsinic acid (DMA), induces lung-specific DNA damage in multiple species, is a multiorgan tumor promotor in rats and mice, and a complete bladder carcinogen in rats. In this study, the time-course tissue distribution of IAs and its methylated metabolites was determined in blood, liver, lung and kidney of female B6C3F1 mice given a single oral dose of 0, 1, 10, 30 or 100 µmol/kg AsIII. Blood levels of IAs and its metabolites were uniformly lower across both dose levels and time points compared to other organs. IAs was the predominant form of arsenic (As) in liver at all doses at 6 hrs post dosing and at all times up to 24 hrs post dosing with 100 µmol/kg AsIII. DMA was the predominant form of As in kidney at all doses at 6 hrs post dosing and at all times from 2 to 24 hrs post dosing with 100 µmol/kg AsIII. Three- to 4-fold higher levels of IAs were achieved in liver compared to kidney, whereas the concentration of DMA occurring in kidney was and 2- to 3-fold higher compared to liver. Monomethyl-arsonic acid (MMA) was the least abundant form of As in all tissues, never exceeding more than 20% of the total As present. DMA was the predominant form of As in lung across all doses at 6 hrs post dosing and concentrations of DMA present were 3- to 4-fold higher than either IAs or DMA at doses of 10 µmol/kg and higher. The concentration of DMA in lung was significantly greater than other organs, except kidney, from 4 to 16 hrs post dosing with 100 µmol/kg AsIII. These data demonstrate distinct organ-specific differences in the distribution and methylation of IAs and its metabolites that will be important to consider when investigating mechanisms of arsenic-induced toxicity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

259 STEADY-STATE DISPOSITION OF ARSENATE IN THE MOUSE.

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Inorganic arsenic (iAs), a human carcinogen, is a common contaminant in air, food, soil, and water. Therefore, exposure to iAs occurs everyday. An unanswered toxicological question with iAs is the steady-steady tissue levels from continuous exposure to it. The objective of this study was to determine the steady-state disposition of arsenate after oral administration. Adult female B6C3F1 mice (N=10) were orally administered [^{73}As]-arsenate (0.5 mg As/kg) daily for nine days. Whole body [^{73}As] levels were determined before and immediately after dosing by whole body radioassay. Urine and feces were collected daily and assayed for [^{73}As]. Twenty-four hr after the last dose, all 10 animals were whole body radioassayed. Five mice were then killed and tissues were removed and analyzed for [^{73}As]. Whole body [^{73}As] levels were determined in the remaining mice for 9 more days. The mice were then killed and tissue levels of [^{73}As] were determined. Whole body retention of As 24 hr after the first dose was 2.2 μg; this increased to 7.1 μg 24 hr after the last dose. Nine days after the last dose, the levels of As decreased nonlinearly to 2.3 μg. [^{73}As] was excreted primarily in the urine, with approximately 76% of the daily dose was eliminated by this route. The daily dose excreted in feces was approximately 15%. Treatment did not affect urinary and fecal elimination of [^{73}As]. In mice killed 24 hr after the last dose, As tissue concentrations (μg As/gm tissue) were greatest in bladder (0.09), kidney (0.07), skin (0.07), and liver (0.05). Levels in blood, muscle, lung, and carcass ranged from 0.01-0.04 μg As/gm. Tissue concentrations of As decreased considerably in mice killed 9 days after the last dose. The concentrations were greatest in skin (0.04), bladder (0.01) and carcass (0.01). The other tissues had <0.01 μg As/gm. Accumulation of As in organs of the mouse correlates with the known human target organs for iAs-induced carcinogenicity. (Supported in part by CR824915.) (This abstract does not necessarily reflect EPA policy.)

260 METABOLISM AND PHARMACOKINETICS OF RODA IN ALBINO AND PIGMENTED RATS.

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1, 3-bis(4-aminophenoxy) benzene (RODA) is an aromatic ether diamine structurally similar to certain other aromatic diamines known to produce ocular lesions. Following a single oral administration to male and female Crl:CD®BR albino and male HsdBlu:LE pigmented rats, plasma kinetics of parent RODA for both sexes and strains were best described by an open one-compartment model ($t_{1/2}$ = 2-11 h) while total radioactivity in plasma was best described by an open two-compartment model ($t_{1/2}$ = 39-61 h); clearance of ^{14}C-residues associated with red blood cells was prolonged ($t_{1/2}$= 76-142 h). RODA was biotransformed primarily to *N,N'*-diacetylated monohydroxylated-RODA and excreted in the feces (83-93%). Urinary excretion was a minor elimination pathway for RODA metabolites (3.5 7.5%) which were excreted as sulfoconjugates of 4-acetamidophenol, 3-phenoxy-4-acetamidophenol, and *N,N'*-diacetylated-monohydroxylated RODA. At terminal sacrifice (120 h), most tissue ^{14}C-residue levels were comparable between albino and pigmented rats except for the amount in the skin and eyes. Samples of skin and whole eyes from pigmented rats contained ~3-fold higher ^{14}C-residues than albino rats at an equivalent dose. Histoautoradiography of eyes established that a greater abundance of radioactivity was sequestered within the choroid region of eyes from pigmented rats as evidenced by increased gain density in the pigmented uvea, and pigmented rats exhibited less severe retinotoxicity than albino rats. These data suggest that pigment may bind RODA or a metabolite, thereby affording some degree of protection from retinal injury.

261 DISTRIBUTION OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN THE RAT AFTER LOW-DOSE, CHRONIC ADMINISTRATION.

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The tissue distribution of 2,4-D was examined in young adult rats after chronic dosing. Male Fisher-344 rats, approximately 12 weeks of age, were lightly anesthetized with isoflurane and kept at surgical anesthesia with 1.8% isoflurane/oxygen. Alzet osmotic pumps (Alza Corp., model 2ML) filled with [14C]2,4-D (12.5 uCi/kg/day) and unlabelled sodium 2,4-D (1 or 10 mg/kg/day) were implanted subcutaneously between the scapulae. The incision was closed with wound clips and the rats were returned to their cages. After 7, 14 or 27 days of 2,4-D infusion, the rats were sacrificed (n=7/dose/group) and blood, peripheral tissues and brain regions were collected. Tissues were analyzed for radioactivity by liquid scintillation spectrometry with automatic quench correction, and the concentration of 2,4-D was calculated from the known specific activity of the radioisotope in the infusate. The highest concentration of 2,4-D was observed in the kidney. This was consistent with both dosing groups (1 and 10 mg/kg/day) over time. The plasma levels of 2,4-D indicated that steady-state concentrations were reached between 7 and 14 days of dosing. Although the 2,4-D level in the kidney did not change over time, the levels in other peripheral tissues and all brain regions examined increased between 7 and 27 days, with brain concentrations of 2,4-D increasing dramatically between 14 and 27 days. These data suggest that 2,4-D levels accumulate in tissues over time with chronic dosing, and the primary route of elimination (kidney) becomes quickly saturated. These data will be used to validate the previously developed physiologically-based pharmacokinetic (PBPK) model for a single, high-dose of 2,4-D, for chronic exposure to low-doses of 2,4-D.

262 BIOTRANSFORMATION AND KINETICS OF EXCRETION OF *TERT.*-AMYL METHYL ETHER IN RATS AND HUMANS.

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Tert.-Amyl methyl ether (TAME) may be widely used as an additive to gasoline in the future. Therefore, widespread human exposure to TAME may occur. To contribute to the characterisation of potential adverse effects of TAME, its biotransformation was compared in humans and rats after inhalation exposure. Human volunteers (3 males and 3 females) and rats (5 males and 5 females) were exposed to 4 (3.8±0.2) and 40 (38.4±1.7) ppm TAME for 4 hours in a dynamic exposure system. Urine samples were collected for 72 h in 6 h intervals and blood samples were taken in regular intervals for 48 h in humans. In urine, the TAME-metabolites tert.-amyl alcohol, 2-methyl-2,3-butane diol, 2-hydroxy-2-methylbutyric acid and 3-hydroxy-3-methylbutyric acid were quantified, TAME and tert.-amyl alcohol were determined in blood samples. After the end of the exposure period, blood concentrations of TAME were 4.4±1.7 μM in humans and 9.6±1.4 μM in rats after 40 ppm TAME and 0.6±0.1 μM in humans and 1.4±0.8 μM in rats after 4 ppm. TAME was rapidly cleared from blood in both rats and humans. The blood concentrations of tert.-amyl alcohol were 9.2±1.8 μM in humans and 8.1±1.5 μM in rats after 40 ppm TAME and 1.0 ± 0.3 μM in humans and 1.8±0.2 μM in rats after 4 ppm TAME. Tert.-amyl alcohol was also rapidly cleared from blood. In urine of humans, 2-methyl-2,3-butane diol, 2-hydroxy-2-methylbutyric acid and 3-hydroxy-3-methylbutyric acid were recovered as major excretory products in urine. In rats, 2-methyl-2,3-butane diol and its glucuronide were major TAME-metabolites. tert.-Amyl alcohol and its glucuronide were minor TAME-metabolites in both species. All metabolites of TAME excreted with urine in rats were rapidly eliminated. Metabolite excretion in humans was slower. The obtained data indicate differences in TAME-biotransformation and excretion between rats and humans. In rats, TAME-metabolites are rapidly excreted. In humans, metabolic pathways are different and metabolite excretion is slower. (Supported by the Health Effects Institute, agreement No. 96-3.)

263 THE EFFECT OF CO-EXPOSURE TO GASOLINE VAPOR ON THE TOXICOKINETICS OF METHYL-*TERT.* BUTYL ETHER.

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The purpose of these studies was to determine the effects of coinhalation of the volatile fraction of unleaded gasoline (LFG) on MTBE toxicokinetics. Groups of male F344/Crl rats were acutely exposed nose-only for 4 hr to 4, 40, or 400 ppm ^{14}C-MTBE and to 20 and 200 ppm LFG containing 4 and 40 ppm ^{14}C-MTBE, respectively. To evaluate the effects of repeated inhalation of LFG on MTBE toxicokinetics, rats were exposed for 7 d to 20 and 200 ppm LFG followed on the 8th day by a similar exposure to LFG containing ^{14}C-MTBE. Endpoints included respiratory parameters, initial body burdens, rates and routes of excretion, and tissue distribution and clearance. MTBE and its metabolite, tertiary butyl alcohol (TBA), were quantitated in blood, and 2-hydroxyisobutyric acid (IBA) and 2-methyl-1,2-propanediol (2MePD) quantified in urine. The rate of MTBE uptake (μmole/kg body weight/hr) was lin-

ear over the 4-400 ppm MTBE. Acute and repeated inhalation of 200 ppm LFG significantly decreased the rate of MTBE uptake. Exposure to the higher MTBE concentrations and to LFG altered the distribution of MTBE equivalents immediately after the exposure. Repeated inhalation of 200 ppm, but not 20 ppm, LFG increased the rate of clearance of MTBE equivalents from blood, lung, liver, kidney, and testes. Exhaled air and urine were the major routes of excretion of MTBE equivalents. Inhalation of 20 ppm LFG increased the relative percentages of MTBE equivalents excreted in exhaled air and decreased the percentage excreted in urine compared to that when 4 ppm MTBE was inhaled. Concentrations of TBA in blood were consistently higher than that of MTBE. TBA was cleared from blood more slowly than MTBE. TBA comprised 70-88 percent of the total urinary metabolite excretion. Results of our studies indicate that acute and repeated coexposure to 20 ppm LFG has similar effects on the toxicokinetics of MTBE compared to that when 4 ppm MTBE is inhaled alone. Repeated inhalation of 200 ppm LFG appears to increase the rate of clearance of MTBE compared to that occurring when 40 ppm MTBE was inhaled alone. This may be due to the induction of cytochrome P450s metabolizing MTBE and TBA. (Research funded under DOE Cooperative Agreement No. DE-FC04-96AL76406.)

264 UPTAKE AND ELIMINATION OF METHYL *TERT.* BUTYL ETHER (MTBE) AND *TERT.* BUTYL ALCOHOL (TBA) IN HUMAN SUBJECTS BY THE ORAL ROUTE OF EXPOSURE.

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The production of MTBE, about 3 billion gallons in 1998, for use as an oxygenating fuel additive, has resulted in contamination of the ground and drinking water supplies in various parts of the country by leaks, spillage, and atmospheric deposition. As a result there is a potential for wide-spread exposure to MTBE by the oral route. In an effort to examine the pharmacokinetics of MTBE and one of its metabolites, TBA, human subjects rapidly drank 6.7 microliters of MTBE in 250 ml(20mg/L) lemon-lime Gatorade after fasting for at least 4 hr. The male subjects were selected to have a body mass index of 22-24 and be within the age range of 18-35. Over a 24-hr period 14 blood samples were obtained and analyzed for MTBE and TBA by a purge and trap technique coupled with GC/MS. The results show that MTBE was absorbed into the blood, but not as rapidly as via inhalation, and declined with an initial half life of about 1-3 hr. The peak levels of MTBE ranged from 5-15 ng/ml. Blood TBA rose more slowly, plateaued, and had a half life of about 13 hr. TBA exhibited peak blood concentrations of 15-20 ng/ml and were above baseline after 24 hr. Double exponential curves described the data well and had a correlation of greater than 0.95, implying that disposition was into two compartments. The AUCs for MTBE and TBA were ~1300-~1700 and ~13000-~17000 ng/ml*min, respectively. The residence time of TBA in the blood may be dependent on its water solubility or slow metabolism. These data demonstrated that MTBE was readily absorbed when administered orally and that TBA persisted with an extended half life. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

265 1,1,1,3,3,3-HEXACHLOROPROPANE: METABOLISM AND DISTRIBUTION IN MALE AND FEMALE SPRAGUE-DAWLEY RATS.

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Short-term inhalation exposure of rodents to 1,1,1,3,3,3-hexachloropropane (HCP) results in adverse effects in multiple organs. These studies were conducted to determine the metabolism and disposition of HCP. Male and female Sprague Dawley rats were exposed for 6 hr to 25 or 100 ppm [14C/13C]HCP by inhalation or administered ip 150 mg/kg [14C/13C]HCP. Expired air, and excreta (collected for up to 24 hr following exposure termination), and tissues (collected at 24 hr) were evaluated for radioactivity distribution. Urinary metabolites and expired volatiles were characterized using 13C NMR spectroscopy. Most HCP-derived radioactivity (49-69%) remained in the rats 24 hr following termination of exposures. HCP was eliminated via metabolism to 14CO2 (15-24 %), urinary metabolites (10-17%), fecal metabolites (1-3%), or expired as volatiles (3-9%). A slightly greater urinary and fecal elimination occurred for males compared with females. Following inhalation exposure, the recovered dose (per kg body weight) was greater in females as compared with males. The greater retained dose for females is largely attributed to a greater accumulation of radioactivity in body fat. Both sexes had a relative decrease in the recovered dose with an increase in exposure concentration, suggesting saturation in the uptake or metabolism of HCP. The relative distribution was similar following inhalation exposure to HCP at 100 or 25 ppm. Evaluation of NMR spectra of urine indicated the presence of 3,3-dichloroacrylic acid (DCAA) and metabolites derived from glutathione replacement of one chlorine from DCAA. NMR spectra of extracted charcoal traps indicated the presence of pentachloropropene as an expired volatile. Mechanisms to produce DCAA may involve reductive dechlorination, hydroxylation, elimination of HCl, and acid chloride hydrolysis. (This study was funded by Vulcan Chemicals.)

266 TOXICOKINETICS OF HEXACHLOROBENZENE IN FEMALE RATS.

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Hexachlorobenzene (HCB) is a chlorine-saturated aromatic that is the parent compound in the substituted aromatic class of pesticides. It has been suggested that HCB could account for more dioxin-like activity in human milk than polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls together. The toxicokinetics (TK) of HCB were assessed after a single oral administration to female Sprague Dawley rats at two dose levels (0.03 or 30 mg/kg body weight). TK parameters estimated included maximum blood concentrations (C_{max}) and time-to-peak (T_{max}), absorption, distribution, and elimination rates (k_a, α, and β) and associated half-lives, systemic clearance (Cl_s/F), apparent volumes of distribution (V_c/F and V_β/F), and dose normalized areas under the concentration-time curves ($DNAUC_{0-\infty}$). Selected tissues (liver, fat, and lung) were also analyzed for HCB. Kinetic parameters estimated for tissues include mean residence time (MRT), C_{max}, T_{max}, $t_{1/2}$ and $AUC_{0-\infty}$. HCB was rapidly absorbed (mean absorption $t_{1/2}$=1.4 hr) and absorption was linear over the dose range. However, the terminal elimination half-life (β-$t_{1/2}$) decreased from 176 hr to 48 hr for rats in the 0.03 and 30 mg/kg dose groups, respectively, suggesting non-linear kinetics. Clearance and volume terms, Cl_s/F, V_c/F, and V_β/F, increased with increasing dose. This increase in systemic clearance was associated with a decrease in the $DNAUC_{0-\infty}$ from 0.65 to 0.14 (µgohr/mL)/dose for rats receiving 0.03 or 30 mg/kg of HCB, respectively. Tissue data indicated HCB is highly concentrated in fat, thus creating a deep compartment where the compound is released slowly into the systemic circulation. The observed C_{max} in fat was 0.23 and 168 µg/g of tissue for rats treated with 0.03 and 30 mg/kg of HCB. HCB kinetics in liver behaved in much the same way. Lung tissue contained significantly lower amounts of HCB than fat or liver, with linear kinetics. One possible explanation for dose-dependent or non-linear kinetics is that tissue retention may be saturated at the higher dose level. A disproportionate increase in the unretained (systemic) fraction of HCB could increase the rate of renal or biliary excretion. However, this can not explain the observed increase in V_c/F and V_β/F with increasing dose.

267 DISPOSITION OF OCTAMETHYLCYCLOTETRASILOXANE (D_4) IN FEMALE FISCHER 344 AND SPRAGUE-DAWLEY RATS FOLLOWING NOSE-ONLY INHALATION EXPOSURE.

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Female Fischer 344 and Sprague-Dawley rats were exposed to 700 ppm ^{14}C-D_4 for a single 6 hr exposure. Immediately following exposure, body burden animals were solubilized and total ^{14}C body burden was determined. Excreta animals were placed in metabolism cages for 168 hr post exposure for collection of urine, feces and expired air. Additional animals were euthanized at predetermined time points for blood and tissue collection. Radioactivity was measured in all samples. Fischer 344 rats retained a significantly higher amount ($p<0.05$) of radioactivity (8.3±0.44%) than Sprague-Dawley rats (5.9±0.26%) at the end of the 6 hr exposure. Excretion of radioactivity was similar in both strains, with similar amounts being excreted in urine (~30%), feces (~20%), and expired volatiles (~30%). Concentration of radioactivity over time in blood, liver and lung was similar over the 168 hr post exposure period while differences were seen in fat (AUC in µg equivalent D_4/g•hr was 24164 and 15894) for Fischer 344 and Sprague-Dawley rats respectively. Analysis of blood, liver, lung and fat samples for parent D_4 demonstrated differences in the percent of radioactivity that could be attributed to metabolites in blood (60 vs. 80%), liver (30 vs 51%) and lung (84 vs. 91%) for Sprague-Dawley vs. Fischer 344 rats respectively. Fischer 344 rats consistently showed a higher amount of radioactivity present as metabolites as compared to D_4. Radioactivity present in the fat for both strains was predominately D_4. These kinetic differences between female Sprague-Dawley and Fischer 344 rats suggest that there may be important biochemical differences leading to a

decreased metabolism of D_4 in the female Sprague-Dawley rat. (Supported in part by the Silicones Environmental Health and Safety Council.)

268 USING PHARMACOKINETIC MODELING AND MONTE CARLO METHODS TO IMPROVE ESTIMATED BIOCONCENTRATION FACTORS AND HALF-LIVES FOR SUBSTITUTED DIPHENYL *P*-PHENYLENE DIAMINES IN CARP.

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A one-compartment pharmacokinetic (PK) model was developed for three substituted diphenyl p-phenylene diamines (DPPDs). The PK model was fit simultaneously to fish tissue concentration and water concentration data collected at two test concentrations during bioaccumulation and depuration studies for each DPPD. Bioconcentration factors (BCFs) were calculated from steady state estimates of fish tissue concentrations as predicted by the PK model. BCFs were also calculated using each individual fish tissue data point, for comparison purposes. Similarly, half-lives were estimated for each DPPD using model predictions for the depuration phase based on: (1) the bioaccumulation and depuration data used together; and (2) the depuration data alone. Monte Carlo methods were used to assess how variability in the fish tissue and water concentration data impact the estimates for the BCFs and half-lives. For each DPPD, the BCF calculated using the maximum fish tissue concentration far exceeded the upper 95th percentile of the BCF distributions calculated from steady-state model predictions for the entire data set. Similarly, the maximum half-lives calculated using the depuration data alone far exceeded the upper 95th percentile of the half-live distributions calculated from the combined bioaccumulation/depuration data set. Because the shape of the bioaccumulation phase (i.e., time to reach steady-state) contains information important to half-life, the importance of including bioaccumulation data when determining half-lives is emphasized.

269 MATERNAL AND FETAL DISPOSITION OF A PHOSPHOROTHIOATE OLIGONUCLEOTIDE, ^{3}H-ISIS 2105, FOLLOWING 3-HOUR OR 7-DAY INTRAVENOUS INFUSION TO PREGNANT RATS.

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Fetal exposure was evaluated following i.v. dosing of ^{3}H-ISIS 2105 to timed-pregnant rats. ISIS 2105 is a 20-base phosphorothioate oligonucleotide (ODN); selected thymidine residues were nonexchangeable ^{3}H-labeled. To maximize the potential for fetal exposure, ^{3}H-ISIS 2105 was administered as either a 3-hr infusion of 6.6 mg/kg/hr, total dose 20 mg/kg, or as a continuous 7-day infusion at 0.3 mg/kg/hr, total dose 59 mg/kg. Infusions were delivered via jugular vein canula by syringe pump on GD 18/19 (3-hr) or by osmotic pumps implanted s.c. on GD 12 (7-day). Reported concentrations are based on measured radioactivity. Following a 3-hr infusion, maternal and fetal plasma conc of ^{3}H-ISIS 2105 were > 100 μg/ml and < 5 μg/ml, respectively. Maternal and fetal regions of the placenta had similar ODN conc (7 to 15 μg/g). Concentrations of ODN in fetal kidney were less than 1% of maternal kidney. Following a 7-day continuous infusion, maternal plasma levels were approx 12 μg/ml; fetal plasma levels were approx 10 μg/ml. Concentrations were similar for maternal and fetal placenta approx 3 μg/g. Fetal kidney concentrations were approx 2-3% of those in maternal kidney. Relative organ distribution in fetuses was similar following either dose regimen (kidney > GI ≈ brain > liver) and different from dams (kidney > liver > spleen). These data indicate a minimal transfer from dam to fetus, consistent with a limited ability for these relatively large polyanionic compounds to traverse the placenta.

270 GF120918 ENHANCES THE CNS PENETRATION OF AMPRENAVIR IN SPRAGUE-DAWLEY RATS.

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The HIV virus is able to survive in extravascular tissues even when protease and reverse transcriptase inhibitors are administered. It has recently been cited in literature that amprenavir, a relatively new protease inhibitor, is actively transported by mdr (P-glycoprotein). Mdr is expressed in normal tissues including the skin, testes, uterus, liver, kidney, intestine, and at the blood-brain barrier. Whole body autoradiograms have shown that the CNS concentration of amprenavir is significantly lower in wild type mice mdr1a/b +/+ when compared to double knock out mice mdr1a/b -/-. The purpose of this study is to determine the extent of mdr modulation in Sprague-Dawley rats treated with GF120918 (250 mg/kg for four days) using microdialysis. A microdialysis probe was placed in the jugular vein and in the frontal cortex of Sprague-Dawley rats. Amprenavir was infused (8.04 mg/hr) for 5.25 hours and samples were taken from the blood and brain to determine the unbound brain to blood concentration ratio (BBR). The BBR for rats in the absence of GF120918 was determined to be 0.10+0.03 (n=5). The BBR for amprenavir in Sprague-Dawley rats pretreated with GF120918 was 0.67+0.3 (n=5). It is clear from this study that GF120918 significantly increases the penetration of amprenavir into the CNS. The use of mdr inhibitors may offer a new strategy to increase the effectiveness of antiviral therapy directed at reducing the HIV load in the brain. (This work was supported by GlaxoWellcome and NIEHS training grant ES07266.)

271 DOSE DEPENDENT METABOLISM OF BENZOFLEX®2-45 PLASTICIZER IN RATS.

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Benzoflex®2-45 (diethyleneglycol dibenzoate; [ring-U-^{14}C] labeled), a plasticizer, was administered orally to rats in corn oil at doses of 50 and 750 mg/kg. Both doses were rapidly absorbed and excreted. Within the first 24 hours, > 97% of the administered radioactivity was excreted in the urine, while < 1% was excreted in feces and no radioactivity was detected in the expired air. Plasma and whole-blood radioactivity measurements, as indicated by the total area under the curves (AUC_t; μg equivelents.h/g) suggested that the 750 mg/kg dose was disproportionately greater than the proportionate increase in dose (from 3 to 4x). Furthermore, the urinary metabolite profile changed from the low to high dose. At 50 mg/kg essentially all the urinary metabolite was composed of hippuric acid, the normal glycine conjugate of benzoic acid. At 750 mg/kg an additional metabolite was observed in the urine. Upon hydrolysis the second urinary metabolite was identified by radio-TLC to be consistent with the glucuronide conjugate of benzoic acid. Thus, doses within the range of 500 to 750 are suggestive of saturating the normal conjugation and excretion mechanism of diethyleneglycol dibenzoate. Any toxicity observed in this dose range or higher needs to be considered carefully since the normal metabolism of this substance is altered. The tissue distribution of the ^{14}C labeled substrate was variable but not remarkable at either dose. Most important was the dose dependent metabolism observed at a dose of 750 mg/kg; and that Benzoflex®2-45 plasticizer was rapidly absorbed and eliminated within 24 hours.

272 ROLE OF AH RECEPTOR ON HEPATIC SEQUESTRATION AND DISPOSITION OF DIOXIN—STUDIES USING THE AH RECEPTOR-KNOCKOUT MICE.

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Dioxin (TCDD, 2,3,7,8-tetrachlorodibenzo-π-dioxin)is the prototype and most potent member of the highly lipophilic polyhalogenated aromatic hydrocarbons (PHAHs) which are persistent and ubiquitous environmental contaminants. TCDD acts as a ligand and binds to the aryl hydrocarbon receptor (AhR which results in a wide range of adverse biological responses. Body burdens associated with these effects are dependent on the disposition of the ligand. Disposition of TCDD and dioxin-like compounds is influence by their lipophilicity, binding affinities to the AhR, and binding affinities to the CYP1A2 which is regulated by the AhR. In both acute and subchronic animal studies, there is a specific accumulation of TCDD in liver compared to adipose tissue. Hepatic sequestration is dose-dependent, and the inducible hepatic binding protein, CYP1A2, has been shown to be responsible for this hepatic sequestration. The objective of the present study was to investigate the role of the AhR on the hepatic sequestration and disposition of TCDD in homozygous AhR knockout (KO) mice (AhR-/-)compared to age-matched heterozygous (AhR+/-) mice (both groups were on the C57BL/6N lineage). The mice were dosed orally with 25 Mg [3H]TCDD/kg and at termination (four days after dosing), tissues were quantitated for TCDD-derived radioactivity. Results demonstrated differential effects on disposition within the two groups

of mice. These differences were reflected by liver-to-fat (L/F) concentration ratios (sensitive indicators for hepatic sequestration of TCDD). The L/F concentration ratios were <0.1 for AhR-/-mice. Previously, studies have demonstrated L/F concentration ratios of 0.2 in the CYP1A2 KO mice and 3.6 in the parental strains (C57BL/6N and 129/Sv) at the same dose and time. This study demonstrates the importance of the AhR on the pharmacokinetic behavior and mechanistic issues for TCDD and related compounds. (This abstract does not necessarily reflect EPA policy.)

273 DISTRIBUTION OF 2,2-BIS(4-CHOROPHENYL)-1,1,DICHLOROETHYLENE (p,p'-DDE) IN FETAL RATS.

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The environmental contaminant 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) is a stable and persistent metabolite of DDT. p,p'-DDE acts as an antiandrogen in both *in vitro* and *in vivo* systems. Male rats exposed perinatally to high doses of p,p'-DDE display feminized external morphology in the form of reduced anogenitial distance and retained thoracic nipples. A recent study investigated the transplacental transfer of p,p'-DDE to the whole fetus, fetal liver, brain, and blood (You et. al., 1999). Here we investigate, in addition to the distribution of p,p'-DDE in fetal liver and blood tissue, the distribution of p,p'-DDE in maternal adipose, liver, blood, and placenta, and the distribution in various fetal urogential tissues including adrenals, kidneys, ovaries, testis, and the external sex papilla. Six time-pregnant Long Evans rats were dosed daily via gavage during GD14-18 with 100mg p,p'-DDE spiked with 3.19*u*Ci 4,4'-DDE-Ring-UL-^{14}C/ mL dosing solution /kg body weight. Each animal, on average, received approximately 2.7*u*Ci ^{14}C-DDE/day. On GD 19, the aforementioned tissues were harvested and the distribution of p,p'-DDE determined. Partitioning of p,p'-DDE in maternal tissues paralleled tissue lipid content with adipose containing approximately 1350 ppm followed by liver (79 ppm), placenta (34 ppm) and blood (22.9 ppm). p,p'-DDE distribution in fetal tissues were: adrenals (118.5 ppm), ovaries (61.9 ppm), liver (33.9 ppm), testis (21 ppm), external sex papilla (13.9 ppm), kidney (9.8 ppm), blood (7.9 ppm). The consequences of p,p'-DDE accumulation in fetal adrenals warrants further investigation. (Funded by a National Research Council-USEPA/NHEERL Research Associateship. This abstract does not reflect USEPA policy.)

274 COMPARATIVE DISPOSITION OF PROPARGYL ALCOHOL (PAL) IN MALE F-344 RATS AND B6C3F1 MICE.

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PAL, a moderately volatile acetylenic alcohol, is a high production volume chemical used as a chemical intermediate, a corrosion inhibitor, and a solvent stabilizer. There is potential human exposure to PAL in the occupational environment by inhalation and dermal contact. We have conducted a series of studies to compare the disposition of [^{14}C]PAL in male rats and mice after a single iv (1 mg/kg), oral (50 mg/kg), inhalation (1 ppm or 100 ppm for 6 h), or dermal (5 mg/kg) exposure. By 72 h following an iv or oral dose, 80-90% of the dose was excreted (urine, feces, volatile organics, and $^{14}CO_2$). The majority of the iv and oral doses was excreted in the first 24 h post-dosing, primarily in urine (40-60%) and as exhaled CO_2 (19-26%) by both species, with <1% of the dose exhaled as volatile organics. In the inhalation studies, 30-55% of the radioactivity to which animals were exposed was absorbed. The primary route of excretion of the absorbed dose was in urine (23-53%), and a significant portion was exhaled as volatile organics (15-20%) by 18 h after cessation of the inhalation exposure. In rats, elimination in bile (62% in 4 h) was much greater than elimination in feces (6% in 72 h), indicating that PAL metabolites undergo extensive enterohepatic recycling. Dermal exposure studies conducted in rats demonstrated that dermal absorption of PAL was minimal due to its inherent volatility; <5% of the applied dose was excreted in urine and feces or exhaled as CO_2. In summary, our studies show that after a single iv, oral, or inhalation exposure to PAL, the majority of the administered radioactivity is rapidly eliminated from the body. Further, systemic exposure after a brief dermal exposure to PAL is minimal due to its volatility, however significant absorption may occur with prolonged inhalation exposure or dermal contact.

275 PHARMACOKINETICS OF INHALED MANGANESE PHOSPHATE IN MALE SPRAGUE-DAWLEY RATS FOLLOWING SUBACUTE (14-DAY) EXPOSURE.

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The goal of this pilot study was to determine the exposure-response relationship for inhaled manganese phosphate in adult male CD rats. Manganese phosphate is the primary respirable ($PM_{2.5}$) tailpipe emission product arising from the combustion of methylcyclopentadienyl manganese tricarbonyl (MMT). Rats were exposed 6-hr/day for either 5 days/week (10 exposures) or 7 days/week (14 exposures) to manganese phosphate at 0, 0.03, 0.3, or 3 mg Mn/m^3 (MMAD=1.5 μm). The following tissues were collected following the last inhalation exposure: plasma, erythrocytes, olfactory bulb, striatum, cerebellum, lung, liver, femur, and skeletal muscle (n=6 rats/exposure group). Intravenous $^{54}MnCl_2$ tracer studies were also conducted following the 14th exposure (n=6 rats/concentration), and whole-body gamma spectrometry was performed immediately after injection and at 1, 2, 4, 8, 12, and 16 weeks after $^{54}MnCl_2$ administration. Increased olfactory bulb and lung manganese concentrations were observed only following exposure to 0.3 (14 exposures) or 3 mg Mn/m^3 (10 or 14 exposures). Increased manganese concentrations were observed in cerebellum, striatum, femur, and skeletal muscle only following exposure to 3 mg Mn/m^3 (10 or 14 exposures). Red blood cell manganese concentrations were increased only in rats exposed to 3 mg Mn/m^3 (10 exposures). Rats exposed to 3 mg Mn/m^3 also had faster initial and terminal manganese elimination rates when compared to air-exposed control animals. Our results suggest that the rat olfactory bulb may accumulate more manganese than other brain regions following inhalation exposure. (Funded by Ethyl Corporation in support of registration testing requirements arising under Section 211 (a, b) of the Clean Air Act.)

276 TOXICOKINETICS OF DOSE DEPENDENT THIOBENZAMIDE HEPATOTOXICITY AND TISSUE REPAIR RESPONSES.

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Our recent mechanistic studies of thiobenzamide (TBZ) hepatotoxicity have demonstrated that at LD100 doses liver function returns to near normal prior to animal death. The lethal-dose repair, response is however, delayed and less robust than responses at sublethal but hepatotoxic doses. Accumulating evidence suggests that TBZ hepatotoxicity arises from the production of at least one reactive metabolite. In an attempt to further explain the observed differences in magnitude and time course of tissue repair response, plasma concentrations were quantitated for TBZ, TBZ-S-oxide (sulfine), and benzamide at various time intervals during 28 h after oral dosing. Preliminary results for 175-200 g (1.5-2 month old) Sprague Dawley rats indicate that at higher TBZ doses, S-oxide concentrations remain elevated for an extended period of time (30% of cmax at 10 h for a 1050 mg/kg dose vs. <1% of cmax at 10 h for 450 mg/kg; and <<1% of cmax at 6 h for 150 mg/kg). For benzamide, which is presumed to be formed from S-oxide via the reactive (sulfene) TBZ-S,S-dioxide, the differences were even more pronounced. At the highest dose, benzamide concentrations were 50% of cmax at 20 h, and still >25% cmax at 28 h. Although the benzamide AUCs increased roughly in proportions to the dose, cmax was approximately unchanged over all three dose levels. These data are consistent with either saturation or capacity reduction in the conversion of the S-oxide to benzamide at higher doses of TBZ, translating to much longer time frames during which tissues are being exposed to reactive intermediates. This scenario may partially account for the delayed onset and decreased aggressiveness of tissue repair at high doses, and therefore further explorations are in progress. (Board of Reagents Support Fund.)

277 HETEROLOGOUS EXPRESSION OF AND THE DEVELOPMENT OF ASSAYS FOR INTERACTION OF DRUGS WITH HUMAN P-GLYCOPROTEIN.

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P-Glycoprotein (PGP or MDR1) is a member of the ABC transporter superfamily and is expressed in the human intestine, liver and other tissues. Intestinal expression of PGP may affect the oral bioavailability of drug molecules and other xenobiotics that are substrates for this transporter. Interaction with PGP can be studied using either direct assays of drug transport in polar-

ized cell systems or with indirect assays such as drug-stimulated ATPase activity and inhibition of the transport of fluorescent substrates. We have expressed human PGP cDNA using both stable mammalian cell and baculovirus/insect cells expression systems. With the insect cell expressed material we have applied a colorometric assay for the ATPase activity to the detection of interaction of drugs with PGP. Known drug substrates stimulate vanadate-sensitive ATPase activity in PGP-containing insect cell membranes. These interactions typically demonstrated saturation kinetics. With the stable mammalian cell system we have examined the kinetics of drug transport across cell monolayers. Again, known drug substrates typically demonstrated saturation kinetics. Several xenobiotics stimulated ATPase activity but were not detectably transported in the mammalian cell system. In contrast, all xenobiotics that were transported also stimulated ATPase activity. For this latter class of xenobiotic, there was good agreement between the systems in the concentration that gave half maximal response. The ATPase assay is a useful screen for interaction with PGP. However, positive responses should be further investigated in transport assays. Concentration response data from the ATPase assay appears to be useful for the design of such transport assays.

278 EFFECTS OF PERCHLORATE ON THYROIDAL UPTAKE OF IODIDE & CORRESPONDING HORMONAL CHANGES.

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The production and storage of ammonium perchlorate (AP) has resulted in contamination of soil, ground and drinking water in a number of states. The perchlorate ion interferes with iodide uptake in the thyroid resulting in reduced thyroidal hormone synthesis. In rodents, this leads to lowered T_3 and T_4 blood levels and TSH activation to stimulate production of thyroid hormones. A series of experiments were conducted to investigate the temporal relationship between perchlorate-induced inhibition of iodide uptake in the thyroid and its effects on the synthesis of thyroid hormones. ^{125}I uptake inhibition studies were carried out in naïve rats given single intravenous perchlorate doses (0 - 3 mg/kg) and in rats given drinking water containing perchlorate for 2 weeks (0 -3 mg/kg/day). In other studies rats were given drinking water containing perchlorate for two weeks (0 - 10 mg/kg/day) and TSH, total T_3, total T_4, and free T_4 in serum were measured on days 1, 5, and 14 of treatment. TSH, T_3, T_4, and free T_4 time course data were collected in rats intravenously dosed with 3 mg/kg of perchlorate. Under conditions when iodide inhibition was about 70% (from 2-24 hours after a single 3 mg/kg intravenously dose of perchlorate), total T_4 serum level decreased and TSH serum level increased. TSH remained elevated for 48 hrs, while total T_4 returned to control levels by 48 hrs. In the drinking water studies, dose dependent thyroid activation was evident by temporal changes in inhibition of iodide uptake in the thyroid and accompanying thyroid hormone changes. TSH remained elevated for the 1, 3, and 10 mg/kg/day dose groups. In the 0.1 mg/kg/day dose group, TSH was elevated only on day 1 of treatment.

279 PREDICTION OF DOSE-RESPONSE FOR PERCHLORATE INHIBITION OF THYROIDAL IODIDE UPTAKE IN HUMANS USING A SIMPLIFIED PHARMACOKINETICS MODEL AND PERFUSED-ORGAN DATA FROM RATS.

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Recent improvement in measurement sensitivity has led to widespread detection of ppb perchlorate levels in groundwater. Populations throughout the Southwest potentially consume drinking water containing perchlorate at levels of approximately 10-20 ppb, leading to doses estimated as 0.0003-0.0006mg/kg-day. It is therefore of importance to determine a safe level of perchlorate exposure. The most sensitive site of perchlorate action is the sodium/iodide symporter. The concentration-dependence of perchlorate inhibition of iodide uptake by the symporter into the thyroid has been measured in perfused rat thyroid lobes (Greer *et al.* 1966. *Endocrinol.* 79: 237-247). On the assumption that the concentration-dependence in the isolated rat thyroid predicts that in the human thyroid *in vivo*, a model was developed for predicting the inhibition resulting from a given daily dose. Published perchlorate pharmacokinetics data in rats and humans were analyzed; assuming first-order kinetics, the elimination half-life ($t_{1/2}$) was estimated as 16-18 hr in humans and 20-24 hr in rats. Based on the model developed for estimating steady-state serum levels, a perchlorate dose of 0.03mg/kg-day (*e.g.*, consumption of drinking water containing 1 ppm perchlorate) was predicted to inhibit iodide uptake by about 50%. Recent data in humans suggest that inhibition of uptake by this amount requires a dose more than five-fold higher (Lawrence *et al.* 1999, ATA meeting abstract). Two hypotheses for this discrepancy can be put forward: (1) the rat symporter *in vivo* is more sensitive than the human symporter *in vivo*, and/or (2) the perfused thyroid is more sensitive than the thyroid *in vivo*.

280 HEPATIC SULFOTRANSFERASE ACTIVITY IN MALE RATS CHRONICALLY EXPOSED TO ETHANOL AS PART OF HIGH AND LOW CARBOHYDRATE DIETS INFUSED VIA TOTAL ENTERNAL NUTRITION (TEN).

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There is good evidence that sexually dimorphic patterns of growth hormone (GH) secretion regulate the expression and activity of many sex-dependent hepatic enzymes in rat liver. We have shown that chronic ethanol exposure demasculinizes the pattern of GH secretion in male rats and as a consequence, significantly decreases expression and activity of the male specific hepatic P450 enzyme CYP2C11 (Badger et al. JPET 264: 438,1993). Moreover, the severity of CYP2C11 suppression increases as dietary carbohydrate is lowered. In addition to P450s, a number of rat hepatic phase II enzyme systems including the sulfotrasferases are sex-dependent and appear to be GH regulated. Therefore, the current study was designed to investigate the effects of ethanol and diet on hepatic sulfotransferase expression. Four groups of 250g male Sprague-Dawley rats (N=6-8) had IG cannulae surgically inserted and were placed on total enteral nutrition (TEN) using liquid diets. In two groups of rats, 36% of the carbohydrate calories were replaced with ethanol. The final carbohydrate content of one ethanol diet was 37.5% while the second was 3%. After 50 days of ethanol infusion, the rats were sacrificed, livers collected and cytosol prepared. Sulfotransferase activities towards the phenolic substrate b-napthol; the estrogens 17b-estradiol and estrone and hydroxysteroid substrate dehydroepiandrosterone (DHEA) were measured. No effects of either diet or ethanol were observed on b-naphthol sulfotransferase activity. In contrast, sulfotransferase activities towards both estrogens was increased 2-fold ($p<0.05$) and that towards DHEA was induced 2-4 fold ($p<0.05$) by chronic ethanol treatment. This effect of ethanol was independent of dietary carbohydrate concentration. These data are consistent with the predicted effects of suppressed and demasculinized GH secretion by ethanol. Phenolic sulfotransferase ST1A1 has been reported to be unaffected by GH manipulation. In contrast, the major estrogen sulfotransferase ST1E2 has been shown to be negatively regulated by GH, while the major hydroxysteroid sulfotransferase ST-40/41 has been reported to be selectively suppressed by the male pattern of GH secretion in rats. NIAAA08645.

281 ROLE OF PREGNENOLONE-16α-CARBONITRILE (PCN)-INDUCED EXPRESSION OF MRP2 IN THE ENHANCED PLASMA DISAPPEARANCE AND BILIARY EXCRETION OF DIBROMOSULFOPHTHALEIN (DBSP).

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Previous studies have shown that PCN increases the plasma disappearance and biliary excretion of DBSP in Sprague-Dawley (SD) rats. PCN also increases rat multidrug resistance protein 2 (Mrp2), a canalicular organic anion transporter protein responsible for excretion of DBSP. We hypothesize that up-regulation of Mrp2 by PCN is responsible for the enhanced plasma disappearance and biliary excretion of DBSP. This was resolved by measuring plasma disappearance and biliary excretion of DBSP in control and PCN-treated SD and Mrp2-deficient Eisai hyperbilirubinemic rats (EHBR). Male SD and EHBR rats were injected *ip* for four days with PCN (75 mg/kg/day) or vehicle control. On day five, DBSP (100 mg/kg) was injected *iv* and measured in blood and bile. Plasma DBSP disappearance was the same in non-induced SD and EHBR rats. PCN enhanced the plasma disappearance of DBSP in both SD and EHBR rats, but disappearance slowed at later time intervals in EHBR rats. The maximal rate of biliary excretion in non-induced EHBR rats was markedly reduced and delayed compared to non-induced SD rats. PCN increased DBSP biliary excretion in SD rats, but decreased the biliary excretion of DBSP in EHBR rats at later time intervals. These results suggest that the enhanced plasma DBSP disappearance produced by PCN is not due to Mrp2, as shown by the EHBR data. The low rate of biliary DBSP excretion in EHBR rats may to be due to an additional canalicular transporter. Furthermore, a PCN-inducible sinusoidal transporter may efflux DBSP into plasma. In conclusion, up-regulation of Mrp2 by PCN is not responsible for the increased uptake of DBSP, but is responsible for the enhanced biliary

excretion of DBSP. (Supported by NIH grants ES-03192 & ES-07079, and AHA grant KS-98-GS-11.)

282 DISPOSITION AND SEROTONERGIC EFFECTS OF CYHALOTHRIN IN CENTRAL NERVOUS SYSTEM.

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Cyhalothrin, type II pyrethroid insecticide, is used topically for the control of ectoparasites. It is also widely used in agriculture and in home desinsection. Pyrethroids are neurotoxic compounds. In humans, sensory facial irritation is a very common syndrome (paraesthesia and dysaesthesia). Cyhalothrin when injected peripherally to rat produced a severe syndrome characterized by tremor, salivation and choreoathetosis. The aim of this work was to determine cyhalothrin disposition and effects on the levels of 5-hydroxytrytamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) in nervous tissues. Two experiments were carried out. Experiment 1: male Wistar rats were treated with cyhalothrin, single i.v. dose of 3 mg/kg and killed at different time periods. Plasma and nervous tissue samples were isolated, homogenized and extracted in acetonitrile to determine cyhalothrin concentrations by HPLC. Experiment 2: rats were divided into two groups. Group 1 rats received cyhalothrin (8 mg/kg for 6 days) orally. Group 2 rats (control group) received 0.5 ml corn oil/rat orally. All animals were killed 24 h after last dosing, brain regions were isolated and the contents of 5-HT and 5-HIAA quantified by HPLC-ED. The results indicated an accumulation and a slow final disappearance of cyhalothrin from tissues as well as a lower activity of the 5-HT-ergic system. (Work supported by Projects No. PB97-1236 (DGICYT), No. 08.8/0002/98 (CAM) and 99/0936 (FIS), Spain.)

283 ALTERATION OF METABOLISM FOR PERCHLOROETHYLENE AND 1,1,1,2-TETRACHLOROETHANE AFTER CARBON TETRACHLORIDE PRETREATMENT IN B6C3F1 MICE.

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Perchloroethylene (PCE) and 1,1,1,2-tetrachloroethane (1,1,1,2-TC) are both metabolized by P450 2E1, ultimately yielding TCA as a major metabolite. Carbon tetrachloride (CT) has been shown to induce and inhibit P450 2E1 metabolism. To understand better the impact of CT on P450 2E1 mediated metabolism male B6C3F1 mice were dosed by bolus gavage with either 0, 0.1, 1, 5, 20, 50 or 100 mg/kg CT. Then, 1 hr later the mice were given a bolus gavage of 100 mg/kg PCE or 200 mg/kg 1,1,1,2-TC. At 4 hr after the challenge dose blood and liver were taken and analyzed for parent solvent concentration and for metabolites. TCA concentration in blood after the 0 mg/kg CT/ PCE challenge was 35.5±2.5 μg/g and decreased to 1.2±0.1 μg/g for the 100 mg/kg CT/ PCE challenge. TCA concentration in blood after the 0 mg/kg CT/ 1,1,1,2-TC challenge was 38.5±5.9 μg/g and decreased to 4.5±0.8 μg/g for the 50 mg/kg CT/ 1,1,1,2-TC challenge. Interestingly, the level of TCA increased in blood with a 0.1 mg/kg CT pre-treatment before both PCE and 1,1,1,2-TC as compared to the 0 mg/kg CT level, suggesting the possibility of induction of the P450 2E1 enzyme by CT at this dose. The pharmacokinetic data for these chlorinated solvents and their metabolites can be used to describe the possible interactions between solvents in a mixture that can alter toxicity.

284 INHIBITION OF FLAVIN-CONTAINING MONOOXYGENASE AND CYP1A2 ACTIVITIES IN HUMAN BY GRAPEFRUIT JUICE.

Y. N. Cha, J. H. Kang, C. S. Park and W. G. Chung. *Inha University, Inchon, Republic of Korea.*

CYP1A2 and flavin-containing monooxygenase (FMO) are known to oxidize various procarcinogens like aromatic and heterocyclic amines to cause colorectal and bladder cancers. Inhibition of these enzymes would lead to cancer prevention. Quercitrin, a flavonoid contained in grapefruit juice and red wine, is known to inhibit CYP1A2 and 3A4 activities *in vitro* and is considered to have cancer preventive effects. Thus, we have determined whether the administration of grapefruit juice would inhibit the CYP1A2 and FMO activities *in vivo* by assessing the molar concentration ratios of urinary caffeine (CA) metabolites after administering a cup of coffee. Intake of grapefruit juice inhibited the *in vivo* FMO activity (theobromine/CA) by 79% and the *in vivo* CYP1A2 activity (1,7-dimethylurate+paraxanthine/CA) by 32%, but not xanthine oxidase activity (1-methylurate/1-methylxanthine). Results of this study suggested that grapefruit juice would inhibit CYP1A2 and FMO activities *in vivo* and would prevent the metabolic activation of aromatic and heterocyclic environmental procarcinogens causing bladder and colo-rectal cancers. (Supported by an intramural grant from Inha University.)

285 INHIBITION OF LIVER MICROSOMAL FLAVIN-CONTAINING MONOOXYGENASE ACTIVITIES BY FLAVONOIDS.

W. G. Chung, S. D. Ryu, C. S. Park and Y. N. Cha. *Inha University, Inchon, Republic of Korea.*

Grapefruit juice, containing several flavonoids, has been known to inhibit the CYP3A4 and 1A2 activities *in vivo*. We found that intake of grapefruit juice inhibited also the flavin-containing monooxygenase (FMO) activity *in vivo*. Thus, we determined whether several bioflavonoids known to be contained in grapefruit juice would inhibit liver microsomal FMO activity *in vitro*. The thiobenzamide S-oxidation was inhibited in a dose dependent manner by flavonoids up to 80% (quercitrin), 64% (kaempferol) and 40% (naringin). The Ki values were 6.2, 12.0 and 13.9uM, respectively. Quercitrin (noncompetitive-uncompetitive type) and kaempferol (competitive-noncompetitive type) provide mixed inhibition pattern while naringin exhibited uncompetitive pattern. In addtion to the thiobenzamide S-oxidation, quercitrin, kaempferol and naringin inhibited the oxidation of N,N-dimethylaniline, another FMO assay, by 52-58%. This study indicated that quercitrin, kaempferol and naringin among bioflavonoids known to be contained in grapefruit juice are the ingredients responsible for the inhibition of *in vivo* FMO activity by intake of grapefruit juice in human. (Supported by an intramural grant from Inha University.)

286 TOXICOKINETIC-TOXICODYNAMIC RELATIONSHIPS IN TWO CASES OF CYANIDE POISONINGS.

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The value of PK-PD relationships in clinical pharmacology is now well recognized. However, the potential interest of Toxicodynamic-Toxicokinetic (TK-TD) relationships in medical toxicology has been poorly investigated. The aim of this study was to correlate the plasma lactate concentrations and the corresponding blood cyanide concentrations. **Materials and Methods:** Plasma lactate concentrations (PLC) were measured using an enzymatic method. Blood cyanide concentrations (BCC) were measured using a colorimetric assay. Non-linear regression was used for modeling TK-TD relationships. **Results:** TK-TD relationships were studied in 2 cases of life-threatening cyanide poisonings (patients 1 and 2). The PLCs at the time of hospital admission were 53.0 and 47.7 mmol/L in patients 1 and 2, respectively (Nal < 2 mmol/L). The corresponding BCCs were 256 and 239 μmol/L, respectively. In patient 1, there was a plateau of PLCs corresponding to BCCs ranging from 256 to 100 μmol/L. Unfortunately, in patient 2 only one measurement was made at the highest PLC. In both cases supportive treatment was associated to the infusion of high doses of hydroxocobalamin. Both patients eventually died. The TK-TD relationships can be fitted with the sigmoidal Emax model in both patients (r2 = 0.946 and 0.824, respectively). The Hill coefficients (value + std error) were 3.15 + 0.95 and 4.75 − 3.06, respectively. The C50 (value + std error) were 60.0 + 5.3 and 46.2 + 7.5 μmol/L, respectively. **Discussion:** Our data showed that in patient 1 a plateau of PLCs was associated with a wide range of BCCs. During the course of severe cyanide poisoning, the relationship between the PLCs and the corresponding BCCs can be described by a sigmoidal model. The high value of the Hill coefficient showed that a small decrease in BCCs near the C50 was associated with a dramatic decrease in PLCs.

287 TOXICOLOGY IN THE NEXT MILLENNIUM: TOXICOGENOMICS AND PROTEOMICS.

I. Y. Rosenblum[1] and J. Barrett[2]. [1]*Schering-Plough Research Institute, Lafayette, NJ and* [2]*National Institutes of Environmental Health Sciences, Research Triangle Park, NC.*

Progress in the Human Genome Initiative has opened the doors to opportunities in toxicology never before imagined. The identification of large numbers of expressed genes has driven the development of technologies to investigate global changes in the expression patterns of both the messages and protein products from those genes. These emerging areas of genomics and proteomics, respectively, hold the promise of unraveling the complexity of the regulation of gene expression in normal tissues in normal states of growth and development, and differentiating those expression patterns from gene expression patterns found following exposures to various agents associated with specific mechanisms of cellular toxicity. The identification of such information could allow for truly powerful, rapid, high throughput *in vitro* screens for toxicity as well as identification of specific patterns diagnostic of specific mechanisms of toxicity associated with *in vivo* exposures to animals and humans. This rapidly emerging area of science is extremely timely for all toxicologists in all venues, academic, industrial, pharmacological, and regulatory, and should be of utmost interest to all the members of the Society of Toxicology.

288 MICROARRAY TECHNOLOGY IN MOLECULAR TOXICOLOGY.

R. Paules. *National Institute of Environmental Health, Research Triangle Park, NC.* Sponsor: J. C. Barrett.

One major challenge facing investigators in environmental health research is to assess exposures, and in doing so to identify intrinsic and extrinsic hazards and identify mechanisms of action underlying toxicity. Traditionally, toxicologists use rodent bioassays to evaluate potential hazards. These assays are expensive and take years to complete. cDNA microarray technology allows one to study changes in genome-wide patterns of gene expression, analyzing the expression of thousands of genes in one experiment. Assuming that exposures to different classes of toxicants result in distinct patterns of altered gene expression, in addition to common changes associated with the subsequent toxic response, microarray technology can be utilized to categorize and classify compound effects through the direct comparison of gene expression signatures in exposed and control samples. This approach may be useful to identify toxic substances individually or in mixtures, to determine whether toxic effects occur at low doses, to determine susceptible tissues and cell types, and to extrapolate effects from one species to another. We have developed a custom DNA microarray, ToxChip, that is a human cDNA clone array oriented toward the expression of genes involved in responses to toxic insult, including xenobiotic metabolizing enzymes, cell cycle components, oncogenes, tumor suppressor genes, DNA repair genes, estrogen-responsive genes, oxidative stress genes, and genes known to be involved in apoptotic cell death. Our initial efforts to build a toxic response data base include results from human cell culture systems exposed to representative agents from various classes of toxicants (including oxidant stressors, DNA damaging agents, and estrogenic compounds). Additional studies analyzing gene expression responses to toxicant exposures in mouse and yeast model systems are ongoing.

289 USE OF GENE EXPRESSION PROFILING IN PREDICTIVE TOXICOLOGY.

D. Mendrick. *Gene Logic, Inc., Gaitherburg, MD.*

Gene Logic is using the combined power of open and closed gene expression systems to develop large-scale expression databases. Working together with pharmaceutical companies we are building a ToxExpress™ reference database to identify patterns of toxicity from animal models that are predictive of human toxicity. The goal is to produce a high throughput assay to assist pharmaceutical companies in their prioritization of proprietary lead compounds. Gene Logic's closed system approach uses the GeneChip™ microarray technology to obtain quantified abundance data on known genes. Our open system, which confirms such markers and allows us to discover novel genes, is a proprietary differential display technique called READS™. Validation of both open and closed systems is performed with quantitative PCR. We are focusing initially on liver gene expression profiles using rat liver samples exposed *in vivo* to well-recognized, non-proprietary toxins and rat and human primary hepatocytes exposed *in vitro* to the same toxins. These studies will allow us to correlate the response between *in vivo* and *in vitro* exposure and across species. These expression profiles can be coupled to well-recognized pathways so that we can detect patterns of gene expression that are indicators of toxicity thus leading to the development of a higher throughput assay for the testing of proprietary compounds.

290 STRENGTHS AND WEAKNESSES IN THE CURRENT APPLICATIONS OF EXPRESSION PROFILING.

S. H. Friend. *Rosetta Inpharmatics, Kirkland, WA.* Sponsor: I. Y. Rosenblum.

Drug discovery rests upon an ability to identify effects of compounds on their intended targets, but it also requires an ability to monitor other activities of compounds that are unintended. Over the past several years, individuals have recognized that it is possible to use transcript arrays to follow the levels of expression for many of the genes within cells. The emphasis has been placed on monitoring transcriptional units to understand the transcriptome. This is quite helpful in understanding biology, but does not provide information regarding the activity or function of various proteins within the cell. This lecture will focus on the ability of expression profiling to follow protein function. The emphasis here is on using matrix approaches and pattern recognition to move beyond the analysis of transcript levels to being able to follow the function of intended and unintended targets within the cell. The strategy that we have employed involves building up large coherent sets of data and developing algorithms and other tools by which to successfully compare new profiles to those existing within libraries of profiles.

291 THE USE OF PROTEOMICS IN MOLECULAR TOXICOLOGY.

M. J. Cunningham[1], G. Holt[2], C. Moyses[2] and M. Egerton[1]. [1]*Incyte Pharmaceuticals, Palo Alto, CA and* [2]*Oxford GlycoSciences, Oxford, United Kingdom.*

Proteomics technology potentially offers a new approach to evaluating and prioritizing lead compound candidates. This technology involves high throughput separation, imaging and analysis of expressed proteins or Protein Expression Maps (PEMs). PEMs allow identification of known and novel proteins, analysis of posttranslational modifications and protein-protein interactions. Approximately 2000 proteins can be separated at the same time by MW and pI on a 2D gel, robotically excised and prepared for analysis and annotation by mass spectrometry. In a study of the rat liver proteome, the effects of compounds with known toxicities were analyzed resulting in over 58,000 curated features. More than 16,000 features have been identified from livers of clofibrate-treated Sprague-Dawley rats and of these, 600 features showed greater than 2-fold differential expression. Many enzymes including those involved in the beta-oxidation pathway were annotated, confirming decades of research. Analysis and management of the large volume of data which results from proteomics experiments has enabled the development of the powerful software tool, LifeProt™. Coupling proteomics technology with gene expression microarrays (GEMs) results in an integrated technology platform which may aid in prioritizing lead compounds.

292 PHARMACEUTICAL PROTEOMICS.

S. Steiner. *Large Scale Biology Corp., Rockville, MD.*

Recent progress in genomics and proteomics technologies has created a unique opportunity to significantly impact the pharmaceutical drug development processes. The perception that cells and whole organisms express specific inducible responses to stimuli such as drug treatment implies that unique expression patterns, molecular fingerprints, indicative of a drug's efficacy and potential toxicity are accessible. While genomic-based approaches capture fingerprints at the mRNA level, molecular analysis of drug effects by proteomics creates snapshots of gene regulation at the level of the protein and hence closer to ultimate cell function control. The integration into state-of-the-art toxicology of protein expression profiling promises new insights into mechanisms of drug action and toxicity. The benefits will be improved lead selection, and optimized monitoring of drug efficacy and safety in pre-clinical and clinical studies based on biologically relevant surrogate markers.

293 VALUES AND LIMITATIONS OF TRANSGENIC ANIMALS IN TOXICOLOGY.

D. L. Laskin[1] and A. E. Silverstone[2]. [1]*Rutgers University, Piscataway, NJ and* [2]*SUNY Health Science Center, Syracuse, NY.*

During the past few years there has been increasing use of transgenic animals in elucidating mechanisms of cellular function and more recently in evaluating the potential toxicity and/or carcinogenicity of xenobiotics. While the availability of these animals has advanced our understanding of a variety of biochemical, cellular, and physiological processes, the results need to be interpreted with caution. Null mice lacking particular genes or transgenics overexpressing genes often develop compensatory mechanisms that alter their responsiveness and confound the interpretation of normal function and response. This is particularly true in the immune system where there are multiple and cooperative pathways mediating host defense. Also important is the concept that the response of transgenic animals to a xenobiotic may vary with the genetic background of the animal. This symposium is focused on looking at diverse models of transgenic animals including mice lacking or overexpressing inflammatory and growth factor genes encoding for mediators such as tumor necrosis factor-α, nitric oxide synthase, superoxide dismutase, and transforming growth factor-βs that have been developed and utilized for evaluating tissue injury, apoptosis, receptor activity and carcinogenesis. In each of these systems the values and limitations of the technology will be discussed. Attempts will also be made to compare and contrast the results obtained with transgenic animals to those generated using pharmacologic antagonists, antisense expression, and monoclonal antibodies.

294 STUDIES ON THE ROLE OF INFLAMMATORY MEDIATORS IN CHEMICALLY-INDUCED TOXICITY, TRANSGENIC MODELS *VERSUS* PHARMACOLOGIC APPROACHES.

D. L. Laskin. *EOHSI, Rutgers University, Piscataway, NJ.*

Our research has focused on analyzing the role of macrophages and inflammatory mediators in chemically induced tissue injury. For these studies, two model systems are being used: the lung and the liver. In both of these tissues we have found that xenobiotic exposure is associated with localized accumulation of macrophages. We hypothesize that these cells become activated following toxicant exposure and release mediators that contribute to injury. In support of this hypothesis are our findings that macrophages isolated from the lung or liver of animals treated with toxicants such as ozone or acetaminophen, respectively, are "activated" to release increased amounts of tumor necrosis factor alpha (TNFα) and nitric oxide. To analyze the role of these cytotoxic mediators in toxicity, both pharmacologic inhibitors and knockout mice are being utilized. Whereas in some model systems, both approaches result in similar reductions in others such as hepatic necrosis induced by acetaminophen administration, opposing results are obtained. Thus, in this model, anti-TNFα antibody prevented toxicity, while TNFα knockout mice were more sensitive to acetaminophen. These data suggest that there are limitations in the use of transgenic animals which may be related to compensatory alterations in cytotoxic mediator production. (Supported by NIH Grants ES04738, GM34310 and the Burroughs Wellcome Fund.)

295 RADIATION CHIMERAS AND TRANSGENIC KNOCK-OUTS AS TOOLS TO DEFINE IMMUNE SYSTEM TARGETS FOR DIOXIN RECEPTOR AND ESTROGEN RECEPTOR ACTIVATION.

A. E. Silverstone. *SUNY Health Service Center, Syracuse, NY.*

The historical approach to determining how chemical agents affect the immune system was one of coincidence. For example, thymic epithelial (TE) cells were suggested to be the target for TCDD induced thymic atrophy because (1) levels of the AhR (TCDD receptor) in developing thymic lymphocytes was very low, but in TE cells was high; (2) exposure of TE cultures *in vitro* to TCDD made them less capable of supporting thymocyte proliferation in response to mitogen; (3) TE cell lines treated with TCDD produced IL-1,often associated with cell loss; and(4) TE cells *in vivo* demonstrated altered morphology after TCDD treatment. Our approach instead was to utilize AhR Knock-Out (KO) mice either as a source of hemopoietic stem cells or as recipients for normal stem cells in lethally irradiated recipients. Only in mice with AhR positive hemopoietic cells (even if all other tissues were AhR-KO) did TCDD cause thymic atrophy. While this result was particularly clear,studies with estrogen receptor (ER)a KO mice using a similar approach suggested both tissues were targets for estradiol induced thymic atrophy. However, these chimeras established a role for ERa in non hemopoeitic tissues as being essential for full thymic development. This approach leads to accurate understanding of the cellular targets for immunotoxic agents. Subsequently, one can determine which gene modulated products are actually produced to cause the observed effects. (Supported by ES07216 and ES04862 from NIEHS.)

296 USING TRANSGENICS AND OTHER APPROACHES TO STUDY REGULATION OF ANTIGEN-SPECIFIC T CELL APOPTOSIS BY GLUCOCORTICOIDS.

J. D. Ashwell. *Laboratory of Immune Cell Biology, NIH, Bethesda, MD.* Sponsor: A. Silverstone.

Activation-induced death of thymocytes (negative selection) and antigen-dependent rescue of thymocytes from a default death pathway (positive selection) determine the spectrum of antigenic specificities that T cells. Occupancy of the T cell antigen receptor (TCR) and the glucocorticoid receptor (GR) are potent means of inducing apoptosis in immature thymocytes. Surprisingly, T cells simultaneously signaled via the TCR and the glucocorticoid receptor survive, leading to the speculation that the balance between TCR and GR signaling might regulate antigen-specific T cell selection. Indeed, epithelial cells in the murine thymus were found to produce steroids. Using TCR transgenic mice and inhibitors of glucocorticoid biosynthesis, it was demonstrated in fetal thymic organ culture that thymocyte death caused by blockade of corticosterone biosynthesis requires TCR engagement. The role of glucocorticoids in thymocyte development was also explored in transgenic mice expressing antisense transcripts to the GR under the control of the proximal lck promoter. Data will be presented with these two model systems regarding the effect of inhibition of local glucocorticoid synthesis or responsiveness on murine models of autoimmune disease and the generation of the peripheral T cell antigen-specific repertoire.

297 IDENTIFYING CHEMICAL CARCINOGENS AND ASSESSING POTENTIAL RISK IN SHORT-TERM BIOASSAYS USING TRANSGENIC MOUSE MODELS.

R. W. Tennant, J. E. French, C. S. Trempus, R. E. Cannon and J. W. Spalding. *Laboratory of Environmental Carcinogenesis and Mutagenesis, NIEHS, USEPA, Research Triangle Park, NC.* Sponsor: A. E. Silverstone.

Significant progress in understanding human risk can be achieved by development of models in which the mechanistic process or sequence of events leading to malignant tumors can be studied.Several transgenic and knockout (haploinsufficient) mouse models have been developed and are being assessed for their responses to selected chemicals. Our laboratory has focused on the p53 haploinsufficient (p53±) and Tg.AC (zetaglobin promoted v-Ha-ras) models for carcinogen identification and mechanistic studies. Data will be presented that demonstrates the p53± model preferentially responds to mutagenic carcinogens via loss of function of the wildtype allele. Such loss can occur via deletion, as in the case of phenolphthalein induced thymic lymphomas or via a point mutation as seen in p-cresidine-induced bladder tumors. In addition, p53 function could be altered by an indirect mechanism involving over-expression of a regulatory protein such as mdm2. With the Tg.AC model, transcriptional activation of the transgene leads to papilloma development (reporter phenotype) by altering keratinocyte differentiation. This activation appears to depend upon a unique arrangement of two transgenes linked promoter to promoter. Spontaneous deletions that remove DNA sequences centered around the promoter junction of these transgenes completely eliminate transgene expression and papilloma development. Investigations into the role of this region in the tumorigenic process will be presented.

298 LESSONS FROM TGFβ1-DEFICIENT MICE: STRAIN DIFFERENCES AND GENETIC MODIFIERS OF RESPONSIVENESS.

T. Doetschman, S. Kallapur, S. Engle and I. Ormsby. *University of Cincinnati Medical Center, Cincinnati, OH.* Sponsor: A. E. Silverstone.

TGFβ1-/- mice have multiple defects that vary considerably with genetic background. Whether or not all null mice die of embryonic lethalities, as well as the type of embryonic lethality they die of, are all dependent on genetic background. On the C57BL/6 background, all TGFβ1-/- animals die before implantation. If the genetic background is a 50:50 mixture of 129 and C57BL/6, about half of the null mice survive to birth with no apparent abnormalities, but subsequently succumb to an autoimmune-like inflammatory dis-

ease around weaning age. On the other hand, if TGFβ1-/- mice are an equal mixture of 129 and CF-1 backgrounds, all null embryos undergo implantation, but about half of them die of hematopoietic and vascular defects at the yolk sac stage, and the other half die at weaning age of the inflammatory disease. Genetic background also determines the organs affected by inflammation, particularly in the gastrointestinal tract. Finally, if TGFβ1-/- mice are rescued from the inflammatory disease by genetic combination with mutations that eliminate functional T-cells, the null animals survive to 4-6 months of age, at which time they die of colon cancer. However, they develop colon cancer only if they are on a 129 background. The degree to which these phenotypes are the result of genetic modifiers will be discussed.

299 AIRBORNE PARTICULATE MATTER: PHYSICO-CHEMICAL CHARACTERISTICS AND HUMAN EXPOSURE ISSUES RELATED TO HEALTH EFFECTS RESEARCH AND ASSESSMENT.

J. A. Graham[1] and J. L. Mauderly[2]. [1]*USEPA, Research Triangle Park, NC and* [2]*Lovelace Respiratory Research Institute, Albuquerque, NM.*

Exposure to particulate matter (PM) is associated with excess mortality and morbidity, especially in individuals with cardiopulmonary disease. These epidemiologic findings are the cornerstone of EPA's revision of the PM National Ambient Quality Standards to include PM less than 2.5 microns. Uncertainties in the available information caused the US Congress to stimulate research by having the National Academy of Sciences identify key information needs and by significantly increasing research budgets. Of the several areas of research needed, the ones on mechanisms of the effect(s) and characterization of the causal PM (and perhaps co-occurring gases) are crucial. Key hypothesis for causative factors are: mass, size, number, surface area, chemistry, co-occurring gases, or some combination of them. Many toxicologists are engaging in research on these topics. One major difficulty is understanding the complex nature of PM as a prelude to designing the most effective studies. This workshop is designed to provide background on the nature of PM and exposures. Both aspects are important. Knowing the physico-chemical nature of key classes of PM is basic. For example, there are major differences between ultrafine, fine, and coarse mode particles in addition to size. Biological components might also have important influences. Information on co-occurring gasses is also a major element. Even if the ambient air were perfectly understood, it still is essential to characterize what fractions people are exposed to.

300 AIRBORNE PARTICULATE MATTER: WHAT DO WE KNOW ABOUT WHAT'S REALLY OUT THERE?

G. R. Cass. *California Institute of Technology, Pasadena, CA.* Sponsor: J. Graham.

Atmospheric particulate matter (PM) is distributed in size over the range from a few nanometers to hundreds of micrometers in diameter. Depending on their size, these particles will deposit in different parts of the respiratory system. Air pollutant particles also are chemically complex, and can act to transport strong acids, catalytic trace metals, allergenic proteins, and toxic organic compounds to various sites in the respiratory system. This presentation describes experimental methods that can be used to determine the size and chemical composition distribution of PM. Filter-based sampling systems have been used for more than 20 years to determine the bulk chemical composition of PM in serveral size ranges. These filter samples can be analysed for nearly all of the elements in the periodic chart, analysed for organic compounds for GC/MS, and be subjected to a variety of bioassay techniques to determine the presence of mutagens and allergens. Likewise, electrical aerosol analysers, differential mobility analysers and optical particle counters have provided much data on particle number counts as a function of particle size. Cascade impactors make it possible to simultaneously measure both the size and chemical composition distribution of a particle mixture. Time-of-flight MS now allow both particle size and chemical compostion to be mearsured in real time at the single particle level. Using these methods, considerable information has been acquired that describes atmospheric aerosol characteristics of possible importance to the community of toxicologists, including trace metals levels, the concentration of more than 100 organic compounds, mutagenic potency, allergenic protein concentrations, as well as the concentration and in some cases chemical composition of ultrafine PM. Examples of such data will be given based on measurements made in California and in the Northeastern US.

301 WHAT HAVE GASES GOT TO DO WITH IT?

E. Fujita. *Desert Research Institute, Reno, NV.* Sponsor: J. Graham.

Primary emissions from various combustion sources are very complex mixtures containing thousands of organic and inorganic constituents in the gas and particulate phases. Gases include criteria gases (e.g., CO, NO_2, SO_2), hazardous air pollutants (e.g., toxic volatile organic compounds [VOCs]), and VOCs and semi-volatile organic compounds that are precursors to both ozone and organic aerosols. Once released into the atmosphere, primary emissions are subjected to dispersion and transport and to various physical and chemical processes that determine their ultimate environmental fate. Many organic compounds are emitted at elevated temperatures as a result of combustion and condense rapidly upon cooling to ambient temperatures forming ultrafine and nuclei range particles. The "nuclei" range consists of particles less than 0.08 μm in aerodynamic diameter that are emitted directly from combustion sources. In polluted areas, the lifetimes of particles in the nuclei range is usually less than one hr because they rapidly coagulate with large particles or serve as nuclei for cloud or fog droplets. This size range is generally detected only when fresh emissions sources are close to the measurement site. Some of the gaseous species are converted into particles by series of chemical transformation, forming secondary aerosol. Sulfates and nitrates are the most common secondary particles, though a fraction of organic carbon can also result from VOCs via atmospheric reactions. This presentation will summarize recent findings pertaining to the composition of gases that co-occur with PM emitted from combustion sources. Because aerosols are particles suspended in gases and because gases are part of the formation processes for particles and have toxicity in their own right, it is important to understand the predominant classes of gases and how their presence may vary with PM.

302 BIOLOGICAL PARTICLES: TIME TO COME OUT OF THE CLOSET.

R. Jaenicke, S. Matthias-Maser and S. Gruber. *University of Mainz, Mainz, Germany.* Sponsor: J. Graham.

Atmospheric aerosol research has seen much progress in recent years; chemistry resolves particle sizes, vertical distributions emerge, inclusion in GCM is progressing, variability is realized, and so on. This research shows how important the understanding of the sources is: ocean and desert (soil) surfaces, gas-to-particle-conversion (i.e. hydrocarbon effluvia), cloud evaporation (particle transformation). However, it is hard to understand how the biosphere as a source for preformed particles has been so neglected. For example, the most recent monograph on clouds has not included the source strength of biological material. Scientists seem to be content with up to 50% of unidentified aerosol mass. Primary Biological Aerosol Particles (PBAP) consist of more than "classic" objects like pollen, spores, bacteria, and viruses. Broken parts of plants and insects as well as skin abrasion materials and hair are included. They might be important for the climatic system, acting as cloud condensation nuclei as well as ice nuclei, thus influencing cloud and rain formation. Knowledge about PBAP will be presented. The annual variation of the total concentration is rather small, mostly because resuspension seems to play a role. In the vertical, we see PBAP at elevated layers, mostly because they are prone to long range transport.

303 PERSONAL EXPOSURE TO PARTICULATE MATTER: WHAT DO WE REALLY BREATHE?

P. Lioy and J. Zhang. *Environmental & Occupational Health Sciences Institute, Piscataway, NJ.* Sponsor: J. Graham.

The presentation will provide basic information on exposures to PM and its size fractions that can occur among the general and selected subgroups of the general population. It will examine the quantitative information available on the actual or potential exposures to the coarse, fine and ultrafine fractions, and attempt to illustrate the complexity of the chemical composition in indoor, outdoor and personal air. Included will be the types of situations or activities that can lead to contacts which lead to high exposures. The analysis will use a mass balance approach to describe the current ambient exposure data base and indicate major areas of uncertainty. Specific examples will include well understood and poorly understood components of the PM mass fractions, e.g. sulfates and organic acids, respectively. All these information will be placed into a contextual framework that includes indoor and personal exposures to PM. The discussion will then be focused on illustrative examples of plausible

interaction of outdoor PM and gases with indoor and personal exposures, and the contributions that can be derived from each. Finally, the role of indoor air chemistry on the production of "fresh" particles from gaseous precursors that have both indoor and outdoor sources will be reviewed and placed into a context of daily variation in total exposure.

304 TOXICOLOGY FOR KIDS: THE CLASSROOM EXPERIENCE.

C. A. McQueen[1] and G. S. Yost[2]. [1]*The University of Arizona, Tucson, AZ and* [2]*University of Utah, Salt Lake City, UT.*

One of the long-range goals of the Society of Toxicology is to increase awareness and understanding of toxicology. One effective strategy is to encourage toxicologists to become involved in K-12 and community education. In 1999, the K-12 Education Subcommittee and the Education Committee sponsored a workshop on this topic. This year's workshop builds on the 1999 presentations by focusing on activities and materials that SOT members can use, giving concrete examples of educational do's and don'ts. The program is designed to give the perspective of scientists who have gone into the classroom or community and survived as well as to explore the powerful synergism of the scientist-teacher relationship. The purpose of the workshop is to provide examples of specific approaches, educational tools and demonstrations of proven techniques that will enable toxicologists to give effective presentations.

305 TOXICOLOGY: QUESTIONS AND ANSWERS.

C. A. McQueen and J. K. Norman. *The University of Arizona, Tucson, AZ.*

Toxicologists are becoming more involved in increasing public awareness and understanding of toxicology. This means going into the classroom or community and engaging the audience in a manner that facilitates learning science. One approach is to involve the audience by using an interactive presentation. Scientists are generally most comfortable with slide-based talks which can be easily modified to encourage audience participation. At the University of Arizona, Southwest Environmental Health Sciences Center, a question and answer format based on the web site "Chemicals and Human Health", www.biology.arizona.edu/chh, has been developed. In the oral presentation, a question is raised and the audience is presented with several possible answers. Choices are indicated by a show of hands. The scientific principle, rationale and correct answer are then discussed. Concepts taught in this way include the dose-response relationship, dose calculation, natural and synthetic chemicals as well as organ specific toxicity. This format has been successfully used with both children and adults. It is simple and can be readily adapted to fit the audience and the topic. (Supported by NIEHS, grant ES06694.)

306 GET THE LEAD OUT!

M. O. Dereski. *Wayne State University, Detroit, MI.*

Effective communication of environmental health issues to the public is a fertile ground for the toxicologist. One of the topics that many lay people do not realize still poses a significant health risk is exposure to lead. Lead poisoning remains a prevalent and serious disease for urban as well as suburban children throughout the country. This instructional lesson discusses the history of lead use and exposure, including a hands-on demonstration of qualitative lead testing of household items. The lesson also includes low cost preventative measures that can be taken by an at-risk homeowner to decrease exposure to the sources of lead. This presentation has been effectively given to large groups of school age children (grades 6-12) as well as adult community groups during the previous three years. Materials to supplement the lesson are available to toxicologists who plan classroom visits, or community group presentations. (Supported by grant ES06639-06.)

307 GOING TOXIC IN THE MIDDLE SCHOOL CLASSROOM.

M. L. Haasch. *University of Maryland, Solomons, MD.*

Bringing lessons in toxicology to the middle school classroom provides some excitement and added interest allowing the students to apply textbook knowledge to real-world situations. In order for trained toxicologists to infuse their unique knowledge into the middle school curriculum, two major things must happen. First, the toxicologist must be willing to break down the science into manageable bits that are economically feasible. Second, a mentor-teacher relationship must be established. A good approach for making a connection to a teacher is to use existing county- or statewide programs that provide teacher internships to the toxicology laboratory. The best mentor-teacher relationships require contact with an enthusiastic science teacher who is willing to work a little harder and take chances in order to liven up the classroom. Possible problems that can arise when bringing toxicology into the classroom include concerns for teachers about fitting the material into curricular standards, chaos in the classroom, encountering non-packaged results and vertebrate animal testing. For the toxicologist possible concerns include the demand on time and creative energies as well as facing and dealing with the middle school culture, but the rewards of engaging in these kinds of activities are boundless.

308 THE TEACHER—MENTOR RELATIONSHIP: HOW TO BRING TOXICOLOGY INTO THE MIDDLE SCHOOL CLASSROOM.

A. T. Williams. *Northern Middle School, Owings, MD.* Sponsor: M. L. Haasch.

This presentation will outline effective methods of translating "real world" laboratory experiences into classroom lessons. It details the 4-year collaboration between an 8th grade science teacher and a toxicologist. This connection utilizes the expertise of each partner – the toxicologist shares background knowledge and original ideas; the teacher addresses the state standards for lesson objectives and converts the information into a context suitable for 8th grade students. Both teacher and mentor work together to alter and refine the concepts to be taught. The students benefit because they are actively involved, the labs are meaningful and they learn by doing (hands on – minds on). They also have the experience of working with the toxicologist during class visits. The students develop a new appreciation for science and come to the classroom eager for the day's activities. Ongoing classroom projects include observation of developing Japanese medaka embryos, monitoring effects of runoff water, or fertilizers in ground-filtered water, to freshwater invertebrates. The inclusion of the topic of toxicology is appropriate in science classrooms because it provides a better understanding of experimental design. The use of the scientific method gives a framework for solving any problems in a logical manner.

309 SENSITIVITY OF NEURONAL NITRIC OXIDE SYNTHASE TO LINDANE.

D. Desaiah[1], A. Vann[2] and J. Cameron[2]. [1]*Univ. of Mississippi Medical Center, Jackson, MS and* [2]*Jackson State University, Jackson, MS.*

Organochlorine compounds, in general are neurotoxic chemicals and lindane is a known neurotoxic insecticide. The mechanism of action of lindane is suggested to be its ability to modulate (γ-amino-butyric acid receptor and intracellular calcium as well as calcium dependent enzymes. However, there is no information available on its effects on calcium-calmodulin dependent neuronal nitric oxide synthase (nNOS). nNOS is a constitutive enzyme involved in the synthesis of NO, a biologically important messenger molecule. Therefore, the present studies were undertaken to investigate the direct interaction of lindane with nNOS *in vitro*. The source of nNOS was from normal rat brain cerebral cortex and cerebellum. The tissue was homogenized in buffer (pH 7.2) containing 20mM Hepes, 0.32M sucrose, 0.5 mM EDTA and 1mM dithiothreitol. The homogenate was subjected to differential centrifugation and post 20,000 x g supernatant was used as enzyme source after removing endogenous arginine. The enzyme activity was measured by quantitation of ^{3}H-citrulline formed. Lindane (99.9% pure) dissolved in DMSO and further diluted in the reaction mixture to obtain 3-120μM concentrations. DMSO at 2μl had no effect on nNOS. The results obtained showed a concentration dependent inhibition of nNOS activity by lindane both in cerebral cortex and cerebellum. A 50% inhibition was obtained at less than 10μM lindane. These results clearly demonstrate that lindane is a potent inhibitor of nNOS. nNOS

may be one of the site of action for lindane through which many neurotransmitter pathways are altered including the well established GABAergic pathway. (Supported in part by grant NIH/T32 HL 07635.)

310 THE ROLE OF GLUTATHIONE S-TRANSFERASES IN THE METABOLISM OF METHYL PARATHION *IN VITRO*.

E. L. Abel, T. K. Bammler and D. L. Eaton. *University of Washington, Seattle, WA.*

The organophosphate esters are the most popular class of insecticides in use today because of their efficacy and lack of environmental persistence. However, organophosphate pesticides are highly toxic to mammals. Most organophosphates require activation via oxidative desulfuration in order to exert toxic effects by binding to acetylcholinesterase. The focus of this study was to investigate the ability of glutathione S-transferases (GSTs) to metabolize and detoxify the organophosphate pesticide, methyl parathion (MP), in several mammalian species prior to activation. Of particular interest are human GSTM1 and T1, both of which are polymorphic. If these metabolizing enzymes are shown to play a significant role in the delicate balance of activation and deactivation of MP, the polymorphisms could be useful in identifying individuals at heightened risk for MP poisoning. In order to ascertain the role of human GSTs in the metabolism of MP, liver cytosolic fractions from nine adults of known GST genotype and/or phenotype were incubated in the presence of MP. Metabolites of MP were analyzed by high performance liquid chromatography and UV absorbance detection. Rat, mouse, and non-human primate liver cytosolic fractions and recombinant GSTs were also tested. Of the cytosolic fractions tested, BHA-treated mice showed the highest activity (7.05 nmol desmethyl parathion /min/mg), followed by monkeys (2.47 nmol/min/mg), followed by rats (2.30 nmol/min/mg), with the human samples exhibiting the lowest activity (1.06 nmol/min/mg on average). Interindividual variation among the 9 human samples did not correlate with either the GSTM1 or T1 genotype/phenotype. In addition, of the recombinant GSTs tested (hGSTM1-1, hGSTM2-2, hGSTM3-3, hGSTM4-4, mGSTP1-1, mGSTP2-2, hGSTA1-1, rGSTA5-5, mGSTA3-3) only hGSTA1-1 and rGSTA5-5 showed significant activity (68.77 and 48.76 nmol/min/mg respectively). Our data suggest that neither hGSTM1-1 nor hGSTT1-1 is responsible for the majority of the activity seen in humans.

311 PESTICIDE TRANSPORT MEDIATED BY THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN (MRP).

T. E. Tribull and L. J. Bain. *Clemson University, Department of Environmental Toxicology, Pendleton, SC.*

The multidrug resistance-associated protein (MRP) is a 190kD ATP-binding cassette protein involved in the active efflux of glutathione-, glucuronide-, and sulfate-conjugates from cells. It has been proposed that this transporter originated to protect cells and organisms from toxicity of many naturally-occurring compounds, including pesticides. Although the transport of pesticides in plant MRP homologues has been reported, no research has ever been published on pesticide transport by MRP in vertebrate systems. This is a particular deficit in toxicology as many environmentally relevant chemicals, such as pesticides, can be detoxified by conjugation. We have examined the toxicity of twelve different pesticides in human MRP-transfected NIH3T3 cells compared to mock-transfected control cells. Of the twelve, four of the pesticides, methoxychlor, alachlor, fenitrothion, and chlorpropham, all demonstrate between three- and four-fold greater toxicity to mock-transfected cells versus the MRP-transfected cells. This study suggests that MRP aids in protecting these cells from toxic effects of these pesticides. Future work will examine the actual transport of these pesticides as well as MRP's contribution to altering their toxicity to the organism.

312 CHARACTERIZATION OF OLFACTORY MUCOSAL TUMORS INDUCED IN RATS BY CHRONIC DIETARY ADMINISTRATION OF ALACHLOR.

M. B. Genter[1], D. M. Burman[1], M. W. Dingeldein[1] and B. Bolon[2]. [1]*Dept. of Environmental Health, University of Cincinnati, Cincinnati, OH and* [2]*Dept. of Pathology, Amgen, Thousand Oaks, CA.*

Chronic dietary exposure to the chloracetanilide (CA) herbicide alachlor (ALA) has been shown to induce nasal cavity tumors in rats (US EPA, Office of Pesticide Programs. PB85-175503, NTIS, Springfield, VA 1985). These CA-induced tumors had a distinctive distribution in the nasal cavity and apparently arose in the olfactory mucosa (Morgan *et al., Fundam Appl Toxicol* 36(Suppl. 1, pt. 2) 1997). The present studies were undertaken to identify the cell type of origin of ALA-induced olfactory mucosal tumors and to investigate the mechanism of ALA carcinogenicity. The current data are derived from interim 1 mo and 6 mo time points in an ongoing chronic feeding study. Male Long Evans rats (6 wk of age at the inception of the study) were administered ALA (>98% pure, Chem Service, West Chester, PA) in powdered diet at the dietary equivalent dose of 126 mg/kg/day. Following 1 mo of ALA exposure, neither histological abnormalities nor enhanced cell proliferation (by BrdU incorporation into S-phase nuclei) were observed in any region of the nasal cavity. However, six mo of ALA treatment resulted in increases in S-phase basal and non-basal cells in the olfactory mucosa. In addition, nasal masses ranging from small dysplastic plaques to adenomas (including one dysplastic variant) occurred in 6 of 15 ALA-treated rats after 6 mo of exposure. Age-matched control rats displayed no discernible nasal epithelial changes at either time point. The tumors appeared to originate in the olfactory regions of the nasal cavity; however, the masses consisted of low cuboidal epithelium, contained glands, and were associated with adjacent regions of respiratory metaplasia. Immunohistochemistry revealed that the tumors were negative for various olfactory mucosal markers, including olfactory marker protein (OMP, for mature olfactory neurons) and NMa (for cytochromes P450 CPY2A10/11 and 2G1), as well as p53. These data confirm that ALA is carcinogenic and support a metaplastic respiratory epithelial cell as the cell of origin.

313 CHANGES IN CAMP-DEPENDENT KINASE ACTIVITY AND EXPRESSION OF CA^{2+}/CAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) IN THE BRAINS OF NEONATAL RATS EXPOSED TO CHLORPYRIFOS.

D. A. Jett, R. V. Navoa and R. Beckles. *Johns Hopkins University, Baltimore, MD.*

The alteration of cAMP-related cell signaling by organophosphate pesticides such as paraoxon and chlorpyrifos has been suggested by previous work in our laboratory and others. We report here on the activity of cAMP-dependent kinase (PKA) and the expression of the phosphorylated form of Ca^{2+}/cAMP response element binding protein (phospho-CREB) in the brains of developing rats exposed to chlorpyrifos. Increases in transcription of genes that are critical in neuronal plasticity and brain development are positively regulated synergistically by PKA and Ca^{2+}-mediated kinases which phosphorylate CREB. We injected rat pups subcutaneously with 40 mg/kg chlorpyrifos on postnatal days 7, 11, and 15, and collected their brains on day 16. The chlorpyrifos exposures did not result in any observable signs of cholinergic intoxication or weight loss, but caused moderate inhibition of brain acetylcholinesterase (55%). We developed a method for analyzing cytosolic PKA activity and phospho-CREB expression in nuclear extracts from the same tissue sample (cerebral cortex). PKA was determined by following the phosphorylation of Kemptide substrate; phospho-CREB was analyzed by Western blot using a specific antibody. We found that PKA activity, the expression of phospho-CREB, and the ratio of phospho-CREB to total CREB in treated pups were significantly decreased relative to rats treated only with corn oil vehicle. Expression of total CREB and the neuronal protein tubulin were not affected, indicating that the effects were specific and not related to a decrease in overall protein synthesis. These studies suggest that the reported changes in adenylyl cyclase activity and cAMP levels may produce effects downstream in the pathway. These effects may play a role in the developmental neurotoxicity of chlorpyrifos. (Supported by ES03819 JHU Center in Urban Environmental Health.)

314 NON-NEURONAL TARGETS FOR DEVELOPMENTAL EXPOSURE TO CHLORPYRIFOS: HEPATIC CELL SIGNALING.

J. T. Auman, F. J. Seidler and T. A. Slotkin. *Duke University Medical Center, Durham, NC.*

In addition to inhibition of cholinesterase, chlorpyrifos and its active metabolite, chlorpyrifos oxon, have been hypothesized to interact directly with constituents of cell signaling cascades, contributing to adverse effects on cell replication and differentiation. Among the putative targets are the receptors and transduction proteins involved in the production of cyclic AMP, one of the most important mediators of cell development. We studied the effects of neonatal chlorpyrifos exposure on hepatic adenylyl cyclase (AC), as the liver accumulates the highest concentrations of chlorpyrifos and is the site for generation of chlorpyrifos oxon. Newborn rats were given 1mg/kg of chlorpyrifos s.c. on PN1-4. Twenty-four h after the last dose, there was induction of AC

catalytic activity, measured with manganese, whereas activity requiring an interaction of AC with G-proteins (fluoride, forskolin) was impaired. In addition, the response of AC to hormonal signals was altered in a receptor-selective manner, with an enhanced response to glucagon but not a beta-adrenergic agonist. Treatment of older animals (PN11-14) did not impede G-protein-AC interactions, even when a higher dose of chlorpyrifos was used (5mg/kg/day); however, the response to glucagon was still enhanced in this later treatment group. These results indicate that chlorpyrifos targets the AC signaling cascade in non-neuronal tissues, leading to altered neonatal responses to hormonal input. (Supported by USPHS ES-07031.)

315 CHLORPYRIFOS AND CELL SIGNALING: EXPRESSION OF PHOSPHORYLATED CA^{2+}/CAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) IN PC12 CELLS.

R. V. Navoa, R. Schuh and D. A. Jett. *Johns Hopkins University, Baltimore, MD.*

Phosphorylation of Ca^{2+}/cAMP response element binding protein (CREB) activates the transcription of genes known to be critical in brain plasticity and brain development. It is hypothesized that changes in the phosphorylation of CREB could be produced by changes we have previously observed in cAMP synthesis and cAMP-dependent kinase (PKA) activity after exposure to organophosphates. Thus, some of the developmental neurotoxicity of organophosphates could be explained by inhibitory effects on this pathway. In this study we used PC12 cells as an *in vitro* model for studying the effects of chlorpyrifos (CPF), and its more potent anticholinesterase metabolite chlorpyrifos-oxon (CPFO), on forskolin-stimulated expression of the phosphorylated form of CREB (phospho-CREB). Cells were incubated with various concentrations of CPF or CPFO for one hour prior to a 15 min stimulation with 25 μM forskolin. At the end of this period, cells were immediately lysed in 100°C lysis buffer, and the expression of phospho-CREB was determined by Western blot analysis using a specific antibody. We found that the expression of phospho-CREB was significantly reduced in cells treated with CPFO at low nanomolar concentrations, and these effects were not due to non-specific effects since the expression of the neuronal protein tubulin was not affected. We also found that CPF at low micromolar concentrations reduced forskolin-induced phospho-CREB expression, but a similar reduction was observed with tubulin. But surprisingly, nanomolar concentrations of CPF did appear to cause a specific reduction in phospho-CREB expression, and not in tubulin expression. The data suggest that nanomolar concentrations of CPF and CPFO caused a reduction in the expression of phospho-CREB, possibly through disruption of the cAMP-signaling pathway. Micromolar concentrations CPF also appeared to alter phospho-CREB expression, but it may have resulted from non-specific effects, e.g., cytotoxicity, decreased cell adhesion, or by the reported inhibitory effects of CPF on overall protein synthesis in this cell system. (Supported by ES03819, JHU Center in Urban Environmental Health.)

316 MECHANISM FOR DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS: REACTIVE OXYGEN OR GENE TRANSCRIPTION?

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Chlorpyrifos is rapidly replacing other organophosphate insecticides because of its relative safety and persistence. There is increasing concern about effects on brain development and studies suggest that targets other than cholinesterase inhibition could contribute to such effects. We used PC12 cells, a model for neuronal development which displays adverse effects of chlorpyrifos, to evaluate two proposed mechanisms: production of reactive oxygen species (ROS) and altered gene transcription. ROS production was measured in response to chlorpyrifos and its active metabolite, chlorpyrifos oxon, using an intracellular indicator. First, undifferentiated cells were treated for various times (10 min, 24, 48, 72 h). Next, differentiation was initiated with NGF and cells were examined at 72 h for ROS production. In neither case did chlorpyrifos (up to 50 μg/mL) or chlorpyrifos oxon (10 μg/mL) produce detectable levels of ROS. In contrast, much lower concentrations of chlorpyrifos directly interfered with the binding of AP-1 nuclear transcription proteins to their DNA recognition sites in a cell-free system; addition of chlorpyrifos to undifferentiated or differentiating PC12 cells also altered the expression/function of both AP-1 and Sp1 proteins. These results suggest that chlorpyrifos does not evoke ROS production in the PC12 model but does elicit effects on gene transcription factors that are likely to be involved in adverse effects on cell replication and differentiation. (Supported by USPHS ES-07031.)

317 CHLORPYRIFOS TARGETS MACROMOLECULE SYNTHESIS IN C6 GLIOMA CELLS.

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The major mechanism for systemic toxicity of chlorpyrifos is inhibition of cholinesterase through the active metabolite, chlorpyrifos oxon. However, recent studies indicate that developmental neurotoxicity may also involve the direct targeting by chlorpyrifos itself, of events that are specific to cell replication and differentiation. Earlier, we found that chlorpyrifos exposure of neonatal rats led to delayed CNS cell loss during periods of synaptogenesis and gliogenesis. In the current study, we used cultured rat C6 glioma cells to determine whether chlorpyrifos itself affects cell replication in glial-type cells. DNA and protein synthesis were measured by incorporation of radiolabeled precursors into macromolecules during the log-phase of cell growth. With as little as 2h of exposure, chlorpyrifos caused a robust, concentration-dependent inhibition of DNA synthesis, with a threshold between 0.5 - 5 μg/mL; the effect in the glial line was greater in magnitude than had been seen previously using a neuronal cell line (PC12). Effects were selective for DNA synthesis, as much higher concentrations (50 μg/mL) were required to produce significant inhibition of protein synthesis. The reduction in DNA synthesis caused by chlorpyrifos exposure had an adverse effect on cell replication, as total cell number was significantly reduced in treated cells. Our results indicate that chlorpyrifos itself can exert adverse effects on neurodevelopment, targeting the proliferation of glial-type cells. (Supported by USPHS ES-07031 and the Ford Foundation.)

318 BEHAVIORAL DEFICITS AFTER EXPOSURE OF NEONATAL RATS TO THE INSECTICIDE, CHLORPYRIFOS.

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The widespread use of chlorpyrifos both agriculturally and in the home has led to increased concern over potential consequences of fetal and childhood exposures. Previous studies showed that apparently subtoxic doses of chlorpyrifos nevertheless target brain development, inhibiting mitosis, producing cell loss and altering synaptic activity. To determine whether these biochemical effects are associated with behavioral deficits, we evaluated locomotor activity and coordination skills in developing rats. Administration of 1 mg/kg of chlorpyrifos on postnatal days (PN) 1-4 elicited deficits in reflex righting on PN 3-4 and negative geotaxis on PN 5-8; the effect was gender-specific, with a greater effect on females. However, with the ontogeny of more complex behaviors, adverse effects of chlorpyrifos became more evident in males. Locomotor activity in an open field, measured on PN 21 and 30, was markedly reduced in male rats who had been exposed to chlorpyrifos on PN 1-4, whereas no differences were detected in females. These studies indicate that, even at levels below the threshold for overt toxicity, gender-selective behavioral abnormalities are present during and immediately after chlorpyrifos exposure, but also appear at later stages of development, well after the disappearance of chlorpyrifos from the body. (Supported by a STAR Fellowship from the US Environmental Protection Agency.)

319 ACTIVATION OF THE AH RECEPTOR SIGNAL TRANSDUCTION PATHWAY BY PROSTAGLANDINS.

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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that mediates many of the biological and toxicological actions of a diverse range of chemicals, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Although no endogenous physiological ligand for the AhR has yet been identified, numerous studies support the existence of such a ligand(s). Previous studies indicating an effect of TCDD and the AhR on arachidonic acid and prostaglandin synthetic and metabolic enzymes prompted us to examine whether these chemicals could activate the AhR signaling system. Using a recombinant cell bioassay system, we demonstrate that relatively high concentrations of several prostaglandins can activate a DRE-luciferase reporter gene in a dose- and AhR-dependent manner. In some cases, the magnitude of induction was several time greater than that produced by a maximal inducing dose of TCDD. Those prostaglandins which induced AhR-dependent gene expression also stimulated AhR transformation and DNA binding *in vitro*, suggesting that they are direct-acting. These data coupled with ligand binding analysis indicate that the prostaglandins are relatively weak AhR ligands. The

observed synergistic induction of AhR-dependent gene expression by selected prostaglandins is likely due to their ability to also activate other cell signaling systems which augment AhR signaling. Our results indicate that selected prostaglandins represent a structurally distinct and novel class of activators of the AhR signal transduction pathway (ES07685, ES07072, ES04699).

320 SYNERGISTIC ACTIVATION OF AH RECEPTOR-DEPENDENT GENE EXPRESSION BY ACTIVATORS OF PROTEIN KINASE C.

E. M. Khan and M. S. Denison. *University of California, Davis, CA.*

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which can be activated by a wide variety of structurally dissimilar chemicals. In addition to its role in modulating gene expression, the AhR mediates many of the toxic and biological effects of persistent AhR ligands, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Currently, no endogenous physiological AhR ligand has been identified. Using AhR ligand and DNA binding assays, we have identified several classes of bioactive lipids which can bind to and transform the AhR into its DNA binding form *in vitro*, albeit weakly. However, exposure of cell lines to some of these chemicals results in induction of AhR-dependent gene expression to levels equivalent to or several-fold greater than that produced by a maximally inducing dose of TCDD. The known ability of some of these compounds to activate second messenger systems suggests that the synergistic increase in gene expression is due to the concomitant activation of the AhR and second messenger signal pathways. Co-treatment or pretreatment of cells with the PKC activator, phorbol-12-myristate-13-acetate (PMA), synergistically increases the ability of AhR ligands (TCDD and β-naphthoflavone) to induce AhR-dependent gene expression; addition of PMA two hours after TCDD has no effect. Inhibitors of PKC activity block this induction. Not only do our results indicate that cross-talk between AhR and second messenger signaling pathways can synergistically enhance AhR-dependent gene expression, but they suggest a scenario in which AhR-dependent signaling by weak ligands (endogenous or exogenous) can be dramatically enhanced. (NIEHS ES07072)

321 ROLE OF THE ARYL HYDROCARBON RECEPTOR (AHR) IN THE ANTIESTROGENIC EFFECTS OF TCDD IN THE MOUSE UTERUS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) causes a decrease in mouse uterine (Ut) weight. We determined previously that TCDD inhibits estrogen (E2)-induced Ut epithelial mitogenesis and that this effect requires AhR. The aim of this study was to further characterize the Ut epithelial response to TCDD and the role of AhR. To do this, ovariectomized wild-type (wt) and AhR knockout (AhRKO) mice received either oil, E2 (30 ng) or a combination of 5 μg/kg TCDD followed 24 h later by 30 ng E2. Northern blot analysis indicated that E2 stimulated similar large increases in Ut epithelial mRNA expression for the secretory protein lactoferrin (LF) in both wt and AhRKO mice compared to oil-treated controls. However, in wt mice, Ut LF mRNA was reduced 38% ($p<0.05$) by TCDD+E2, compared to E2 alone. In contrast, TCDD did not alter LF mRNA in E2-treated AhRKO mice. To investigate the tissue-specific role of AhR on TCDD-induced inhibition of epithelial mitogenesis, Ut stroma (S) and epithelium (E) from AhRKO and wt mice were enzymatically separated and recombined into four types of Ut tissue recombinants (wt-S + wt-E, wt-S + AhRKO-E, AhRKO-S + wt-E, AhRKO-S + AhRKO-E). Tissue recombinants were grafted into female nude mice, which were later ovariectomized and treated as above (oil, E2 or TCDD+E2); then epithelial labeling index (LI) was determined by thymidine autoradiography. Epithelial LI in wt-S +wt-E grafts from host animals given TCDD 24 h before E2 was reduced compared to those in E2-treated hosts. Similarly, LI in wt-S + AhRKO-E from hosts exposed to TCDD 24 h before E2 treatment, was reduced 33% ($p<0.05$) compared to E2 controls. Further, epithelial LI in AhRKO-S + wt-E and AhRKO-S + AhRKO-E was not affected by TCDD, even when epithelial AhR was present. We conclude that TCDD inhibits normal Ut epithelial secretory activity in response to E2 and that AhR is necessary for this inhibition. Also, TCDD inhibition of Ut epithelial mitogenesis appears to be mediated indirectly through stromal AhR, suggesting that activation of the AhR by TCDD disrupts the normal Ut stromal E2 signaling pathway. (Supported by an Interdisciplinary Environmental Toxicology Scholarship from Univ of IL to DLB and NIH Grant ES01332 to REP.)

322 THE ROLE OF RETINOIDS IN DIOXIN TOXICITY.

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Retinoids play a continuing role in many aspects of adult life. In addition, retinoids are critically involved in differentiation and embryonic development. An increasing amount of data suggest that retinoid disturbances, could be a general toxic mechanism for a large number of compounds, including environmental pollutants, pharmaceuticals, and alcohol. This study, which demonstrates structure-activity relationships and species and strain variations in the retinoid response, strongly suggest a role for retinoid disruption in dioxin toxicity, and a role for the Ah-receptor in retinoid disruption. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces early, long-lasting, and marked increases in renal retinyl ester levels, renal retinol esterifying lecithin:retinol acyl transferase (LRAT) activity, and serum retinoic acid level in rat studies. Strongly correlating time- and dose-curves suggests a causal relationship between the three parameters. Increased whole body turnover and mobilization of retinoids from the liver by TCDD has been demonstrated by the use of model-based compartmental kinetic analysis. Hepatic retinoid reduction is a common response in all species investigated, which conforms to the assumed additive mechanism of action for dioxinlike compounds. In contrast, effects on serum and renal retinoid levels differ between species and strains. In addition, renal and serum retinoid responses show structure-related differences and non-additive interactions between different model PCB congeners, suggesting that disruption of the retinoid system can occur via different mechanisms. Together, these data strongly suggest a role for retinoid disruption in dioxin toxicity and a role for dioxins as specific tools to further clarify important steps in the regulation of retinoid metabolism.

323 MICROARRAY ANALYSIS OF IMMEDIATE EARLY GENE EXPRESSION IN RESPONSE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Although numerous cellular and molecular processes altered by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been identified the sequella of events ultimately regulating toxicity of this compound remain undefined. In humans, an array of pathological responses ranging from teratogenic events to carcinogenesis have been attributed to exposure to TCDD. Although differential responses to TCDD exposure observed in humans have been attributed to potential genetic polymorphism, the acute chloracne response to high concentrations of TCDD is consistantly observed. Chloracne is believed to result from aberrant functioning within skin hair follicle and endothelial cells. To begin to address this issue we used mRNA isolated from primary cultures of human dermal microvascular endothelial cells in conjunction with microarray techniques to identify immediate early genes whose expression is altered by treatment with TCDD. Specific expression profiles of identified human genes as well as uncharacterized sequences were induced by TCDD. This distinct pattern of gene expression may be useful in determining processes critical for TCDD toxicity. (Supported by NIH grant ES 06897.)

324 DIOXIN INDUCED CHANGES IN GLOBAL EXPRESSION AS MEASURED USING EST FREQUENCY AND CDNA MICROARRAYS: IDENTIFICATION OF A SECOND GENE BATTERY.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin ("Dioxin") is a highly toxic environmental contaminant and one of the most potent liver tumor promoters ever described. Genetic and pharmacological evidence suggests that all of the biological effects of dioxins are mediated through binding to a ligand-activated transcription factor known as the aryl hydrocarbon receptor (AHR). The most widely studied AHR-regulated genes are xenobiotic metabolizing enzymes (XMEs), such as Cyp1a1, Cyp1a2, Cyp1b1 and Udpgt. Induction of the XME gene battery is thought to be part of an adaptive metabolic response to a family of structurally related toxicants and has not been shown to be related to any toxic endpoint. In an attempt to identify a responsive gene battery that might play a role in the hepatocarcinogenicity of dioxin, we initiated a comprehensive survey of dioxin-induced changes in liver gene expression using both expressed sequence tag (EST) frequencies and cDNA microarrays. Based on frequency analysis of nearly 5,000 ESTs derived from the livers of dioxin-

treated (10 mg/kg) and control mice, the overall inventory of liver mRNA transcripts was altered by approximately 30% following exposure to dioxin. In addition, significant changes were identified in a large number of genes not previously shown to be responsive to dioxin. In total, eleven genes were found to be significantly up-regulated by dioxin and nine genes were significantly down-regulated. Analysis of these changes using cDNA microarrays confirmed the EST results and revealed that a second battery of genes paralleled the XME response at all doses investigated (100, 10, 1, 0.3, and 0.05 mg/kg). Experiments in cell culture demonstrated that the changes in the second putative dioxin-inducible battery are mediated by more than one AHR-dependent mechanism. These studies provide molecular evidence that there exists a second battery of dioxin-inducible genes and that the transcriptional inventory of the liver as a whole is significantly altered following dioxin exposure.

325 MECHANISM OF INHIBITION OF TCDD-INDUCED GENE EXPRESSION BY ADENOVIRUS ONCOPROTEIN $E1A_{243}$: ROLE OF COADAPTOR PROTEINS IN AHR FUNCTION.

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The aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, activates gene expression via binding to the dioxin responsive element (DRE) adjacent to target genes such as CYP1A1. Recent *in vitro* evidence suggests that interactions between the AhR and/or Ah receptor nuclear translocator (Arnt) and coadaptor proteins within the nucleus modulate AhR-dependent signal transduction. Here we have investigated the functional significance of the reported interactions of AhR/Arnt with the retinoblastoma (Rb) and p300 coadaptor proteins using the adenovirus oncoprotein $E1A_{243}$ which is capable of binding both Rb and p300. Previously, $E1A_{243}$-dependent inhibition of TCDD-inducible CYP1A1 expression was solely attributed to inhibition of DRE function via disruption of Arnt:p300 complex formation. Using a combination of reporter and expression plasmid constructs containing discrete regions of the CYP1A1 gene as well as wild type and mutant $E1A_{243}$ proteins which vary in their ability to bind either Rb or p300 respectively, we show that high levels of $E1A_{243}$ directly inhibit CYP1A1 promoter function in a manner independent of $E1A_{243}$ binding to Rb or p300. However, low levels of $E1A_{243}$ expression have minimal effects on the promoter and appear to negatively regulate AhR-dependent signaling in a Rb- and p300-dependent manner. These studies suggest a functional role for both Rb and p300 in AhR-dependent gene transcription and demonstrate the importance of examining both enhancer and promoter function when evaluating the role of coadaptors in inducible gene transcription. (Support: NIEHS grant ES07072.)

326 TRANSIENT EXPRESSION OF CYP1A1 IN RAT EPITHELIAL CELLS CULTURED IN SUSPENSION.

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CYP1A1 is known to be induced in human epidermal cells by suspension in methylcellulose-containing medium. The induction is Ah receptor-dependent, but other aspects of the mechanism remain to be elucidated. In present work, CYP1A1 was induced in cultured epithelial cells from a variety of rat epithelia (epidermis, esophagus, vagina, endometrium) upon suspension. The induction was dramatic but transient, where CYP1A1 mRNA reached substantial levels by 1 hr, was maximal by 4-5 hr, and disappeared by 8-10 hr. In addition, suspension induced transcription from a transfected luciferase reporter gene driven by a fragment containing the dioxin-response elements from the mouse CYP1A1 promoter. Although no exogenous Ah receptor ligand was added, induction was strongly inhibited by addition of alpha-naphthoflavone, consistent with generation of an endogenous ligand during suspension. Suspension resulted in activation of the Ah receptor as determined by mobility shift assay using nuclear extracts from suspended cells, but the methylcellulose medium itself failed to activate Ah receptor in cytosolic extracts of rat keratinocytes or mouse Hepa cells, indicating that this medium was not a source of ligand. Addition of TCDD to the suspension medium did not prolong the period of induction, but the protein synthesis inhibitor cycloheximide extended maximal induction beyond 12 hr. The kinetics of the induction process in cultured rat cells in suspension thus are consistent with generation first of an endogenous ligand and then of a relatively unstable protein acting to repress transcription.

327 MAXILLARY AND MANDIBULAR OSTEOINVASIVE PERIODONTAL SQUAMOUS PROLIFERATION IN MINK FED 3,3',4,4',5-PENTACHLOROBIPHENYL OR 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN.

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Polychlorinated aromatic hydrocarbons, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), are persistent environmental contaminants. Mink (Mustela vison) are extremely sensitive to the toxic effects of these compounds and serve as sentinels of environmental contamination. In a study designed to examine the effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on growing mink, an unexpected lesion, maxillary and mandibular osteoinvasive periodontal squamous proliferation, was observed in all 20 animals that were fed 24 ppb PCB 126 beginning at 12 weeks of age. After receiving the PCB-treated diet for 31 days, the first mink was observed having difficulty chewing. Gross examination revealed mandibular and maxillary lesions consisting of swelling of the upper and lower jaws with nodular proliferation of the gingiva and loose teeth with increased gingival surface area. Radiographs indicated periodontal osteolysis of the maxilla and mandible. As the trial progressed, mink were euthanized as the clinical condition became apparent. The last mink was euthanized on day 69 of the trial. A subsequent trial was conducted to verify the lesion. Six-week-old and 12-week-old mink were administered either 24 ppb PCB 126 or 2.4 ppb 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) via the feed. Within 2 weeks, maxillary and mandibular osteoinvasive periodontal squamous proliferation was detected histologically in both PCB 126- and TCDD-treated mink. These results document that certain polychlorinated aromatic hydrocarbons cause periodontal disease.

328 DIOXIN CONGENERS IN TISSUES OF VIETNAMESE LIVING IN THE NORTH AND SOUTH OF VIETNAM FROM 1970 THROUGH 1999 FROM AGENT ORANGE AND OTHER SOURCES.

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The largest dioxin (TCDD) contamination known was from spraying Agent Orange in Vietnam 1962-71. TCDD milk analyses in 1970 and 1973 showed extremely high TCDD levels, up to 1,850 and 400 parts per trillion (ppt) lipid respectively. Epidemiological studies have usually been conceptualized assuming most exposure was from the original spraying and that dose could be backcalculated using a half life of about 7-9 years. Blood dioxin levels from 1999 samples suggest elevated levels are still present and may sometimes be from new contamination. Blood TCDD levels of as high as 271 ppt (TEQ 300) have been found in blood from residents living near a US storage base in Binh Hoa which had known Agent Orange leakage. Also, a family of four which had moved from the north of Vietnam to the south after Agent Orange spraying ended was found to have TCDD blood levels of 65-85 ppt consistent with exposure from a recently contaminated food supply, especially fish from the Binh Hoa airbase potential TCDD contamination into nearby waterways. Average south Vietnamese blood TCDD is about 4 ppt and northerners have about 2 ppt. These recent finding complicate epidemiology studies because of the varying times of dose intake but uniquely provides large populations with recent as well as older TCDD exposures. This unfortunate set of circumstances may provide opportunity for toxicokinetic studies in addition to health studies of men, women, children, and of several generations.

329 MITOCHONDRIAL MECHANISMS OF DEVELOPMENTAL TOXICITY: CRITICAL EVENTS AND INTERVENTION.

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Mouse embryos lacking the *Trp53* gene (p53) are prone to spontaneous neural tube defects and altered teratogenicity. Differential display of p53-deficient mutant embryos revealed under-expression of 16S mitochondrial ribosomal RNA (16S rRNA), confirmed by metabolic profiling, as the early embryo

switches from anaerobic (glycolytic) to aerobic (oxidative) metabolism. In the present study, we tracked 16S rRNA during the critical period following teratogen exposure. Pregnant mice were injected intraperitoneally with 5 mg/kg 2-chloro-2'-deoxyadenosine (2CdA) on GD8 or 5 mg/kg methylmercury (MeHg) on GD9; expression PCR analysis used primers specific for 16S rRNA and β-actin to control for input RNA. Levels of 16S rRNA were significantly altered in either treatment scenario. 2CdA raised 16S rRNA levels by about 40% ($P < 0.01$) whereas MeHg lowered 16S rRNA by about 75% ($P < 0.01$) versus respective control embryos. Embryos were rescued from either teratogen-induced alteration in 16S rRNA when pregnant dams were injected with 4 mg/kg PK11195, an isoquinoline carboxamide specific for the mitochondrial peripheral-type benzodiazepine receptor (PBR). PK11195 protected embryos from developmental toxicity. For example, it protected fetuses from 2CdA-induced microphthalmia and fetal weight reduction. Rescued embryos did not display 2CdA-induced changes in p53 protein at 4.5h post-treatment, nor was therapy successful once p53 protein induction had occurred. These results suggest that: (1) deregulation of 16S rRNA expression represents a broad-based physiological response to chemical exposures; and (2) the mitochondrial peripheral-type benzodiazepine receptor may provide a critical link between toxicant effects and cellular responses. (Supported by grants RO1 ES09120 and T32 ES07282 from the NIEHS.)

330 ONTOGENY OF NAD KINASE IN THE RAT CONCEPTUS.

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NAD^+ kinase (NADK) is the only known enzyme in living systems to catalyze the formation of $NADP^+$ from NAD^+. The capacity to maintain an adequate supply of NADP(H) has important implications for developmental biosynthesis and embryonic biotransformation. Therefore, we believe it is important to determine the activity of this enzyme during development. No detectable activity was observed in GD 10 conceptuses. By GD 11 specific activities of 1.8 and 7.0 pmoles $NADP^+$/min/mg protein were measured in embryo and VYS, respectively. The VYS activity decreased thereafter with an increase in gestational age. By GD 18, the specific activity in the VYS had dropped to 0.5 pmoles $NADP^+$/min/mg protein. Developmental ontogeny profiles were generated for several other tissues at gestational times where adequate tissue amounts were available. The specific activity of NADK in the placenta increased from 1.3 pmoles $NADP^+$/min/mg protein on GD 11 to 32.7 pmoles $NADP^+$/min/mg protein on GD 15. Specific activities in the heart decreased with increasing gestational age (3.75 pmoles $NADP^+$/min/mg protein on GD 13 to 0.14 pmoles $NADP^+$/min/mg protein on GD 21). In contrast, the activity in the liver increased from 1.7 pmoles $NADP^+$/min/mg protein on GD 15 to 51.1 pmoles $NADP^+$/min/mg protein on GD 21. The specific activity was also determined in the brain where it peaked at 5.4 pmoles $NADP^+$/min/mg protein just before birth. In the lung, the activity increased from 0.9 pmoles $NADP^+$/min/mg protein on GD 17 to 5.9 pmoles $NADP^+$/min/mg protein on GD 21. However, it dropped in the kidney from 2 pmoles $NADP^+$/min/mg protein on GD 17 to 1.12 pmoles $NADP^+$/min/mg protein on GD 21. These results demonstrate time-dependent and tissue-specific variations in the activity of NAD^+ kinase. Variations in enzyme activities reflect alterations in functional needs for cofactors during differentiation and may reflect a cooperation between tissues to optimize detoxification capacity. This is of particular importance when chemical exposure during pregnancy disrupts the pyridine nucleotide redox status and the conceptus must then rely on NADK to provide additional NADP(H). (This research was supported by ES05235.)

331 ALTERATIONS IN ESTROGEN RECEPTOR (ER) ALPHA, ER BETA, AND LACTOFERRIN (LF) EXPRESSION IN REPRODUCTIVE TRACT TISSUES FOLLOWING TREATMENT WITH GENISTEIN OR DIETHYLSTILBESTROL (DES) DURING DEVELOPMENT.

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Permanent alterations result in both male and female mice following developmental exposure to synthetic and natural occurring estrogenic compounds. Reports from our laboratory have shown that perinatal exposure of mice to DES results in impaired fertility and reproductive tract lesions. Long-term consequences of DES exposure on days 1-5 include a 95% incidence of uterine carcinoma. Similar long-term effects have been shown with other estrogens suggesting the developing reproductive tract is uniquely sensitive to compounds with estrogenic activity. To study the developmental effects of the phytoestrogen, genistein, we tested the possibility that neonatal exposure to this compound would alter estrogen signaling pathways in target tissues; these changes were compared to tissues similarly exposed to DES. The ontogeny of ERα, ERβ, and LF in differentiating reproductive tracts of control CD-1 mice (fetal day 14 through PND 26) was determined using a ribonuclease protection assay and immunohistochemical analysis. ERα was present in both ovary and reproductive tract; ERβ was in the ovary but not uterus. LF wasn't detected in uterine tissue until approximately postnatal day 19. Following neonatal treatment on days 1-5 with genistein (500-50,000 μg/kg) or DES (0.01-1,000 μg/kg), reproductive tract tissues were compared to controls to determine if treatment altered the expression of these proteins. Genistein or DES-exposed mice showed an increase in LF in the uterus on day 5, the last day of treatment. Further, data from genistein or DES neonatally exposed mice showed alterations in ERα and ERβ expression. The dose response and time course for ERα, ERβ, and LF expression is being determined, as well as, the relationship of altered protein expression to long-term adverse consequences.

332 MOLECULAR AND CELLULAR PATHOGENESIS OF 5-AZA-2'-DEOXCYTIDINE-INDUCED MURINE HINDLIMB DYSMORPHOGENESIS.

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5-AZA-2'-deoxycytidine (d-AZA) elicits defects and altered gene expression in mouse hindlimbs. Segmental formation of limb regions (stylopod, zeugopod, and autopod) is partially dependent upon Hox gene activation. The objective of this study was to understand the early pathogenesis and molecular etiology of d-AZA-induced hindlimb defects. Semi-quantitative RT-PCR was used to analyze hox gene expression (Hox A and D homologs, paralogs 9 - 13). Untreated and treated fore and hindlimb buds were collected 12 and 24 hrs after i.p. treatment (1mg/kg of d-AZA at 9 am on gestational day (GD) 10) and processed for RT-PCR. Other pregnant mice were treated similarly and whole embryos collected 12 and 24 hours post-treatment and processed for histopathological analysis. No changes in hox gene expression were detected in the forelimb tissue. There was a two-fold down-regulation of hox a-11 in the hindlimb bud tissue. No changes in the hoxd series were detected in the hindlimb bud tissue. The 12 and 24 hr. untreated mice exhibited findings consistent with physiological apoptosis. The treated mice exhibited necrosis and inflammation in most embryonic tissues. The hindlimb buds of the 12 hr. treated tissue exhibited axonal vacuolation. Thickening of the apical ectodermal ridge was evident in the 24 hr. treated limb buds (more severe in the hindlimb buds). Further, 6-aza-2'-deoxycytidine does not induce developmental toxicity at doses up to three times that of d-AZA. The data supports the hypothesis that altered gene expression via alterations in DNA methylation is associated with d-AZA-induced dysmorphogenesis. Further work is ongoing to determine the interplay of altered expression cascades and to better define the genotype/phenotype relationship in d-AZA-induced limb alterations.

333 MATERNAL IMMUNOSTIMULATION PROTECTS AGAINST URETHANE-INDUCED CLEFT PALATE.

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Non-specific immunostimulation has reported efficacy in humans for reducing spontaneous early resorptions and abortions. This technique has further been reported to decrease malformations in mice caused by teratogenic chemicals. In the present study, ICR mice were exposed to urethane alone or together with Freund's complete adjuvant (FCA) or IFNγ. Urethane alone induced cleft palate in 70% of fetuses. Immunostimulation with IFNγ or FCA decreased cleft palate occurrence to 45% and 27%, respectively. Severity of the defect was reduced in fetuses from IFNγ-treated mothers compared to FCA-treated mothers. On gd 14, expression of genes bcl2, p53, and pkC was lower in fetal palate from urethane-exposed mothers compared to control. The bcl2/p53 ratio after urethane exposure was shifted in favor of p53. FCA immunization did not alter fetal bcl2 or pkC expression, but resulted in a further decrease in p53 expression which tended to normalize the bcl2/p53 expression ratio. Maternal IFNγ injection restored fetal palate bcl2 and pkC expression without changing p53 expression. Thus, with IFNγ treatment the bcl2/p53 expression ratio exceeded control. Urethane downregulated placental TGFα, TGFβ3, IGF II, wee-1 and bcl2 genes. However, FCA and IFNγ treatments normalized these gene expression profiles in placenta. These data

suggest that maternal immunostimulation may protect against urethane-induced cleft palate in rodents by normalization of critical gene expression during development. (Supported by NIH RO1-ES 09642-01.)

334 SECALONIC ACID D INHIBITS PROTEIN KINASE C ACTIVITY AND THE FORMATION OF AP-1 COMPLEX IN THE DEVELOPING MURINE PALATE.

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Secalonic acid D (SAD), a teratogenic mycotoxin, causes cleft palate (CP) in the offspring of exposed mice in association with inhibition, *in vitro*, of protein kinase C (PKC), an essential component in signaling pathways mediating cell proliferation and differentiation. In this study, we investigated whether the inhibition of PKC by SAD also occurs *in vivo* and if so what other down stream events are altered. Protein kinase C activity assays involving the transfer of g-phosphate from [32P] ATP to the substrate, using palatal tissue extracts from control and SAD-exposed litters, showed that SAD inhibited the activity of Ca^{2+}-dependent PKCs significantly more than the Ca^{2+}-independent ones. Activation of PKC is known to stimulate the formation of AP-1 (containing c-jun and c-fos in general and CREB/CREM in select tissues), which binds to the TPA responsive elements (TRE) on the DNA to influence gene transcription. Electrophoretic mobility shift assays performed with nuclear extracts from control and treated palatal tissues using the [32P] labeled AP-1 consensus oligonulceotide, demonstrated that the palatal AP-1 complex contains CREB but not CREM and that SAD inhibited the AP-1 complex formation at all time points tested compared to controls. These results suggest that SAD-induced PKC inhibition in the developing mouse palate affects downstream events and is likely to alter gene expression. (Supported by NIH grant # DE/OD 11822.)

335 EVALUATION OF THE PEROXYNITRITE PATHWAY IN PHENYTOIN EMBRYOTOXICITY USING INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) KNOCKOUT MICE IN EMBRYO CULTURE.

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Existing evidence indicates that teratogencity of the antiepileptic drug phenytoin involves reactive oxygen species (ROS), such as superoxide anion ($O_{2\cdot-}$), which oxidatively damage embryonic cellular macromolecules. $O_{2\cdot-}$ can react with nitric oxide generated by NOS forming peroxynitrite, which also causes macromolecular damage. To test whether the embryonic peroxynitrite pathway contributes to phenytoin embryotoxicity, B6129PF2 iNOS knockout and congenic wild-type mouse embryos were cultured on gestational day 9.5 (vaginal plug=day 1) for 24 hr with either a therapeutic concentration of phenytoin (20 μg/ml, 80 μM) or its vehicle (0.002N NaOH). Compared to wild-type controls, iNOS knockout embryos were partially protected from phenytoin embryopathies, with increases of 2.4-fold in anterior neuropore closure ($p<0.05$), 3.0-fold in turning ($p<0.05$), 4.4% in yolk sac diameter ($p=0.001$) and 15% in somite development ($p<0.001$). These results suggest that iNOS is constitutive during organogenesis, unlike in adult tissues where it is generally nonconstitutive, and that the embryonic peroxynitrite pathway interacts with ROS in the mechanism of phenytoin embryotoxicity. The partial nature of the protection may be due to peroxynitrite-independent ROS damage and/or induction of other embryonic NOSs. (Support: Medical Research Council of Canada.)

336 REACTIVE OXYGEN SPECIES IN DEVELOPMENTAL METHYLMERCURY NEUROTOXICITY.

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The developmental neurotoxicity of methylmercury (MeHg) has been well studied, yet the underlying mechanisms remain largely unknown. In the developing brain, MeHg-induced injury involves both the forming cerebellum (CEL) and cerebral cortex, and can be ameliorated by vitamin E. Recent data suggest that MeHg increases the production of reactive oxygen species (ROS) in the brain of adult rodents, but the role of ROS in developmental MeHg neurotoxicity has not been determined. This study was undertaken to determine whether MeHg increases ROS production in the developing brain, and whether the effect varies according to brain region and/or subcellular fraction. Balb/c mouse pups were injected s.c. with 4.0 mg/kg MeHg or PBS on postnatal days (PND) 6, 8, and 10 and killed 24 hours after the final dose. CEL was separated from the rest of the brain (ROB); synaptosomes and mitochondria from both regions were prepared by differential centrifugation and ROS determined using the DCF assay. Dichlorofluoroscin diacetate (DCFH-DA), a stable non-fluorescent molecule, readily crosses cell membranes and is hydrolyzed by esterases to DCFH, which is trapped intracellularly. Subsequent oxidation to the fluorescent DCF was determined at excitation 488 nm and emission 525 nm. ROS production in brain was variable, with only moderate elevations in CEL mitochondria and ROB synaptosomes and no significant change in CEL synaptosomes or ROB mitochondria. Results suggest that acute MeHg treatment during the perinatal period does not yield marked and widespread production of ROS in the brain. Scavenging of ROS by antioxidant systems such as glutathione or ascorbate, or the immature pattern of lipids in the perinatal brain may also limit the detection of ROS. Assessment of antioxidant systems in these animals is currently under way. (Supported by NIH ES 04976 and ES-05022.)

337 ALTERED LEVELS OF APOPTOTIC AND NEUROTROPHIC FACTOR MRNA IN DEVELOPING RAT BRAIN FOLLOWING EXPOSURE TO MERCURY VAPOR OR LEAD ACETATE.

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Both apoptotic and neurotrophic factors play essential roles in the development of the brain and formation of the neural networks. Due to evidence suggesting apoptosis as a mode of action following developmental exposure to mercury and lead, we examined the temporal and regional profiles of mRNA levels for various pro- and anti- apoptotic factors following exposure to either mercury vapor or lead. The mRNA levels for bax, caspase 3, caspase2, bcl-x, and BDNF (brain derived neurotrophic factor) were examined in the postnatal rat brain following inhalation (nose-only) exposure to mercury vapor ($2mg/m^3$) at gestational days 6-15 or postnatal (PND 1-21) exposure to lead acetate (0.2%). Our results indicate an elevation in mRNA levels of both apoptotic factors and BDNF that were metal and brain region specific. Following exposure to mercury vapor, mRNA levels for the above factors increased in the cerebellum at PND 21while levels in the frontal lobe remained unchanged. Following lead acetate exposure, the hippocampus was most affected with significant elevations in caspase 3 and bax observed at PND 12 and post-weaning while bcl-x and BDNF were increased at PND12. In the cerebellum, mRNA levels for caspase 2, 3, and bax were elevated at PND 9. In the cortex, bcl-x and BDNF mRNA levels were decreased at PND 15. These data indicate both time and region specific responses of the developing nervous system following low level exposure to mercury vapor or lead acetate. This abstract does not necessarily reflect US EPA policy.

338 NEUROTOXIC HEAVY METALS LEAD AND MERCURY DISTURB N-CADHERIN EXPRESSION IN EMBRYONIC CHICK CORTICAL CELLS.

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Exposure to heavy metals during embryogenesis disturbs brain development and results in decreased neurological potential. Morphogenesis of the central nervous system is mediated, in part, by the developmentally-regulated expression of the cell adhesion molecule N-cadherin. Appearance of this 130 kDa transmembrane glycoprotein and its associated cytoskeleton-linking proteins at neuronal cell surfaces mediates homophilic adhesion and bi-directional cell signaling during all stages of neurodevelopment. One prominent mechanism by which neural N-cadherin is turned over is by divalent-cation dependent proteolysis, resulting in the generation of truncated membrane-associated forms and soluble N-cadherin (NCAD90) fragments. Experiments were undertaken to determine whether the toxic metals lead and mercury disturb N-cadherin expression in chick cortical cells following exposure to metals using *in vitro* cell cultures and in purified synaptosomal membrane preparations. Incubation of cultured cortical cells with either 2 or 20 mM mercury chloride for 24 hours resulted in the dose-dependent increase in membrane-associated N-cadherin isoform expression, with significant elevation in levels of both the 130 kDa (at 20 mM) and 110 kDa isoforms (2 and 20 mM) and generation of

soluble NCAD90. Lead exposure of cortical cell cultures for one week in either 1 or 5 mM lead chloride resulted in reduced membrane-associated N-cadherin expression, along with significant loss of the N-cadherin cytoskeletal linker protein beta-catenin. Alteration in the developmentally-regulated expression of N-cadherin by lead and mercury may disturb N-cadherin-mediated brain morphogenesis, and could contribute to the neurological defects observed in developmental metal neurotoxicity. (Supported by NIH training grant T32 ES-07282.)

339 FEMALE PREDOMINANCE AMONG CD-1 MOUSE FETUSES WITH ARSENIC-INDUCED EXENCEPHALY.

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For comparison with the sex ratios of exencephalic fetuses reported by Machado *et al.* (Tox. Sci. 51:98-107, 1999) in arsenite treated C57BL/6J and SWV/Fnn mice, we examined exencephalic fetuses from both arsenite- and arsenate-exposed mice of a third strain (CD-1). These fetuses were derived from an ongoing study of the influence of methylation on arsenic teratogenicity. Groups of 20 female mice were mated (plug = gestation day 1) and given one of these treatments on GD 9: sodium arsenite (As III), 7.5 mg/kg, ip, with a 100 μM/kg dose of the methylation inhibitor periodate oxidized adenosine (PAD), ip, or with a 5, 10, or 20% protein diet; sodium arsenate (As V), 17.9 mg/kg (as Na_2HAsO_4), ip, with 100 μM/kg periodate oxidized adenosine (PAD), ip, or with a 5, 10, or 20% protein diet. The highest proportions of exencephalic female fetuses were from dams exposed to As(III) combined with PAD (34/21, 62%) or with the 10% protein diet (9/4, 69%) or to As(V) combined with PAD (21/14, 60%) or with the 10% protein diet (18/8, 69%). As(III) or As(V) plus the 20% diet was suggestive of a female preponderance, but the differences were smaller, while neither arsenical was associated with such an effect when given with the 20% protein diet. Due to the relatively low numbers of exencephalic fetuses, however, only the female preponderance of the total of As(III)-treated fetuses was statistically significant by the binomial test ($p \leq 0.02$), although the value for all As(V)-exposed fetuses closely approached significance ($p \leq 0.055$). These results thus support the findings of Machado *et al.* (1999), who reported a predominance of females among exencephalic fetuses from arsenite-exposed mice. (Supported by a grant from Sigma Theta Tau.)

340 RECENT TRENDS IN CHILDHOOD BLOOD LEAD LEVELS.

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Blood lead levels for children in the United States are known to have declined over the last few decades through at least 1994, the date of the most recent National Health and Nutrition Examination Survey (NHANES). The analysis presented here was performed to determine whether blood lead levels for children under age six have changed since 1994, and to quantify the magnitude of any change. This study evaluates blood lead levels from 12 longitudinal data sets from 11 states and one city. Geometric mean (GM) blood lead levels declined between four and 16 percent per year in ten of the 12 data sets. No differences in decline rates were observed between data sets from states that had universal screening or included repeat measures for an individual child, and those data sets from states that did not. Based on the quality of the data sets and the reproducibility of the decline rates, the average decline rate for blood lead levels since 1994 in children under age six is estimated as four to seven percent per year. This is comparable to the decline rate observed in earlier years of six to seven percent per year based on the NHANES II and III surveys. Using the decline rates estimated here, this analysis suggests that the GM blood lead level for U.S. children today is between approximately 2.0 and 2.3 μg/dL.

341 UPDATED VERSION OF CAL/EPA'S LEADSPREAD MODEL FOR PREDICTING BLOOD LEAD IN CHILDREN AND ADULTS.

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The California Department of Toxic Substances Control has revised and updated its lead risk assessment spreadsheet model (LeadSpread) for predicting distributions of blood lead (PbB) for adults and for children 1-2 years old. Inputs to LeadSpread are central tendency values; output is converted to a lognormal distribution via an assumed geometric standard deviation. We increased this geometric standard deviation to 1.60, according to White et al. (1998). We decreased food consumption to 1.1 kg/day for children and 1.9 kg/day for adults (Bolger, 1996) and decreased our estimate of lead in the diet to 2.8 μg/kg of food for children and 1.6 μg/kg of food for adults (USFDA, 1996-97). Based on USEPA (1997), we increased soil ingestion rates to 100 mg/day for children and 50 mg/day for adults, decreased the ventilation rate for children to 6.8 m3/day, and changed exposed skin surface to 2,900 cm2 for children and 5,800 cm2 for adults. Using recent guidance on dermal risk assessment (USEPA, 1998), we decreased soil-to-skin adherence from 1 mg/cm2 for children and adults to 0.2 mg/cm2 for children and 0.07 mg/cm2 for adults. Using data from California Air Resource Board (Cal/EPA, 1999), we decreased our estimates of lead in air to 0.028 μg/m3 and airborne dust to 1.5 μg/m3. Assuming 20 mg Pb/kg in soil and 15 μg Pb/L in drinking water, these revised inputs to LeadSpread predict a geometric mean PbB of 1.7 μg/dL for children 1-2 years old, with a 99th percentile of 5.0 μg/dL. The National Health and Nutrition Examination Survey III, Phase 2 (NHANES III; USDHHS, 1996) found the geometric mean PbB in the Western U.S. to be 2.17 for children 1-6 years old and 2.64 in children 1-2 years old. Restricting the data from NHANES III to children living in post-1973 housing, geometric mean PbB decreases to 1.7 and 1.9 μg/dL for children 1-6 and 1-2 years old, respectively. Thus, LeadSpread with its revised inputs agrees well with NHANES III data for children either 1-2 or 1-6 years old in post-1974 housing. We also present predicted PbB using LeadSpread with various combinations of possible site-specific inputs.

342 COMPARISON OF UPDATED LEADSPREAD TO INTEGRATED UPTAKE, EXPOSURE, AND BIOKINETIC MODEL (IEUBK) FOR PREDICTING BLOOD LEAD IN CHILDREN.

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California Department of Toxic Substances Control has revised LeadSpread, its spreadsheet model for predicting distributions of blood lead (PbB) in adults and children. Here we compare revised LeadSpread to USEPA's IEUBK model (ver. Lead99d). Structurally, the two models are very different. IEUBK is a multi-compartment, time-dependent model, while LeadSpread uses empirical relationships under steady-state assumptions. IEUBK allows a user-specified maternal blood lead concentration, indoor/outdoor ratios for dust, activity, and air, and optional inputs for additional sources of lead, such as paint. LeadSpread does not offer these features, but it has a plant uptake pathway. IEUBK estimates PbB for children up to 7 years old, while LeadSpread provides estimates for 1-2 year old children, with or without pica, and for adults. Operationally, IEUBK is a compiled model run in DOS, while LeadSpread is a spreadsheet model run in Microsoft Excel®. Slope factors describing change in PbB/unit of lead content in food, drinking water, or environmental media are explicit in LeadSpread, and they can be derived from IEUBK by running the model iteratively. For change in PbB vs. lead in air, LeadSpread has a discernible slope, while IEUBK does not. For drinking water and food, IEUBK shows faster rises in PbB per unit concentration of lead than does LeadSpread. For ingestion of soil, IEUBK includes a saturable and a non-saturable process. Because the saturable process is only slightly non-linear, we describe two slope factors for IEUBK, one for 0-700 and one for 0-7,000 mg Pb/kg soil. The one slope factor in LeadSpread for soil ingestion vs. change in PbB is about equal to the larger of the two for IEUBK. We compared outputs of the two models for children 1-2 years old. Using recommended defaults, 20 mg Pb/kg of soil, and 15 μg/L of drinking water, IEUBK predicted a geometric mean PbB of 1.9 μg/dL with a 99th percentile of 5.6 μg/dL, while LeadSpread predicted 1.7 and 5.1 μg/dL. A large national data base (NHANES III) reports a geometric mean PbB of 2.7 μg/dL for all children 1-6 years of age and 2.0 μg/dL for children 1-6 years of age living in post-1973 housing. The latter value is comparable to both models.

343 INVESTIGATION OF LEAD-BASED PAINT IN SOILS IN NON-RESIDENTIAL AREAS AT CLOSING MILITARY BASES.

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Lead contamination in soils from the use of lead-based paints has been an environmental concern in residential areas for some time and has been the focus of legislation and various remediation efforts. This investigation was to

ascertain the extent of lead contamination around buildings in non-residential settings that had previously used leaded paints and have no operational history of lead usage. Several closing military bases in California representing the major branches of military service were investigated, affording a variety of building types, operational histories, construction materials, maintenance practices, and ages. Buildings were selected for investigation if they currently had lead-based paint, as indicated by hand held x-ray fluorescence, or were of the age that could have had leaded paint. Sampling was further based on the extent of painted surfaces and paint condition. Since the contribution of lead in soil from lead-based paint would be expected to be deposited in the near surface, soil samples were collected at 0 to 1 inch below ground surface at the building dripline and at distances of ½, 2 and 4 times. Samples were collected wherever there was soil at approximately 50-foot intervals around the selected buildings. There was no attempt to include or exclude paint chips from the sampling. Visible gravel, pebbles, and plant material were excluded from the samples. On selected sites that demonstrated lead in soil, composite samples to 6 inches were also collected to reveal lead impact with depth. Results yielded a few individual samples with percent-by-weight levels of lead in soil. In general, concentrations drastically decreased with distance from the building and with depth. The overall volume of soil impacted was small. Building material was weakly correlated with lead in soil with metal and wood structures having the highest burdens. Building age was also weakly correlated with soil burdens. High visibility buildings that receive more frequent maintenance had higher soil burdens. These results may be used to evaluate the risk from and risk management decisions needed for dealing with lead in soils in other then residential areas to effectively speed military base closure property transfers.

344 RECONSTRUCTION OF LIFETIME LEAD EXPOSURE FROM QUESTIONNAIRE, κ-XRF MEASUREMENTS, AND BIOKINETIC MODELING.

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Exposure to lead is typically assessed by measurement of lead in blood, bone, or urine, and by use of risk factor questionnaires. In an effort to improve upon estimates of lifetime lead exposure, we sought to integrate historical exposure assessments and κ x-ray fluorescence (κ-XRF) bone lead measurements using biokinetic modeling. An algorithm was constructed to convert information derived from a Lead Exposure Questionnaire (LEQ) to a daily lead input to ICRP biokinetic model for lead to reconstruct the adult lifetime internal lead dose (blood and bone lead). All occupational and nonoccupational activities at risk for lead exposure were considered. The LEQ and κ-XRF measurement of tibial and calcaneal bone lead were assessed in 34 men and women, aged 62 to 86 yr, considering all potential lead exposures from age 18 yr onward. κ-XRF measured tibial and calcaneal lead was 17.6 +/- 15.1 and 19.8 +/- 17.5 microg Pb/g bone mineral, respectively. For all subjects considered together, the difference between bone lead stores predicted by biokinetic modeling at the time of κ-XRF measurement, and the κ-XRF measurement itself, was 2.9 +/- 2.2 microg Pb, weighted to cortical bone. This novel methodological approach provides the opportunity to construct and test hypotheses regarding the timing, duration, and intensity of adult lifetime lead exposure as a risk factor for chronic neurodegenerative, cardiovascular and skeletal diseases. (Supported by NIH ES06418 [JMG] and ES04040 [JGP].)

345 INCORPORATING SITE-SPECIFIC INFORMATION IN THE DETERMINATION OF LEAD REMEDIAL REQUIREMENTS AT CLOSED SMALL-ARMS FIRING RANGES.

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The Air Force Center for Environmental Excellence and Parsons Engineering Science have implemented a risk-based approach to determine remedial requirements at closed small-arms firing ranges on Air Force facilities. The purpose of this nationwide initiative was to establish a cost-effective, site-specific, technically sound, and unified approach to firing range site investigation, risk evaluation, and remediation. USEPA-approved biokinetic exposure models were used to assess site-specific risks from exposure to lead in soil and derive remediation goals. Bioavailability of lead has been shown to decrease with increasing particle size. There is evidence suggesting that soil particles <100 μm are more likely to be incidentally ingested because they adhere more readily to skin. Lead concentrations in the soil fraction containing particles with diameters ≤ 250 μm were used to estimate potential exposure. Lead bioavailability is expected to vary dependent on the chemical species. Lead carbonate and lead/metal oxides were the species most frequently encountered in soil samples. An *in vitro* method for estimating the relative bioavailability of lead in humans has been developed by researchers at Colorado University in Boulder and USEPA Region 8. *In vitro* relative bioavailability results for soil samples ranged from 70 to 100 percent. Site-specific information on particle size, chemical species, and *in vitro* bioavailability suggested that the bioavailability of lead in soils at small-arms firing ranges was not significantly different than the USEPA-recommended default. Results of exposure modeling incorporating the above site-specific information to assess potential risks and develop risk-based remediation goals in soils at small-arms firing ranges in Alaska, California, and Texas will be presented. In addition, lessons-learned in applying site-specific risk-based decision-making in the overall remedial process will be summarized.

346 SUBCHRONIC HEALTH EFFECT LEVELS FOR CHILDHOOD EXPOSURE TO ARSENIC.

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The recent U.S. EPA policy to evaluate health risks to children has directed attention toward short-term exposures to chemicals in young children, even for chemicals regulated as long-term carcinogens. This paper reviews available data for a subchronic health effect level for arsenic exposure in ages 0-6, the focus of EPA assessments for children. A number of epidemiological studies have examined arsenic exposures primarily via drinking water. Studies noting health effects involving children in these populations were from Chile, China, Hungary, India, Japan, Mexico, Singapore, Thailand, and Taiwan. The following observations were noted: (1) dermatoses are consistently reported in both adults and children with hyper pigmentation reported at the lowest exposure levels (i.e., arsenic concentration in water); (2) the prevalence and severity of disease increase with age and exposure level; (3) studies tend to report mean exposure levels for the population rather than the level for those with health effects; (4) few studies give a detailed breakdown of disease prevalence with exposure level and age group. A study from West Bengal best documents prevalence of dermatoses by exposure level and age and indicates a no adverse effect level (NOAEL) below 100 ppb for children age 0-9 years. The other studies, although not as precise, are consistent with this level. Depending on drinking water intake rate and body weight assumed, this level would result in a subchronic NOAEL dose of approximately 0.008 mg/kg/day for hyperpigmentation. This dose is 10 times higher than the chronic NOAEL for skin and other effects used by EPA to develop the chronic reference dose for arsenic exposure.

347 DETERMINATION OF A HUMAN ACUTE NO OBSERVED ADVERSE EFFECT LEVEL (NOAEL) FOR COPPER.

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This prospective, double blind controlled study was designed to determine the acute NOAEL of nausea in an apparently healthy population of individuals who drink copper containing water as the sulfate salt. Sixty adults, 30 of each sex and 18-60 years of age, were recruited at three different international sites (Coleraine, Santiago, and Grand Forks). Individuals with occupational copper exposure, at pregnancy or with gastrointestinal (GI) illness were excluded. Pharmaceutical grade copper was used to prepare testing solutions containing 0, 2, 4, 6, and 8 mg Cu/L (as copper sulfate) daily 30 minutes before administration by dissolving copper sulfate into freshly made room temperature, deionized water and maintained at the same temperature until dosing. Each subject was given a blind, randomly selected dose in a bolus of 200 ml (final total copper was equivalent to 0, 0.4, 0.8, 1.2, and 1.6 mg) over a consecutive 5 week period. Following dosing all subjects were monitored by health care personnel for 1 hour. All subjects completed questionnaires at 0 time, 15 minutes, 1 hour and 24 hours that screened for positive GI effects (nausea, vomiting, abdominal pain, and diarrhea). Nausea was the most frequently reported effect and was reported within the first 15 min. All other GI effects were reported within 1 hr. For the combined tri-site population (n = 180), 8, 9, 14, 25 and 44 subjects responded to one or more GI symptoms at 0, 2, 4, 6 and 8 mg Cu/L, respectively. Chi-square analysis of the data demonstrated a clear dose response to the combined positive GI effects and to nausea alone. Statistically significant greater reporting of effects occurred at 6 and 8 mg Cu/L. Therefore, an acute NOAEL and LOAEL of 4 and 6 mg Cu/L, respectively is determined in a combined international human population.

348 HOW USEFUL IS REGRESSION ANALYSIS IN DETECTING DOSE-RESPONSE IN EPIDEMIOLOGY STUDIES WHEN ONLY A FRACTION OF THE POPULATION IS SENSITIVE? THE CASE OF METHYLMERCURY.

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The existence of a dose-response in epidemiologic studies is generally determined from the linear regression slope after controlling for covariates. This approach assumes the entire population is equally sensitive to the toxicant and that response is a function only of dose and a random error function. However, sensitive sub-populations have been identified for a variety of toxicants, possibly including the neurodevelopmental effects of methylmercury (MeHg). The study of MeHg exposure in the Seychelles Islands has failed to find significant effects (dose-response slope not significantly different from zero). Using data on the error function in developmental test scores and MeHg exposure distribution from that study, and assuming plausible dose-response relationships for sensitive subpopulations, we conducted Monte Carlo simulations of the power of linear regression analysis to detect a dose-response relationship in the total population (n = 700), and to compare dose-response slopes in the total and sensitive populations. Linear regression did not reliably detect a dose-response relationship for most scenarios when sensitives were 5% of the total and for some scenarios when sensitives were 10% of the total. We also found that the dose-response slope for the total population underestimated the sensitive dose-response slope in all cases by about an order of magnitude. These findings may have important implications for detection and quantification of dose-response relationships from epidemiologic studies.

349 NONCANCER RISK ASSESSMENT FOR ARSENIC BASED ON ITS VASCULAR EFFECTS.

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Considerable controversy has centered on whether there is epidemiological or mechanistic evidence for a "threshold" for the carcinogenic effects of arsenic (As), or at least for a highly nonlinear dose-response. If a nonlinear or MOE approach were to be chosen for the cancer risk assessment for As, a noncancer effect could well become the primary determinant of an acceptable water contamination limit for As. The current USEPA oral Reference Dose (RfD) for As is 0.0003 mg/kg/day, which equates to a drinking water concentration of approximately 0.01 mg/L, assuming a 70-kg person drinks 2 liters of water per day. Thus the current Maximum Contaminant Limit for As in drinking water, which is 0.05 mg/L, is roughly a factor of 5 higher than the concentration that would be consistent with the RfD. This RfD was based upon skin changes and possible vascular complications observed in a Taiwanese population exposed to a mean concentration of As in drinking water of 0.17 mg/L; these effects were not observed in a control population exposed to a mean concentration of 0.009 mg/L. Unfortunately, there is nearly a 20-fold difference between the control concentration, which was used as a No-Observed-Adverse-Effect-Level (NOAEL) in the derivation of the RfD, and the concentration at which the effects of As were observed. In order to provide a more precise estimate of the threshold for the noncancer effects of As, the Benchmark Dose method was applied to a number of published epidemiological studies investigating the vascular effects of As. Endpoints examined included cerebrovascular disease, ischemic heart disease, peripheral vascular disease, diabetes mellitus and hypertension. The Benchmark Doses obtained from these studies provide a robust basis for identifying a NOAEL for the noncancer effects of As.

350 IMMUNOCHEMICAL DETETCTION OF TRICHLOROACYLATED PROTEINS IN TETRACHLOROETHENE-TREATED AUTOIMMUNE-PRONE MICE.

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The extensive use of perchloroethene (tetrachloroethene, PCE) combined with improper disposal has lead to it becoming a common environmental contaminant. Recent studies have linked PCE exposure to autoimmune diseases (ADs) such as lupus and scleroderma, which could be attributed to binding of PCE or its metabolites to cellular macromolecules, forming adducts which act as haptens and elicit an autoimmune response. Therefore, we determined the formation and distribution of protein adducts in tissues after PCE treatment as a basis for examining its potential to cause ADs. In the present study, we used MRL lpr and MRL +/+ mice as a model of a population susceptible to ADs, since both strains spontaneously develop a type of murine lupus; although the lpr strain develops ADs much faster then the +/+. Mice were given either acute (1 dose) or subacute (1 dose every third day for 8 weeks) treatments of PCE (5 mmol/kg in corn oil by gavage) and were sacrificed 24 hours after the last dose. Immunohistochemistry was done on paraffin embedded tissue sections using an antisera that was raised in rabbits to trichloroacylated keyhole limpet hemocyanin and recognizes trichloroacylated proteins. Very intense reactivity was seen in the hepatic centrilobular areas of both acutely and subacutely treated mice of both strains, while no staining was seen in vehicle-treated controls. No differences in apparent staining intensity or tissue distribution were observed in males compared to females and adducts were not found in any other tissue examined. We conclude that the formation of hepatic trichloroacylated protein adducts in PCE-treated mice is not dependent on duration of dosing, strain or sex of the subject animal.

351 TRICHLOROETHYLENE INDUCED FORMIC ACID EXCRETION LEADS TO KIDNEY DAMAGE IN THE MALE FISCHER RAT.

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Trichloroethylene is both nephrotoxic and a weak renal carcinogen in rats in 2 year studies. Its two major metabolites, trichloroethanol and trichloroacetic acid, have been shown to induce the sustained excretion of large amounts of formic acid in urine which may account for the observations in the 2 year studies. The consequences of formic acid excretion on the rat kidney have been investigated in male Fischer rats given trichloroethanol in drinking water at concentrations of 0, 0.35 and 1.0 g/l for 52 weeks. The rats excreted large amounts of formic acid in urine reaching a maximum after 12 weeks (~65 mg/24hr at 1 g/l) and thereafter declining to reach an apparent steady state at 40 weeks (15-20 mg/24hr). Urine from treated rats was more acidic throughout the study and urinary methylmalonic acid and plasma N-methyltetrahydrofolate concentrations were increased, indicating an acidosis, vitamin B12 deficiency and impaired folate metabolism respectively. The rats treated with trichloroethanol developed kidney damage over the duration of the study which was characterised by increased urinary NAG and protein excretion (from 4 weeks), increased basophilia, protein accumulation and tubular damage (from 12 to 40 weeks), increased cell replication (at week 28) and evidence of focal proliferation of abnormal tubules at 52 weeks. Overall, these findings provide a potential explanation for the results of the two year chronic studies.

352 TRICHLOROETHYLENE INDUCED VITAMIN B12 AND FOLATE DEFICIENCY LEADS TO INCREASED FORMIC ACID EXCRETION IN THE RAT.

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Trichloroethylene is both nephrotoxic and a weak renal carcinogen in rats in 2 year studies. Whilst investigating the mechanism(s) involved in the development of nephrotoxicity and renal cancer it was observed that trichloroethylene induced a sustained excretion of large amounts of formic acid in urine. Both of the major metabolites, trichloroethanol and trichloroacetic acid, were found to induce this response, but not the minor metabolite S-(1,2-dichlorovinyl) cysteine. Addition of folic acid either to diet or drinking water modulated the response indicating that these rats were folate deficient. Two markers of vitamin B12 deficiency, methylmalonic acid and 5-methyltetrahydrofolate, were also markedly increased in urine and plasma respectively. The

increase in 5-methyltetrahydrofolate is consistent with a folate deficiency caused by an inhibition of the vitamin B12 dependant methionine salvage pathway. Other polychlorinated solvents, including carbon tetrachloride and chloroform, also increased urinary formate excretion and since both vitamin B12 and polychlorinated carbon atoms readily form free radicals, it is suggested that trichloroacetic acid and trichloroethanol interact with vitamin B12 through a free radical mechanism inducing a B12 deficiency and, as a consequence, a folate deficiency. As a result of the folate deficiency, excess formic acid, which is normally utilised through this pathway, is excreted in urine.

353 TOXICOKINETICS OF MONOCHLOROACETIC ACID IN ADULT MALE SPRAGUE-DAWLEY RATS AFTER DERMAL APPLICATION.

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Male Sprague-Dawley rats were dosed with a nontoxic dose of 10 mg/kg and a toxic dose of 125 mg/kg (~LD_{20}) dermally. An area of about 3-4 cm^2 was shaved slightly posterior to the scapulae of the rats and ^{14}C-MCA (2.5-3.0 μCi/kg) in acetone was applied onto the skin at 0.5 ml/kg. Acetone was allowed to evaporate. Thereafter, the dosing site was covered with parafilm which was then secured with masking tape. Three animals were euthanized at 15, 45 min, 2, 4, 8, 16, and 32 h after dosing and tissues collected by gross dissection. Radioactivity in tissue aliquots and excreta was determined by scintillation counting after digestion. Absorption was rapid through the skin (>50% within 45 min and about 80% within 4 h). About 93% dose penetrated through the skin within 32 h. Concentration of MCA in plasma and all other tissues peaked by 45 min after application (0.17% of absorbed dose), with the exception of thymus which peaked at 2 h. Radioactivity in brain and thymus declined very slowly from peak concentrations. Within 45 min, >10% of the absorbed dose was metabolized and cleared via bile followed by reabsorption. At the toxic dose, concentration in plasma peaked at 0.36% of the absorbed dose by 45 min and declined thereafter. Concentration in almost all of the other tissues peaked by 4 h with the exception of lung, muscle and skin where peak concentrations were found at 2 h after application. Again, similar to the nontoxic dose, 9% of the absorbed radioactivity appeared in small intestine within 45 min followed by reabsorption within 8 h. Fecal excretion was negligible. A total of 32 and 64% of the absorbed dose was recovered in urine by 32 h after the nontoxic and toxic dose, respectively. The plasma half-life of elimination of MCA after dermal application was about 2 h. Dermal application of MCA did not cause any overt toxicity (coma and/or death) up to 100 mg/kg. Doses 125, 150, and 175 mg/kg caused 20, 60, and 100% coma and death, respectively.

354 RENAL MITOCHONDRIAL ACONITASE FROM TETRAFLUOROETHYLCYSTEINE-TREATED RATS IS COVALENTLY MODIFIED BY DIFLUOROTHIOACETYL FLUORIDE AND POSSESSES DECREASED ACTIVITY.

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The site-selective nephrotoxicant tetrafluoroethylene (TFE) is bioactivated through sequential metabolism by glutathione-S-transferase, γ-glutamyl transpeptidase, cysteinylglycine dipeptidase, and cysteine-S-conjugate β-lyase. The ultimate toxicant, difluorothioacetyl fluoride (DFTAF), is a potent thioalkylating agent. In order to better understand the pathological sequalae of TFE-mediated toxicity, studies were performed to identify and characterize the DFTAF target previously designated P99. We utilized differential centrifugation, a DEAE anion exchange column, and SDS-PAGE with silver staining to isolate and visualize mitochondrial proteins from the renal proximal tubular epithelium of tetrafluoroethylcysteine (TFEC) -treated rats. Immunoblot analysis utilizing an anti-trifluoroacetyl antibody with confirmed selectivity for difluorothioamidyl lysine (DFTAL) moieties detected a DFTAL protein adduct with an apparent molecular mass of 99kD. Silver-stained bands containing this target protein were excised from polyacrylamide gels, destained, and subjected to in-gel digestion with trypsin. Tryptic peptides were analyzed by tandem mass spectrometry using a microcapillary liquid chromatography interface. The software Sequest™ was used to match collision-induced dissociation spectra against theoretical spectra of tryptic peptides in the OWL protein sequence database. The parent protein was found to possess a very high degree of sequence similarity to bovine mitochondrial aconitase (m-aconitase). (A sequence for rodent m-aconitase was not available.) Whole kidney homogenates from TFEC-treated rats were found to have significantly less aconitase activity than control tissue homogenates. We propose that DFTAF adduction of m-aconitase in the rat proximal tubular epithelium compromises turnover of citrate and constitutes an early event in the pathologic sequelae arising from exposure to TFE. (Supported by NIH GM51916 [SAB], P30 ES07033 [SDN], and the Merck Research Scholar Program [EAJ].)

355 POLYCHLORINATED BIPHENYLS ENHANCE INTRACELLULAR CALCIUM THROUGH PURINERGIC RECEPTOR ACTIVATION IN HUMAN MACROPHAGES.

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Polychlorinated biphenyl compounds (PCBs) are environmental neurotoxicants shown to exert their toxicity through increased intracellular calcium $[Ca^{2+}]_i$ in a number of cells. The mechanism of the disruption of calcium homeostatis by PCBs is not well understood. However, the non-planar but not co-planar PCBs were shown to disrupt calcium dependent signalling pathways. For many of the calcium homeostasis regulating purinergic receptor sub-types including P2X and P2Y adenosine trisphosphate (ATP) is a natural ligand. Human macrophages are known to express both $P2X_7$ and $P2Y_2$ receptors. Therefore, the objectives of this study were to determine whether PCBs enhance the $[Ca^{2+}]_i$ in human macrophages and, if so, is it due to the activation of purinergic receptors. Human monocyte derived macrophage cultures were treated with different concentrations of ATP, PCBs and iNOS modulators. The $[Ca^{2+}]_i$ imaging was conducted using Fura-2 as fluorescent probe. The results obtained showed that the $[Ca^{2+}]_i$ was increased as a function of ATP concentration with an EC_{50} of 7.1 μM. PCB 47, a non-planar PCB enhanced the ATP mediated $[Ca^{2+}]_i$ increase as evidenced by a shift of ATP dose-response curve to the left reducing the EC_{50} of ATP from 7.1 to 1.5μM. PCB 77, a co-planar PCB had no effect on purinergic receptor activity. N^G-amino-L- arginine (NAA), an inhibitor of iNOS increased the ATP mediated $[Ca^{2+}]_i$. SN-acetyl-L-,D-amino-pencillate (SNAP), a NO donor reduced the effects of both PCB 47 and NAA. Immunohistochemistry showed that PCB 47 induced iNOS. This effect was completely abolished by benzoyl-ATP, a specific $P2X_7$ agonist. These data clearly demonstrate for the first time that PCBs increase $[Ca^{2+}]_i$ by the modulation of purinergic receptors in human macrophages. These data further suggest that NO regulation depends on $P2X_7$ receptors in human macrophages. (This work was supported by Deutsche Forschungsgemeinschaft.)

356 COPLANAR PCB CONGENERS ARE UTEROTROPIC IN PRE-PUBERTAL RATS.

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There is considerable controversy whether dioxin-like coplanar polychlorinated biphenyl (PCB) congeners are estrogenic or anti-estrogenic. Determining the effects of these contaminants is important because, in addition to possible effects on reproduction, alterations in the hormonal milieu of the immature brain induced by these PCB congeners may negatively affect normal development leading to long-lasting changes in neurochemical function and behavior. We exposed prepubertal rats intra-peritoneally on postnatal days (PND) 21 and 22 to one of three coplanar PCB congeners: 3,4,3',4'-tetrachlorobiphenyl (TCB), at doses of 3 to 27 mg/kg; 3,4,5,3',4'-pentachlorobiphenyl (PtCB), at doses of 16 to 400 μg/kg or 3,4,5,3',4',5'-hexachlorobiphenyl (HCB), at doses from 100 to 800 μg/kg and measured uterine wet weight (UWW) in a blinded fashion on PND24. TCB, at doses ≥ 9 mg/kg and PtCB at doses ≥100 μg/kg significantly elevated UWW. HCB, a PCB congener highly resistant to metabolism, was not uterotropic at any of the doses. The anti-estrogen ICI 182,780 (0.5 mg/kg on PND21 and 22) completely blocked the uterotropic actions of TCB and PtCB, suggesting that these coplanar PCB congeners act through the estrogen receptor (ER). In additional experiments, we exposed prepubertal rats to PtCB and varying doses of 17β-estradiol (E_2). Concentrations of E_2 that did not alter UWW blocked the uterotropic actions of PtCB. These results suggest that coplanar congeners, capable of being metabolized to biphenylols, are uterotropic, act through the ER and appear to bind poorly to the ER. (Supported in part by NIEHS grant ES04913 to RFS.)

357 INSULIN RELEASE FROM RINM5F CELLS PRODUCED BY POLYBROMINATED AND POLYCHLORINATED BIPHENYLS (PBBS and PCBS).

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The structural similarities of PBBs and PCBs are consistent with published reports showing similar toxic actions produced by these two classes of environmental contaminants. However, differences in the physical properties of brominated and chlorinated organic molecules may result in quantitative, if not qualitative differences in their actions. Non-coplanar PCBs are known to stimulate insulin release from RINm5F cells by a calcium and kinase-dependent mechanism. The present study examines the potency of PBBs in producing the hormone-releasing effect. Experiments using a commercial PBB mixture (Firemaster-FF1) indicated that exposure of cells to 10 μg/ml in media produced a slight increase in insulin release compared to control (21.3 ng insulin/μg DNA vs 14.0 ng insulin/μg DNA)during a 120 min exposure period. A comparison of insulin release by identical pure congeners of PBBs and PCBs indicated striking differences. 2,2',4,4',5,5'-Hexabromobiphenyl (30μM) produced a 21% increase over control in media insulin whereas the identical chlorine-containing congener yielded a 342% increase in a 30 min exposure period. The 2,2'-dihalogenated congeners exhibited a different pattern as 30 μM of the dibromo congener provided a 274% increase in media insulin over control and the dichlobiphenyl congener produced a 189% increase. The shapes of the dose-response curves of the dibromo and dichloro biphenyl congeners were identical suggesting similar mechanisms of action. The results indicate that both PBBs and PCBs can elicit insulin release but the structure-activity relationships for this effect appear to be different. Based on results from the limited number of congeners studied, increasing the bromine content of PBBs attenuated activity whereas an increase in chlorine content of PCBs enhanced activity.

358 REACTIVITY OF HALOGENATED AND NON-HALOGENATED DIHYDROXY-BIPHENYLS *IN VITRO* AND IN CELLS IN CULTURE.

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Several non-halogenated and halogenated biphenyls are suspected to be carcinogenic by being metabolized to dihydroxy-biphenyls or their quinones. For example, 2,5-dihydroxybiphenyl is a major metabolite of the fungicide orthophenylphenol that induces bladder cancer in rats. The leukemogene benzene is metabolized to phenol and further by peroxidases to 4,4'- and 2,2'-dihydroxybiphenyl, making both of them and their quinones possible candidates as the ultimate carcinogens of benzene. Also, chlorinated 4,4'-dihydroxybiphenyls are formed during industrial processes, but very little is known about these compounds. To learn more about the chemical and biological reactivity of such dihydroxy-biphenyls, and to determine structure activity relationships, we analyzed biphenyls with the hydroxyl groups in the positions 2,5- (2,5-BP), 3,4- (3,4-BP), 2,2'-(2,2-BP), and 4,4'- (4,4-BP), as well as 3,5,3',5'-tetrachloro-4,4' (TCBP), and 3,5,3',5'-tetrabromo-4,4'-biphenol (TBBP). We determined spectrophotometrically *in vitro* spontaneous and enzymatic oxidation and binding to glutathione, analyzed cytotoxicity by measuring 3H-thymidine incorporation and detected depletion of intracellular glutathione with the monobromobimane fluorescence assay. 2,5-BP was so reactive that it readily autoxidized in aqueous solution. The other biphenols were stable. All but 2,2'-BP were oxidized enzymatically by peroxidase/H2O2. The biphenols were cytotoxic at micromolar concentrations. The order of toxicity, 2,2'-BP < 4,4'-BP < TCBP < TBBP < 3,4-BP < 2,5-BP, paralleled the order of intracellular GSH depletion. We conclude that i. the position of the hydroxyl groups strongly influences the reactivity, ii. halogenation increases the cytotoxicity of 4,4'-BP, iii. cytotoxicity may be caused by oxidation to the quinone and binding to cellular sulfhydryl-containing compounds. Redox reactions, GSH depletion, and binding to cellular molecules may play a role in cancer initiation and promotion. (Supported by grants DAMD 17-96-1-6262 from DOD, P42 ES 07380 from NIEHS/EPA, and grant # 85-001-13-IRG from the American Cancer Society.)

359 RELATIVE POTENCY VALUES FOR INDIVIDUAL POLYCHLORINATED BIPHENYL CONGENERS FOLLOWING SUBCHRONIC DIETARY EXPOSURE.

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Polychlorinated biphenyls (PCBs) is a class of man-made chemicals consisting of 209 isomers with variable pattern of chlorine substitution. Although they were banned, they are still widely spread in the environment and many of them accumulate in fatty tissues of living organisms causing a broad range of adverse effects. In this study the potencies of individual chlorinated biphenyl (CB) congeners to modify vitamin A levels in liver and kidney and to increase liver 7-ethoxyresorufin-O-deethylase (EROD) activity, were investigated in Sprague-Dawley rats exposed to CB 126, CB 118, CB 77, CB 153, CB 128, CB 28, CB 105 and CB 156 for 13 weeks in their diets. The dose-response relationships were determined in order to calculate the relative potency (REP) values. Results showed almost the same order of potency of the congeners for the three investigated end points. EROD activity was the most sensitive end point followed by hepatic vitamin A reduction and by kidney vitamin A increase. The most powerful congeners, except CB 126, were CB 156 followed by CB 77 and CB 105. The REP values estimated for these congeners were in good agreement with the Toxic Equivalency Factors (TEFs) estimated for human risk assessment.

360 HEPATIC UPTAKE AND BIOLOGICAL ACTIVITY OF HALOGENATED AROMATIC HYDROCARBONS (HAHS) IN PRECISION-CUT RAT AND HUMAN LIVER SLICES.

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Precision-cut rat and human liver slices in dynamic organ culture were utilized as an *in vitro* model to assess the hepatic uptake of HAHs and expression of dioxin responsive genes, including cytochrome P450 (CYP) 1A1, 1A2, and 1B1. Liver slices were incubated with medium containing HAHs, individually and in defined mixtures, or vehicle alone (0.1% DMSO) for 24 hrs and in HAH-free medium for an additional 72 hrs. The concentration of HAHs in culture medium and/or liver slices was determined at 24 hrs by GC with electron capture detection. The hepatic concentration of HAHs increased in direct proportion with the exposure and remained generally unchanged over the period form 24 to 96 hrs, when the slices were incubated in medium without HAHs. Similar hepatic concentrations of HAHs were also observed in rat and human liver slices following a given *in vitro* exposure. Dose response relationships for CYP1A1 mediated 7-ethoxyresorufin o-deethylase (EROD) activity in rat liver slices support a dioxin toxic equivalency factor (TEF) of 1.0 for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 0.5 for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 0.1 for polychlorinated biphenyl (PCB) 126, 0.0001 for PCB 118 and 0 for PCB 153. Exposures to various binary mixtures of PCB 126 and 153 and tertiary mixtures of TCDD, PeCDF, and PCB 126 support the additive activity (based on EROD) of these HAHs. In general, human rat liver slices respond similarly to these HAHs (within a factor of 10), but exhibit a greater interindividual variability in the potency of a given HAH and the magnitude of EROD activity following *in vitro* exposure. Thus, this *in vitro* model not only provides a better means to extrapolate data to humans, but provides data on the range of responses observed in different individuals and a tool to investigate mechanisms which contribute to this variability. (Supported by US EPA R 825808.)

361 DEVELOPMENTAL EXPOSURE TO AROCLOR® 1254 ALTERS CALCIUM BUFFERING AND PROTEIN KINASE C ACTIVITY IN THE BRAIN.

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Developmental exposure to polychlorinated biphenyls (PCBs) causes cognitive deficits in humans and animals; however, the underlying mode of action is not known. We have hypothesized that altered signal transduction may be associated with the neurotoxicity of PCBs, since second messengers such as Ca^{2+} and protein kinase C (PKC) play key roles in neuronal function and development. Our previous *in vitro* studies indicate that *ortho*-PCBs affected second messenger events at low micromolar concentrations while non-*ortho* PCBs did not. Subsequent repeated exposure studies with Aroclor 1254 in adult rats showed that *ortho*-PCBs preferentially accumulated in brain up to

13-15 ppm (equivalent to 40-50 μM) and decreased Ca^{2+} buffering as well as PKC activity which is in agreement with *in vitro* results. The present study was designed to determine if developmental exposure to Aroclor 1254 causes similar neurochemical changes. Pregnant dams were dosed orally with 0, 1 or 6 mg/kg/day Aroclor 1254 (Lot # 124-191, AccuStandard; contains mostly *ortho*-PCBs) from GD6 through PND21. The brains from pups at PND 4, 7, 14, 21, and 60 were assayed for Ca^{2+} buffering and PKC activity. Both microsomal and mitochondrial Ca^{2+}-uptake in frontal cortex increased during development reaching maximum at PND21, while total PKC activity increased from PND4 to PND14, and remained elevated until PND60. Developmental exposure to Aroclor 1254 increased microsomal Ca^{2+}-uptake from PND4 through PND21 with a decrease at PND60; however, mitochondrial Ca^{2+}-uptake was not altered. Exposure to Aroclor 1254 resulted in a dose-dependent decrease in PKC activity at PND14 and 21, which recovered to control levels by PND60. The distribution of PKC activity between the cytosol and membrane was not altered by Aroclor 1254. Total PCBs accumulated in frontal cortex of PND21 animals were 2.4 ppm and predominantly *ortho*-PCBs. These results suggest that developmental exposure to a PCB mixture altered second messengers in the brain and *ortho*-PCBs were accumulated in the brain at concentrations where significant *in vitro* effects were observed. (This abstract does not necessarily reflect USEPA policy.)

362 STRUCTURE ACTIVITY RELATIONSHIPS BETWEEN SELECTED POLYCHLORINATED BIPHENYL CONGENERS AND METABOLITES TOWARDS ACTIVATION OF RYANODINE RECEPTOR TYPE 1.

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We have previously reported that selected *ortho*-substituted polychlorinated biphenyl (PCBs) congeners alter Ca^{2+} regulation in both isolated microsomes and cultured PC12 cells. In order to further understand the mechanism(s) by which PCBs disrupt Ca^{2+} signaling, we performed a study detailing structure activity relationships between thirty-one PCB congeners and twelve PCB metabolites towards activation of ryanodine receptor type 1 (RyR1, a microsomal Ca^{2+} release channel expressed in skeletal muscle and $CD19^+$ B lymphocytes etc.). The activity of RyR1 with varying concentrations of selected PCB congeners was monitored by a radioligand binding assay using [^{3}H]ryanodine (the conformation selective probe specific for ryanodine receptors) and microsomal preparations enriched in RyR1 isolated from rabbit fast-twitch skeletal muscle. Our results revealed that the *ortho*- and *meta*-chloro-substitutions on the biphenyl structure of PCBs were the most determinants towards RyR1 activity. Among the thirty-one congeners tested, fourteen environmentally relevant congeners were examined. PCB 18, PCB 52 and PCB 187 are highly efficacious towards activation of RyR1, while PCB 41, PCB 101 and PCB 110 are with intermediate efficacy. Of all the tested congeners, PCB 136 (2,2',3,3',6,6'-hexachlorobiphenyl) is the most efficacious and potent congener (EC_{50} = 575 nM). These results enable us to predict the potency or efficacy of other environmentally relevant PCB congeners/metabolites on disruption of cellular Ca^{2+} signaling. Hence the study provides important information on further investigation of the mechanism(s) by which PCBs induce toxicity in humans. (Research is sponsored by NIEHS.)

363 MECHANISM OF INHIBITION OF STIMULATED NEUTROPHIL DEGRANULATION BY 2,2',4,4'-TETRACHLOROBIPHENYL.

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Ortho-substituted polychlorinated biphenyls (PCBs) like 2,2',4,4'-tetrachlorobiphenyl (2,2',4,4'-TCB) can activate neutrophils but also decrease activation in response to the peptide f-met-leu-phe (fmlp). The objective of this study was to examine mechanisms by which 2,2',4,4'-TCB inhibits degranulation. Degranulation of neutrophils exposed to fmlp was associated with activation of phospholipase A2 (PLA2). Degranulation and PLA2 activation were both inhibited by BEL, an inhibitor of calcium-independent PLA2 (iPLA2), suggesting that this isozyme is essential for degranulation. 2,2',4,4'-TCB also activated PLA2, but despite this, 2,2',4,4'-TCB abolished fmlp-induced degranulation. Calcium ionophores failed to restore the degranulation response in the presence of 2,2',4,4'-TCB and fmlp, suggesting that interference with intracellular calcium was not responsible for the inhibitory effect. Mitogen-activated protein kinases (MAPKs) are also important in fmlp-stimulated degranulation, but 2,2',4,4'-TCB increased phosphorylation of MAPK, indicating that this pathway was still functional. Electron microscopic examination of neutrophils revealed granules in close apposition to the membrane in fmlp-treated cells but not in neutrophils cotreated with 2,2',4,4'-TCB. These data suggest that the impairment of neutrophil degranulation observed with 2,2',4,4'-TCB may be related to the prevention of vesicle fusion with the plasma membrane. (Supported by ESO4911.)

364 A COMMON ELECTROTOPOLOGICAL MOTIF AMONG ORGANOCHLORINE COMPOUNDS THAT ACTIVATE NEUTROPHILS.

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Organochlorine compounds (OCs) are some of the main toxicants present in the food web and target different cellular systems including the non-specific immune system. The objective of this study was to test the hypothesis that OCs that activate neutrophils share common structural features. Using activation of phospholipase A2 (PLA2) as a marker of neutrophil activation, isolated, rat neutrophils were exposed to a variety of OCs. The ortho-substituted polychlorinated biphenyl, 2,2',4,4'-tetrachlorobiphenyl, the alpha-, delta- and gamma-hexachlorocyclohexanes (HCCHs), dichlorodiphenyltrichloroethane (DDT), dieldrin, aldrin and chlordane, but not the non-orthochlorinated 3,3',4,4'-tetrachlorobiphenyl or beta-HCCH, induced activation of PLA2 in neutrophils. This activation of PLA2 was reduced by 60-80% by bromoenolactone (BEL), an inhibitor of Ca-independent PLA2 (iPLA2), and by the calmodulin inhibitors trifluoperazine and W7. These results suggest that all of the active OCs activate the same isoform of PLA2 and do so through Ca-calmodulin-dependent processes. Molecular modeling techniques were used to calculate molecular electrostatic potentials. Superimposing three-dimensional structures we have identified an electrotopological motif shared by all of the active OCs, as well as BEL. This motif was absent in the inactive OCs, beta-HCCH and the non-orthochlorinated PCBs. These results suggest that the bioactivity of OCs in neutrophils may be due to the presence of a specific substructure that fits into a receptor-like structure, allowing the activation of the neutrophil. (Supported by ESO4911.)

365 HEMOGLOBIN ADDUCTS OF POLYCHLORINATED BIPHENYLS AS POTENTIAL BIOMARKERS OF EXPOSURE.

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Lower polychlorinated biphenyls (PCBs) are known to form reactive metabolites. Proteins would be likely targets for attack by these electrophiles and covalent binding to the proteins would result in the accumulation of PCBs in both target and non-target tissues. This study investigates the formation of hemoglobin adducts using two PCBs. C57BL/6 mice (4 animals per group) were injected i.p. with phenobarbital (400 umol/kg) and B-naphthoflavone (100 umol/kg) for three consecutive days. On day 4, mice received i.p. either 14C 4-chlorobiphenyl, 14C 3,3',4,4'-tetrachlorobiphenyl, 14C benzo[a]pyrene (B[a]P) or vehicle. A second set of mice were treated with same doses of cold PCBs and B[a]P. Blood was collected at different time points from the tail vein. After removal of plasma, the red blood cells were lysed and the hemolysate was freed from the cellular debris by centrifugation. The total radioactivity in the hemolysate from both the hot PCB and B[a]P treated animals was found to be greater than the control group, suggesting adduct formation with hemoglobin. From the cold B[a]P/PCB treatments, globin was precipitated from the hemolysate using mild acidic conditions (0.1% HCl in acetone). LC-MS (electrospray) was then used to identify the adducts formed with globin. Our preliminary data showed an increase in mass corresponding to the adduct of the oxidized metabolite of at least one PCB. Detection of adducts of PCBs with hemoglobin could provide a valuable tool to evaluate exposure of a population. (Supported by P42 ES07380.)

366 COVALENT BINDING OF BENZO[A]PYRENE, 4-CHLOROBIPHENYL AND 3,3'4,4'-TETRACHLOROBIPHENYL TO NUCLEAR MACROMOLECULES IN FEMALE MICE.

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Polychlorinated biphenyls (PCBs) are environmental pollutants and rodent carcinogens. Covalent binding of PCB metabolites to nucleophilic macromolecules was demonstrated *in vitro*, but few studies of such binding *in vivo* are available. The purpose of this study was to determine the binding affinity (carcinogen binding index, CBI) of benzo[a]pyrene, 4-chlorobiphenyl (4-CBP) and 3,3',4,4'-tetrachlorobiphenyl (3,3',4,4'-TCB) to nuclear macromolecules *in vivo*. C57/BL6 female mice were treated for three consecutive days with phenobarbital (400μmol/kg) and B-naphtoflavone (100μmol/kg), and on the fourth day, with 14C-labeled carcinogens. Mice were euthanized 24 hours later. DNA and chromatin proteins were extracted, separated and purified from whole livers by differential centrifugation and solvent extraction. DNA was further purified by hydroxylapatite chromatography. Radioactivity in DNA and nuclear proteins was determined by scintillation counting. Binding indexes were computed according to the concentration of chemical bound to macromolecules (umol chemical/mol amino acids or DNA) relative to the dose administered to the animals (mmol chem./kg body weight) or to the dose found in the liver (umol chem/g liver). Detection limits were computed based on the background readings of a corn oil-treated group. The protein binding index (PBI) was significantly lower for 3,3',4,4'-TCB (95% C.I.: 13.6±2.85) than for 4-CBP (95% C.I.: 29.6±9.65) and B[a]P (95% C.I.: 48.6±10.06) but all PBIs were significantly greater than detection limits (3.50, 4.85 and 3.09, respectively). Mean DNA binding indexes for B[a]P (95% C.I.: 1.81±1.5), 4-CBP (95% C.I.: 0.95±0.8) and 3,3',4,4'-TCB (95% C.I.: 0.77±1.1) were all greater than detection limits (0.63, 0.74 and 0.68, respectively) but these values were not statistically significant. This study shows that covalent binding of the tested compounds is significant for proteins. Binding to DNA is also suggested but not conclusive. (Supported P42 ES 07380.)

367 IDENTIFICATION AND CHARACTERIZATION OF ARYL HYDROCARBON RECEPTOR POLYMORPHISMS IN ATLANTIC TOMCOD (MICROGADUS TOMCOD) FROM ATLANTIC COAST POPULATIONS.

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Cytochrome P4501A1 (CYP1A1) mRNA induction serves as an easily quantifiable surrogate marker for all biological responses to exposure to aromatic hydrocarbon (AHs), including halogenated AHs (HAHs) such as dioxins and PCBs, and polycyclic AHs (PAHs) such as B[a]P. Hepatic CYP1A1 mRNA induction was observed in adult tomcod from the Hudson River exposed to PAHs (B[a]P, b-NF, 3-MC), but induction was absent or at reduced levels in the Hudson River tomcod treated with HAHs (PCB congeners 77, 126, 169) even after fish were extensively depurinated in clean laboratory water. Both PAHs and HAHs induced hepatic CYP1A1 mRNA in fish from cleaner Canadian rivers (Margaree and Miramichi). We hypothesize that adaptation of the aryl hydrocarbon receptor (AhR) pathway has occurred in the Hudson River population due to chronic exposure to unusually high concentrations of AHs along with mixtures of other genotoxic xenobiotics. Restriction fragment length polymorphism (RFLP) analysis revealed high levels of structural diversity in AhR alleles among fish from four rivers. Four different exon-specific cDNA probes that spanned the entire AhR cDNA from exon 1 through exon 11 were developed to more precisely map the locations of these polymorphisms in tomcod AhR. One probe, which hybridized with fragments exclusively in exon 10, revealed two alleles, 0.86 kb and 0.67 kb. Three different genotypes, homozygotes for both alleles and a heterozygote, were observed. A second probe, which hybridizes with exon 11, revealed at least seven alleles among tomcod from these rivers. These polymorphisms were then characterized by direct sequencing of genomic DNA PCR products. Variation in exon 10 included a 6 nucleotide (nt) addition in Canadian fish starting after nt 3892 downstream of the transcription initiation site of the AhR genomic DNA sequence of tomcod from the Hudson River. These nucleotides are in frame and encode for two amino acids, phenylalanine (F) and leucine (L). Three other point mutations, two G-C transversions at nt 5174 and 5665 and a T-C transition at nt 5359 were found, but do not result in amino acid change. Characterization of exon 11 variation is ongoing.

368 ROLE OF CYTOCHROME P450 INDUCTION AND LIPID PEROXIDATION IN 3,3',4,4'-TETRACHLOROBIPHENYL (PCB-77) AND 2,2',4,4',5,5'-HEXACHLOROBIPHENYL (PCB-153)-INDUCED TOXICITY IN LIVER OF MALE RATS.

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Polychlorinated biphenyls (PCBs) are environmental contaminants that have been widely used for various industrial purposes. In spite of the numerous studies on PCBs, however, the mechanism of toxicity of PCBs remains unknown. The role of cytochrome P-450 in PCBs-induced hepatic lipid peroxidation is controversial. Therefore, the present study was undertaken to study the mechanism of action of two PCBs and their role in cytochrome P450 induction and lipid peroxidation. Two congeneric PCBs were studied: PCB-77, a non-ortho-substituted, coplanar PCB; and PCB-153, a di-ortho-substituted, nonplanar PCB. Groups of male Sprague-Dawley rats were given a single ip injection of one of the two PCBs (low and high doses), both PCBs at the low dose, or vehicle alone. Rats were killed after 2, 6, or 24 h; or 2, 6, or 10 days. Cytochrome P-450 induction occurred as early as 2 h with PCB-77 and 2 days with PCB-153. Significant increases of 1.4-, 2.3- and 2.3-fold and 1.8-, 3.1- and 2.3-fold in TBARS content in liver tissue occurred 2, 6 and 10 days after treatment with 150 umol/kg of PCB-77 and PCB-153, respectively. The results indicate that both PCBs induced cytochrome P-450 enzymes and enhanced lipid peroxidation in liver and subcellular fractions but with different potencies and onsets of action. The results also indicate a time difference between P-450 induction and lipid peroxidation. Thus, both PCB-77 and PCB-153 are toxic to cells, but may act via different mechanisms to induce their effects. (Supported by ES07380.)

369 PCB METABOLITES: CYTOTOXICITY, GLUTATHIONE DEPLETION AND INHIBITION OF TOPOISOMERASE II.

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PCBs are highly persistent contaminants in our environment. We have previously shown that lower halogenated PCBs can be metabolized to dihydroxy compounds that are further oxidized to (semi)-quinones, and this oxidation is associated with the formation of reactive intermediates, such as reactive oxygen species (ROS). In this study, we investigated the reactivity of these metabolites by analyzing 1. cytotoxicity to HL-60 human lymphoma cells 2. effect on GSH in cells & *in vitro* and 3. activity of topoisomerase II *in vitro*. Test compounds were dihydroxy- and quinone metabolites of mono-, di- and tri-chlorinated biphenyls. We found that the PCB metabolites are cytotoxic to HL-60 cells with LC50's ranging from 1 -15 μM. We hypothesized that the mechanisms of toxicity could be through GSH depletion. GSH depletion from HL-60 cells was measured using monobromobimane assay and GSH binding *in vitro* was measured using the method of Tietze. We found that PCB quinones bound to GSH in ratios of 1:2 *in vitro* and depleted GSH in HL-60 cells in a dose-dependent manner. The dihydroxy metabolites did not bind GSH *in vitro* and depleted GSH from cells only at higher concentrations than the quinones. To analyze the effect on other sulfhydryl-containing biological macromolecules, we measured topoisomerase II activity after exposure to the metabolites *in vitro*. The PCB quinones inhibited topoisomerase II while the PCB dihydroxy metabolites did not. Hence binding of the PCB quinones to cellular molecules, oxidation of the dihydroxy PCBs to the corresponding quinone, and oxidative stress may be the major causes for the cytotoxicity of these compounds. (Supported by DAMD 17-96-1-6262, NIEHS P42 ES 07380, and 85-001-12-IRG from the American Cancer Society.)

370 CONTRIBUTION OF RYANODINE-SENSITIVE Ca^{2+} POOL TO METHYLMERCURY (MeHg)-INDUCED CHANGES IN $[Ca^{2+}]_i$ IN RAT CEREBELLAR GRANULE NEURONS.

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Cerebellar granule cells are preferentially targeted during MeHg neurotoxicity, resulting in their death following both *in vivo* and *in vitro* exposure. In granule cells in culture, MeHg causes a multiphasic elevation of intracellular Ca^{2+} ($Ca^{2+}{}_i$) consisting of release of $Ca^{2+}{}_i$ stores ("first phase"), followed by influx of extracellular Ca^{2+} ("second phase") and subsequent cell death. The purpose of the present study was to identify the source of the first-phase Ca^{2+} elevation following MeHg exposure. We used the dye fura-2 and single cell microfluorimetry to monitor changes in $[Ca^{2+}]_i$ of neonatal rat cerebellar gran-

ule cells in culture. The presence of a ryanodine-sensitive Ca^{2+} pool was verified using 10 μM ryanodine to inhibit Ca^{2+}-induced Ca^{2+} release due to release of the IP_3-sensitive smooth endoplasmic reticulum (SER) Ca^{2+} pool. Subsequently we used 10 μM ryanodine to inhibit this Ca^{2+} pool during exposure to 0.5 μM MeHg. The time-to-onset of the two phases of Ca^{2+}_i elevations (intracellular and extracellular) in response to MeHg was measured, as was the amplitude of the first phase as a measure of the amount of Ca^{2+} released from intracellular pools. Inhibition of the ryanodine-sensitive Ca^{2+} pool resulted in delays of both the first phase (from 10.9±1.4 min to 20.2±3.6 min) and second phase (from 19.5±2.4 min to 28.0±4.3 min) Ca^{2+}_i elevations. Further experiments in a low Ca^{2+}, EGTA-containing buffer (to isolate the first phase) indicated a similar delay in the first Ca^{2+}_i phase as well as a 33% reduction in its amplitude, indicating the loss of a portion of the first phase during ryanodine pool inhibition. These results suggest that the SER ryanodine-sensitive Ca^{2+} pool contributes to the first phase elevation of Ca^{2+}_i induced by MeHg. (Supported by NIH grant ES03299.)

371 METHYLMERCURY (MEHG) ALTERS MITOCHONDRIAL CALCIUM HOMEOSTASIS IN RAT CEREBELLAR GRANULE NEURONS.

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The purpose of the present study was to test the effect of acute MeHg exposure on mitochondrial Ca^{2+} (Ca^{2+}_m) homeostasis using single cell microfluorimetry and the dyes fura-2 and rhod-2 to monitor changes in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$, respectively, in neonatal rat cerebellar granule cells in primary culture. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and oligomycin were used simultaneously to depolarize the mitochondria and prevent Ca^{2+}_m buffering. Treatment with CCCP+oligomycin followed by MeHg (0.5-1.0 μM) delayed significantly both the release of Ca^{2+}_i and influx of Ca^{2+}_e. The fluorescence due to Ca^{2+}_i release was almost completely abolished. Thus most of the Ca^{2+}_i released during early MeHg exposure originates in the mitochondria. Cells loaded with rhod-2 and exposed to 0.5 μM MeHg exhibited a multiphasic increase in fluorescence; the initial increase occurred at 7.9±1.5 min, which is before the onset of the first phase observed with fura-2. CCCP+oligomycin abolished this phase, suggesting that the fluorescence increase was from Ca^{2+}_m uptake. When CCCP+oligomycin was used with 0.5 μM MeHg in a low-Ca^{2+}, EGTA-containing media, only two increases in fluorescence were observed: the initial rise at 7.1±1.9 min, with an amplitude similar to that of physiological Ca^{2+}_m uptake; and a second increase several min later, when fluorescence increased above that seen with normal Ca^{2+}_m uptake. The early time-to-onset of the initial elevation suggests mitochondrial Ca^{2+} buffering from another Ca^{2+}_i source, possibly the smooth endoplasmic reticulum Ca^{2+} pools. The second elevation was associated with spreading of the dye from punctate mitochondrial staining to diffuse cytosolic staining, suggesting that the dye had leaked from the mitochondria, perhaps via opening of the permeability transition pore. These results suggest that mitochondria are an early target during low-concentration MeHg exposure, resulting in loss of Ca^{2+}_m buffering which occurs during the initial stages of altered Ca^{2+}_i homeostasis. (Supported by NIEHS grants R01 ES03299 and T32 ES07255.)

372 ASSESSMENT OF INTEGRIN PROTEIN EXPRESSION IN PC-12 CELLS AFTER METHYLMERCURY EXPOSURE.

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We are conducting a pilot project to investigate the use of integrins as early markers of developmental neuronal changes to neurotoxicants. Integrins serve to transduce signals bi-directionally between extra-cellular components such as extra-cellular matrix and cell adhesion proteins and intracellular components such as transduction pathway proteins and the cytoskeleton (Clark and Brugge, 1995). Formed from heterodimers of α and β protein subunits, the composition of the heterodimeric receptor is determined by tissue and cell type as well as specific cellular response (Huhtala et al., 1995). Integrins are intimately involved in a number of processes related to neuronal development, including cell migration (Georges-Labouesse, et al., 1998), maintenance or plasticity of synapses (Einherber et al., 1996), and axonal elongation (Murase and Hayashi, 1998). Currently our efforts are directed at identifying in *in vitro* studies profiles of integrin subunit protein expression using Western Blot analysis of PC-12 cells, which have been used extensively as a model of neurite outgrowth. We are monitoring changes in these profiles after exposure to the known neurotoxicant, methylmercury. PC-12 cells are converted to the neuronal phenotype by 7 days culture in nerve growth factor β (NGF) containing media. Cells are then detached, washed and replated in NGF free media ± 0.2 μM methylmercury and proteins extracted 1, 2, 4, 8, 12, 24 and 48 hrs post-treatment. Levels of integrin protein expression will be correlated with measurements of neurite outgrowth in control and experimental cultures. This abstract does not necessarily reflect US EPA policy.

373 TOXICITY OF THE CO-CONTAMINANTS 137CESIUM AND MERCURY ON THE DEVELOPING NEURON.

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137Cesium (137Cs) and mercury compounds, including methylmercury (MeHg), are co-contaminants found in fish from rivers and streams on and adjacent to several decommissioned nuclear processing facilities around the U.S. The present study explores the potential for both cesium (Cs) and radiocesium (137Cs) to modify MeHg toxicity. CsCl and 137CsCl alone and in combination with MeHg were added to cultures of developing neurons (derived from P19, embryonal carcinoma cells) during neuronal differentiation. After 2 days of differentiation in retinoic acid, P19 cells were exposed to MeHg, CsCl, or 137CsCl individually and in combination for 2 hrs. Cells were stained with an antibody to tyrosinated a-tubulin, and microtubules were examined by fluorescent microscopy as an index of toxicity during early differentiation. Data demonstrated a dose-dependent disruption of microtubules in cells treated exclusively with MeHg. Treatment with CsCl alone did not disrupt microtubules during neuronal differentiation. In addition, CsCl did not modify MeHg toxicity at concentrations up to 275 nM. Cells treated with 137 CsCl alone did not significantly alter microtubules at concentrations up to 10pCi. However, MeHg and 137CsCl treatment increased cell damage, characterized by cytoplasmic blebbing, complex membrane invaginations, and loss of membrane integrity at concentrations of 1.0 μM MeHg and 0.1 pCi 137 CsCl. This effect became more robust with increasing concentrations of radiocesium and mercury. The addition of 137CsCl did not alter the dose-response of microtubules to MeHg. Data suggest that 137Cs and MeHg are not acting through a common mechanism of microtubular disruption. These effects could have implications in established risk estimates associated with ingestion of mercury and 137Cs contaminated fish near Department of Energy sites. (Research funded in part by NIEHS #ES05022,ES04976, and DOE #DE-FC01-95EW550084.)

374 MICE WITH METALLOTHIONEIN-III OVER-EXPRESSION IN BRAIN: COMPARISON TO C57/BL6 MICE IN BEHAVIORAL EFFECTS OF METHYLMERCURY (MEHG).

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The MT-III isoform of Metallothionein, expressed primarily in neurons, may have important roles in protecting the nervous system from exposure to metals as well as a role in regulating neurobehavioral function. Mice with an over-expression of human MT-III (MT+/+) in brain were evaluated as potential models of the functional significance of MT in Neurotoxicology. Repetitive circling behavior was exhibited by 25% of the offspring of MT+/+ mice. This circling occurred in response to handling or other stimulation. MT+/+ mice which did not exhibit the circling were similar to mice of C57B6 and 129SV/LMT strains in growth and locomotor behavior. The mice were then exposed to MeHg in drinking water (8 or 32 ppm) for 14 days. Exposure to 32ppm caused the C57BL6 mice to begin losing weight and begin dragging the hind feet after 14 days of exposure. Early in the MeHg exposure, MT+/+ mice exhibited less habituation to an unfamiliar test cage, than did C57BL6 mice. The reduced habituation during exposure to MeHg was similar to that observed in rats during oral exposure to Pb (Gong & Evans, Toxicol. Appl. Pharmacol. 144:205, 1997). MT+/+ mice which exhibited repetitive circling became even more active during exposure to MeHg. There was no evidence that MT+/+ mice were much less sensitive to MeHg than other "normal" strains. These findings indicate the importance of determining the neurobehavioral baseline of genetic variants before one can interpret the effects of a toxicant. MT+/+ source mice provided by Dr. R. Palmiter (U. Wash.). (Supported by a Center Grant ES-00260.)

375 EFFECTS OF PROTEIN-DEFICIENT NUTRITION DURING PREGNANCY AND DEVELOPMENT ON DEVELOPMENTAL HINDLIMB CROSSING DUE TO METHYLMERCURY INTOXICATION.

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The effects of methyl mercury intoxication on the clinical neurological signs due to protein-calorie malnutrition in children are not known sufficiently. Timed pregnant Sprague-Dawley rats were fed either a control (20% protein) or low (3.5% protein) diet during gestation and lactation. The pups were separated from their mothers of each diet group on postnatal day 21, and were given the same diets as those of their corresponding mothers. The groups of pups from each diet group were treated either on postnatal day 21, or postnatal day 60 with 7.5mg methylmercury chloride (MeHgCl) per kg b.w. once daily by gavage for 10 consecutive days and the development of ataxia (hind limb crossing) was monitored. The offspring from protein-deficient diet were found to be more sensitive to MeHg-induced ataxia than those from protein-sufficient diet. Rats fed 3.5% casein diet accumulated more mercury in the different brain regions than those fed 20% casein diet. The rates of protein synthesis in different brain regions of offspring fed the protein-deficient diet were significantly reduced compared to those fed the protein-sufficient diet. However, MeHg treatment did not significantly modify further the rates of such protein synthesis in protein-deficient rats. Thus, increased inhibition of the rates of protein synthesis plus increased accumulation of mercury in different brain regions due to severe protein deficiency may result in increasing susceptibility of developing rats to MeHg-induced ataxia, or hind limb crossing. (Supported in part by FRSQ, Environmental Health.)

376 INHIBITORS OF ASTROCYTIC EXCITATORY AMINO ACID TRANSPORTER FUNCTION ALSO DECREASE MRNA LEVELS.

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Removal of the potentially neurotoxic excitatory amino acids (EAAs) aspartate and glutamate from the synaptic cleft is accomplished via a family of transporters localized on astrocytes (GLAST and GLT-1) and neurons (EAAC1 and EAAT4). Astrocytes are responsible for the majority of the EAA uptake, and are also responsible for the subsequent metabolism of glutamate to glutamine. Previous studies (Trotti et al, 1997) have shown EAA uptake induced current in astrocytes is decreased by oxidation of thiol-containing cysteine residues with 5,5'-ditho-bis-2-nitrobenzoic acid (DTNB) and increased by reduction of protein thiols with dithiothreitol (DTT). MeHg, a thiol reactive neurotoxicant, inhibits EAA uptake as does the non-thiol reactive competitive analog threo-β-hydroxy-aspartic acid (THA). This study examined if mRNA levels of GLAST, the only EAA transporter constitutively expressed in primary astrocyte cultures, were altered during inhibition or facilitation of EAA transport. Confluent cultures of primary neonatal rat cortical astrocytes were exposed for 6 hours to MeHg (10 μM), the EAA transport inhibitor THA (1 mM), the thiol oxidizing agent DTNB (500 μM), or the thiol reducing agent DTT (1 mM). Steady state levels of GLAST mRNA were assessed by northern analysis and standardized to 28S rRNA levels. Inhibitors of EAA transport, THA and DTNB, significantly decreased steady state levels of mRNA following 6 hours of exposure (-34% and -26% respectively). MeHg, a weaker inhibitor of EAA transport, produced a lesser decrease (-17%), while DTT produced an increase (+17%) in GLAST mRNA levels. In general, mRNA levels of GLAST reflected functional state of the transporter following 6 hours of inhibition or potentation. These data suggest GLAST mRNA levels may be regulated by the activity of the transporter. Conditions producing changes in the function of the transporter, such as over-production of free radicals, may cause not only acute inhibition of function, but also prolonged inhibition of uptake due to decreased mRNA transcription. (This study was supported by NIEHS 07331 to MA. JWA is a NIAAA trainee, T32 AA07565.)

377 FOREIGN METALLOTHIONEIN-I (MT-I) EXPRESSION BY TRANSIENT TRANSFECTION IN MT-I AND MT-II NULL ASTROCYTES CONFERS INCREASED PROTECTION AGAINST ACUTE METHYLMERCURY CYTOTOXICITY.

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The mechanisms associated with metallothionein (MT) gene regulation are complex and poorly understood. Only a modest increase in brain MT expression levels is attained by exposure to metals, MT gene transfection, and MT gene knockin techniques. Accordingly, in the present study, MT null astrocytes isolated from transgenic mice deficient in MT-I and MT-II genes were introduced as a zero background model of MT expression. MT protein levels were determined by western blot analysis. MT proteins in MT-I and MT-II null astrocytes were undetectable. Transient MT-I gene transfection increased the levels of foreign MT expression in MT-I and -II null astrocytes by 2.3-fold above basal levels in wild type astrocytes. Intracellular Na251CrO4 efflux and D-[2,3-3H]-aspartate uptake were studied as indices of acute MeHg (5 mM) cytotoxicity. In MT-I and -II knockout astrocytes MeHg led to significant ($p<0.01$) increase in Na251CrO4 efflux and a significant ($p<0.05$) decrease in the initial rate (1 min) of D-[2,3-3H] aspartate uptake compared to MT-I and -II knockout controls. Transfection of the MT-I gene in MT-I and -II null mice significantly ($p<0.01$) decreased the effect of MeHg on Na251CrO4 efflux in MT null, as well as wild type astrocytes. MT-I gene transfection in MT-I and MT-II null astrocytes reversed the inhibitory effect of MeHg on D-[2,3-3H] aspartate uptake, such that initial rates of uptake in MT-I transfected cells in the presence and absence of MeHg (5 μM) were indistinguishable. These results demonstrate (1) that astrocytes lacking MTs are more sensitive to MeHg than those with basal MT protein levels, (2) that the MT-I gene can be overexpressed in MT-I and -II null astrocytes by transient MT-I gene transfection, and (3) that foreign MT expression endows astrocytes with increased resistance to MeHg. (Supported by NIEHS 07331. J.W.A. was supported by a NIAAA T32 AA07565.0.)

378 METAL AND RADIATION-INDUCED TOXIC NEUROPATHY (TN) IN TWO NAVAJO SISTERS.

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Introduction: "Navajo Neuropathy (NN)" was first reported in the 1970's in some Navajo. It was postulated to be a "genetic" disorder without any scientific proof. Thirty of 37 with this "syndrome" have died from fulminant liver disease or systemic infections. This "syndrome" includes progressive to static neuropathy, cataracts, and subcortical disease (by MRI) with an onset in early childhood. **Purpose and Methods:** Detailed clinical, genetic and environmental risk assessments of two Navajo sisters (27 and 28 years of age), presenting with "NN", were evaluated. **Results:** Extensive genetic testing was negative for both; but environmental health risk assessments revealed a toxic neuropathy from multiple toxicant exposures from abandoned surface mines during year-round herding. They had *in utero* and postnatal exposures to chemotoxic and radiotoxic levels of uranium (U) [and other radionuclides] and toxic levels of lead (Pb) in abandoned U mine pit waters, the exclusive source for drinking water and diluting infant formula. Additional fetal and postnatal toxicant exposures were to Pb, arsenic, and cadmium, in sheep meat from animals contaminated through vegetation and pit waters. The sisters had an *in utero* U/polonium radiation dose sufficient for CNS damage. Modeled *in utero* exposure to highly fetoneurotoxic Pb occurred via maternal blood lead level throughout pregnancy-embryonic and fetal stages of 40μg/dl. Further toxicity arose from other contaminant intakes during pregnancy in (μg/day): Cd, 656; As, 89. These toxic intakes of multiple contaminants continued postnatally. **Conclusion:** We conclude that there is no genetic basis for this disease. Its etiology is far more likely excessive exposure to metal toxicants and radionuclides *in utero* and postnatally thereby resulting in TN and subcortical CNS disease.

379 EFFECT OF NEONATAL LEAD EXPOSURE ON THE DEVELOPMENT OF CORTICAL COLUMNS IN THE RODENT BARREL FIELD.

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To examine the effects of neonatal lead exposure on developing neuronal cir-

cuitry, this study utilized the rodent barrel field model. Rodent somatosensory cortex contains a topographic map of the whisker pad, called the barrel field. Columnar processing units in the barrel field have discrete clusters of neurons in layer IV that receive sensory information from a single whisker. In all areas of mammalian neocortex, less anatomically distinct cortical columns constitute the fundamental unit for information processing and storage. In this study, rat pups were exposed to 0, 0.02%, 0.1% or 0.2% lead acetate dissolved in their dam's drinking water from the day of birth through postnatal day 10. This treatment, which coincides with the development of the barrel field topographic map, produced blood lead levels ranging from 1 to 22 micrograms/dl. On postnatal day 10, barrel field area was measured using cytochrome oxidase histochemistry in sections tangential to the cortical surface. A dose-related reduction in barrel field area was observed (Pearson correlation -0.73, $p<0.01$), and the mean area of the barrel field in rats exposed to 0.2% lead acetate was reduced 13%, compared to controls. In contrast, there was no difference in total cortical area. These data support the hypothesis that childhood lead exposure may disrupt the development of columnar processing units in immature neocortex, leading to permanent impairment of cognition. (Support: NIEHS P01 ES 08131.)

380 TIME- AND REGION-SPECIFIC CHANGES IN AMINO ACID NEUROTRANSMITTERS IN RATS EXPOSED TO LOW LEVEL LEAD.

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The neurobiochemical mechanisms underlying lead(Pb)-induced behavioral alterations, learning/attention deficits and cognitive impairments are largely unknown. But evidence points to disruptions of monoamine and amino acid neurotransmitter function in the mesocorticolimbic system. Strictly from a quantitative standpoint, the amino acids are the major transmitters in the mammalian brain whereas the monoamines account for transmission at only a small percentage of central synaptic sites. This study sought to determine the temporal and regional pattern of amino acid neurotransmitter (i.e., glutamate, GLU, glutamine, GLN, gamma-aminobutyric acid, GABA, and glycine, GLY) level changes produced by subchronic postweaning low Pb exposure on the mesocorticolimbic system (i.e., nucleus accumbens, NA, hippocampus, HIP, and frontal cortex, FC). 21-day old male Long Evans rats exposed to 0 ppm (as sodium acetate in drinking water) and 50 ppm Pb (as lead acetate in drinking water) were sacrificed at 0, 30, 60 and 90 days. Blood lead (BPb) analysis by atomic absorption spectrophotometry showed a significant difference between the control and Pb-treated rats at each time point except at day 0. Moreover, there was no significant difference in BPb level between day 30, 60, and 90 within the control and Pb-treated groups. By day 30, BPb seems to stabilize around 11.8 microg/dl which is comparable to the Center for Disease Control and Prevention's threshold level for children. The evaluation of tissue amino acid contents by high performance liquid chromatography indicated significant decreases in GLU, GABA, and GLY in the FC at day 90 and in the HIP at day 60 whereas significant increases in GLN were observed in these regions at these time points. Moreover, GABA, GLY and GLN were increased in the NA at day 60 and 90. These data suggest that subchronic postweaning Pb produces time- and region-specific changes within the mesocorticolimbic amino acid transmitter system. (Supported by ATSDR Cooperative Agreement # U50/ATU 398948.)

381 BLOCKADE OF NMDA RECEPTORS IN CORE BUT NOT SHELL OF NUCLEUS ACCUMBENS MIMICS LEAD (PB) EFFECTS ON LEARNING.

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Pb exposure selectively impairs learning on a multiple schedule of repeated learning and performance (Cohn et al., 1993). Since Pb inhibits NMDA receptors and nucleus accumbens (NAC; the terminal projection region for mesolimbic dopamine cell bodies) has been shown to be a target region for Pb-induced behavioral toxicity (Cory-Slechta et al., 1999), this study examined whether blockade of NMDA receptors in NAC could mimic Pb effects and if such effects would differ in core and shell subregions of NAC. Intra-NAC administration of the non-competitive NMDA antagonist MK-801 into surgically implanted bilateral guide cannulae, like Pb, decreased accuracy in the learning but not performance components of the schedule at 1.0 but not at 2.5 μg (total bilateral dose). This effect was not altered by chronic postweaning 100 ppm Pb acetate exposure of rats from weaning. Also, like Pb, this effect derived from increases in perseverative errors, while decreases in some skipping errors were detected. The accuracy reduction in the learning component could not be attributable to response rate changes, since rate increases occurred at 2.5 but not 1.0 μg MK-801 in all components. In shell, MK-801 decreased accuracy in both the learning and performance components in both control and Pb-exposed rats, and had no effect on response rates. These findings demonstrate a critical role for mesolimbic systems in the mediation of Pb-induced learning impairments. The relatively small magnitude of the accuracy decreases (10-15%), and their occurrence in core but not shell suggests the potential importance of hippocampal and prefrontal cortical afferent projections to NAC core as primary sites for these effects. ES01247, ES05017, ES05903.

382 SYNAPTIC TRANSMISSION MEDIATED BY GLUTAMATE AND GABA IS DECREASED IN RATS EXPOSED TO LEAD (PB^{2+}) *IN VIVO*.

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The heavy metal, Pb^{2+}, has long been known to affect cognition in children, as well as learning and memory in experimental animals. To determine whether synaptic transmission is altered in rats exposed to Pb^{2+} during gestation and postnatally, we studied the hippocampus of rats chronically exposed to PbAc (0, 750 and 1500 ppm). Hippocampal slices were prepared from control and Pb^{2+} treated rats at 14 and 21 days of age, and whole-cell patch clamp recordings were performed on CA1 pyramidal neurons and interneurons. Currents were evoked either by applying agonists via a U-tube or by stimulating afferent fibers in the presence of selective receptor antagonists. In CA1 interneurons of Pb^{2+}-exposed rats, the peak amplitude of currents evoked by NMDA (50 μM) plus glycine (10 μM) was reduced by ~40% in 750 ppm-treated rats. In CA1 pyramidal neurons of 1500 ppm PbAc-treated rats, the amplitude of NMDA-evoked currents was decreased by ~50%. In addition, Pb^{2+} exposure (i) reduced the amplitude of pharmacologically isolated excitatory postsynaptic currents (PSCs) more than the inhibitory PSCs recorded from CA1 pyramidal neurons, (ii) caused a pronounced attenuation of ACh-evoked GABAergic PSCs, and (iii) enhanced the inhibitory effect of $PbCl_2$ (100 nM) on both glutamatergic and GABAergic synaptic transmission of hippocampal neurons. These findings suggest that multiple receptors associated with synaptic transmission are altered by *in vivo* exposure to Pb^{2+}. An imbalance of activity in different neurotransmitter systems may underlie the effects of Pb^{2+} on learning and memory in developing experimental animals and in humans. (Supported by ES05730 to EXA and ES06189 to TRG.)

383 [^{3}H]-MK-801 AND [^{3}H]-IFENPRODIL AUTORADIOGRAPHY SUGGEST N-METHYL-D- ASPARTATE RECEPTORS IN THE DENTATE GYRUS OF LEAD-EXPOSED RATS DIFFER IN THEIR PHARMACOLOGY.

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We have previously shown that mRNA and protein expression for *N*-methyl-D-aspartate receptor subunit 1 (NMDAR1) are decreased in the hippocampus of rats exposed to 1500 ppm PbAc that exhibit deficits of long-term potentiation (LTP) and spatial learning. (Nihei *et al.*, Toxicol Sci. 48 1-S: 359, 1999). To determine the effect of Pb^{2+} on functional NMDARs in the hippocampus of littermates, we measured the binding of the NMDAR non-competitive antagonist [^{3}H]-MK-801, and the polyamine site antagonist [^{3}H]-ifenprodil. Levels of [^{3}H]-MK-801 binding were significantly higher ($F_{1,16}$=8.3, p=0.01) in Pb^{2+}-exposed dentate gyrus (DG) in the absence of exogenously added agonists (30 μM glutamate and glycine). [^{3}H]-MK-801 binding was significantly increased ($F_{1,16}$=8.3, p=0.01) in the DG of control rats by the addition of exogenous agonists. In Pb^{2+}-exposed rats, binding of [^{3}H]-MK-801 in the absence of agonists was equal to maximal binding in controls and could not be further increased by the addition of agonists. NMDARs composed of NR1/NR2B subunits have a greater sensitivity for both agonist and ifenprodil. [^{3}H]-ifenprodil binding was significantly greater ($F_{1,7}$=11.4, p=0.01) in the DG of Pb^{2+}-exposed rats. These findings suggest that in the DG of Pb^{2+}-exposed rats, either NMDARs are maximally stimulated by agonists under basal conditions, or a greater proportion of functional NR1/NR2B receptors exist in this brain region. (Supported by ES06189 to TRG.)

384 PKC-γ BUT NOT CA^{2+}-DEPENDENT PKC ACTIVITY IS REDUCED IN THE HIPPOCAMPUS OF RATS CHRONICALLY EXPOSED TO PB^{2+}.

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The protein kinase C (PKC) family of serine/threonine kinases is comprised of at least 12 different isoforms. The brain-specific PKC-α requires Ca^{2+} and lipid cofactors for activity and has been shown to be involved in spatial learning and memory and is implicated in activity-dependent neuronal plasticity. In the present study, cytosolic and membrane fractions of hippocampus were prepared from postnatal day (PN) 50 rats exposed chronically to 0, 750, or 1500 ppm lead acetate (PbAc) in their diet. Western immunoblots showed reduction of PKC-α in both the cytosolic ($F_{2,17}=4.8$; $p<0.05$) and membrane ($F_{2,17}=3.3$; $p<0.064$) fractions. The alpha, beta-I, and beta-II isoenzymes were unchanged in either fraction. Ca^{2+}/lipid-dependent activity of PKC was not changed in either the cytosolic or membrane fractions in hippocampi of rats exposed to PbAc. Reductions in PKC expression were present at blood Pb^{2+} levels ranging from 25-35 μg/dl. Littermates to these rats demonstrate impaired spatial learning and *in vivo* long-term potentiation (LTP). Thus, reduced subcellular concentrations of PKC-gamma may be indicative of altered signal transduction and may be important in manifesting Pb^{2+}-induced impairments of learning and memory. (Supported by ES06189 to TRG and ES05869 to MKN.)

385 EFFECTS OF DIVALENT CATIONS ON [^{3}H]MK-801 BINDING TO THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR OF RAT BRAIN.

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NMDA receptor, a subclass of glutamate receptor, plays an important role in a wide variety of physiological and pathophysiological processes. Our previous study have showed that subacute Pb-exposure of adult rats causes a decrease and that chronic Pb^{2+} exposure of developmental rats causes an increase in [^{3}H]MK-801 binding in the brain of rats. The present study investigated effects of several divalent cations on [^{3}H]MK-801 binding to rat brain sections and membrane preparations from adult rats *in vitro*. These cations (1-500 μM) were added into incubation system. Autoradiographic analysis of brain sections indicated that Pb^{2+} and Zn^{2+} enhanced [^{3}H]MK-801 binding at low concentrations (around 10 μM) and inhibited the ligand binding at high concentrations (>100 μM) in the cerebral cortex and hippocampus. However, Mg^{2+} and Ca^{2+} did not show such kind of biphasic effect. At high concentrations (>100 μM), Mg^{2+} induced decrease and Ca^{2+} induced increase in [^{3}H]MK-801 binding in the cortex and hippocampus. Similar effect of these cations on [^{3}H]MK-801 binding was also found in cortical membrane preparations. Hg^{2+} and Cd^{2+}, like Pb^{2+}, produced biphasic effect on [^{3}H]MK-801 binding to brain sections. When brain sections or membrane preparations were pre-treated with 0.8% Triton X-100, Pb^{2+} and Zn^{2+}-induced increase in [^{3}H]MK-801 binding was completely abolished. These results suggest that, besides Zn^{2+}, heavy metals Pb^{2+}, Hg^{2+} and Cd^{2+} may also act on modulatory site(s) on the NMDA receptor. The divalent cations-induced biphasic effects on [^{3}H]MK-801 binding to NMDA receptor rely on existence of the membrane lipids.

386 LEAD INDUCES PKC-MEDIATED PHOSPHORYLATION OF 45 KDA PROTEIN IN ISOLATED NERVE TERMINALS.

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Lead (Pb) is a well-known neurotoxicant. Exposure to relatively low levels of Pb is associated with deficits in cognitive function. The precise molecular mechanism underlying low level Pb toxicity is not understood. The highest affinity action of Pb yet described is its ability to activate calcium/phospholipid-dependent phosphotransferase, PKC. PKC is involved in many signal transduction processes in neurons and is essential for the induction of long-term potentiation, an electrophysiological model for learning. In the present study, we examined the ability of Pb^{2+} to activate PKC in presynaptic nerve terminals isolated from rat brain cortex, synaptosomes. The specific endogenous proteins phosphorylated were resolved by SDS-PAGE. PKC activity was determined in digitonin-permeabilized synaptosomes where the specific [Ca^{2+}] and [Pb^{2+}] were set using 5F-BAPTA as the chelator and the free metal ions confirmed using ^{19}F NMR. Experiments were performed in the presence and absence of the exogenous substrate [^{25}ser] PKC peptide 19-36, in the presence and absence of the PKC activator, PMA, and in the presence and absence of the PKC selective inhibitor, calphostin c. Increases in phosphorylation of exogenous substrate were seen at [Ca^{2+}] from 10^{-6} to 10^{-3} M. A marked increase in phosphorylation was observed at 10^{-9} M Pb^{2+}, corresponding to about 65% of maximal phosphorylation induced by Ca^{2+}. Phosphorylation was inhibited at higher [Pb^{2+}]. Ca^{2+}-induced increases in total phosphorylation of endogenous substrates were seen from 10^{-6} to 10^{-3} M with a specific increase in the phosphorylation of an 84 kDa protein. No significant increase in the total phosphorylation of endogenous substrates was seen with Pb^{2+}, but a specific significant increase in the phosphorylation of a 45 kDa protein was observed a 10^{-10} M Pb^{2+}. This protein corresponds to the localization of growth-associated protein 43 (GAP43, F1, B50), a well-documented PKC substrate whose phosphorylation is essential for the induction of LTP. These data begin to reveal how high affinity interactions of Pb^{2+} with PKC can alter specific molecular mechanisms involved in cognitive function.

387 CHRONIC DEVELOPMENTAL LEAD (PB) EXPOSURE INCREASES PHOSPHORYLATED FORMS OF RAT HIPPOCAMPAL PROTEIN KINASE C (PKC).

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Previous studies have demonstrated that chronic developmental Pb exposure impairs induction of long-term potentiation (LTP) and stimulation of glutamate release in rat hippocampus. Here we sought to identify mechanisms underlying these alterations by examining the expression of GAP-43–a PKC substrate linked to LTP development and transmitter release–and phosphorylation events related to PKC activation. Post-translational activation of PKC involves serial phosphorylation at three sites in order for the kinase to be responsive to stimulation (Dutil *et al.*, 1998). 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 weaned to the same solution. At 60 days of age rats were sacrificed by focused microwave irradiation and dorsal hippocampi were dissected and sonicated. Proteins were separated by gel electrophoresis, transferred to nitrocellulose and immunoblots were probed with various primary antibodies (Ab); signal was detected by enhanced chemiluminescence. Hippocampal expression of GAP-43 did not differ between exposed and control animals. Moreover, an Ab that recognized an epitope common to the conventional α, β and γ isozymes of PKC also did not detect a Pb-related difference. However, blots probed with an Ab raised against a phosphorylated form of PKC (i.e., site 3 above) that recognized the conventional isozymes plus the novel δ isoform exhibited a band of phospho-PKC-like immunoreactivity that was significantly increased ($p < 0.05$) in Pb-exposed animals. Furthermore, blots probed with a phospho-PKCδ-specific Ab also displayed a band of significantly increased phospho-PKCδ-like immunoreactivity in exposed tissue ($p < 0.05$). The mechanism of the phosphorylation of GAP-43 by the δ isozyme has been described (Dekker and Parker, 1997). These results suggest an up-regulation in post-translational activation of some PKC isozymes. Future studies should examine these mechanisms in tissue tetanically stimulated to produce LTP or depolarized to induce glutamate release. (This abstract does not necessarily reflect U.S. EPA policy.)

388 PB EXPOSURE MODULATES EGR-1 DNA-BINDING BOTH *IN VIVO* AND *IN VITRO*.

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Zinc finger proteins (ZFPs) are essential components of developmental gene regulation and transcriptional activation and are the likely targets for disruption by heavy metals such as lead (Pb). We have previously reported alterations in the DNA-binding of ZFP transcriptional factors such as Sp1 and Egr-1 in rat cerebella, following developmental exposure to Pb. We further extended our studies to examine Pb induced alterations in the DNA-binding Egr-1 in different brain regions and in PC12 cells. Rats were lactationally - exposed to 0.2% Pb acetate from birth to weaning and their brain regions were obtained on postnatal days (PND) 3-30. Pheochromocytoma (PC12) cells were exposed to Pb acetate, nerve growth factor (NGF) in the presence or absence of staurosporine, a protein kinase C inhibitor. Nuclear extracts were prepared from brain tissues and PC12 cells and Egr-1 DNA-binding analysis were done using the gel shift mobility assay. Egr-1 DNA-binding in the control cerebellum and hippocampus was low on PND3 with a gradual increae to a peak at PND20. Egr-1 DNA-binding was relatively weak in the neocortex

and brainstem. In Pb-exposed animals, a preamture peak in Egr-1 DNA-binding was observed in the cerebellum and hippocampus. Egr-1 DNA-binding in PC12 cells increased in a dose dependent manner upon exposure to lower concentrations of Pb and NGF and was attenuated by the presence of staurosporine. These data suggest that Pb exposure alters gene expression through perturbations of ZFPs, and that such modulations may be mediated by protein kinase C.

389 INFLUENCE OF LEAD ON NITRIC OXIDE IN THE DEVELOPING RAT BRAIN.

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Nitric Oxide (NO), a neuronal messenger in the brain is synthesized by nitric oxide synthase during the conversion of L-arginine to citruline. Lead (Pb) is neurotoxic. This study was initiated to determine the effects of Pb on NO production in the developing rat brain. Pregnant Sprague Dawley rats in their 5th day of gestation were treated orally with 0.1% and 0.2% lead acetate. The control groups received saline. Treatments were continued during the pregnancy and until 21 post-natal days (PND). The pups were sacrificed at PND 5,10,15, 20 and 25, whole brain tissues were excised and separated into four regions: the cerebellum,the hippocampus, the frontal cortex and the brain stem. Nitrate and nitrite (NOx) levels were estimated by the chemiluminescent method using Sievers 280 - Nitric Oxide Analyzer (NOA). The effects of lead on NOx production were dose and age dependent and region specific. In the cerebellum of the brain at PND 25, 0.1% and 0.2% Pb reduced NOx by 428% and 328% respectively. In the hippocampus, both 0.1% and 0.2% Pb reduced NOx at all PND except PND 15. In the brain stem, there were significant reduction in NOx at PND 20 and 25. Lead (Pb) at both dose levels reduced NOx in the frontal cortex at PND 10, 15, 20 and 25. However, at 5 PND, there were no significant changes at both 0.1% and 0.2% Pb in the NOx of the frontal cortex. These results suggest that Pb reduces NO production in different regions of the rat brain leading to neuronal dysfunction. (Supported by NIH/NIGMS/MBRS #GM55356.)

390 OXIDATIVE STRESS IN EMBRYONIC RAT HIPPOCAMPAL NEURONS INDUCED BY LEAD IS REVERSED BY ASTROCYTE/NEURON INTERACTIONS.

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Inorganic lead is a very potent neurotoxicant that has profound effects on the development and function of the nervous system. This toxicant is very prevalent in the environment today and continues to affect individuals living primarily in urban settings. We performed experiments to evaluate 1)the effects of lead on reactive oxygen species (ROS) generation in both neurons and astrocytes, and 2) if astrocytes co-cultured with neurons conferred protection against the effects of lead on ROS generation in neurons. First, we exposed primary cell cultures of E-18 rat hippocampal neurons or astrocytes to 10μM lead chloride, 200μM hydrogen peroxide, or both lead and peroxide for 24 hours, after which the levels of ROS were measured using the fluorescent probe 2,7-dichlorofluorescine. Levels of ROS in neurons exposed to lead or peroxide increased to 150% of control values. ROS generation in neurons exposed to both lead and peroxide together increased to 210% of controls. Astrocytes showed no increase in ROS generation under any conditions. These data suggest that rat hippocampal neurons are unable to protect themselves against lead or peroxide induced oxidative stress, whereas astrocytes are. Previous studies have shown that heme oxygenase (HO-1, HO-2) is induced in other cell types undergoing oxidative stress. We evaluated the ability of both neurons and astrocytes to synthesize these proteins under lead and hydrogen peroxide induced oxidative stress. Neurons showed no significant increase in HO-1 or HO-2 with exposure to lead or peroxide. Astrocytes however showed a significant increase in HO-1 synthesis. These data suggest that HO-1 may play a role in protecting astrocytes from the effects of oxidative stress. Further experiments were performed to investigate whether or not astrocytes may confer protection to neurons under conditions of oxidative stress. Neurons were cultured both with astrocytes present and in astrocyte conditioned media. In both instances, the lead and peroxide-induced ROS generation seen in neurons alone was reversed. These data suggest a non-cell contact role of astrocytes in preventing oxidative stress in neurons.

391 LEAD (PB) TARGETS GRP78, A MOLECULAR CHAPERONE, IN C6 RAT GLIOMA CELLS.

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Exposure to potentially neurotoxic levels of lead (Pb) occurs in about 9% of American children under six years of age. Astroglia in the brain serve as a Pb depot, sequestering Pb and preventing its contact with the more sensitive neurons. Astroglia have the capacity to adapt to Pb exposure, and as such are able to tolerate relatively high intracellular Pb accumulation. This tolerance mechanism has yet to be defined in biochemical terms. In the present study, we present evidence that glucose-regulated protein (GRP78), a molecular chaperone in the ER, participates directly or indirectly in the tolerance mechanism. Exposure of cultured C6 rat glioma cells, an astroglia-like cell line, to 1 μM Pb acetate for 1 week raised the intracellular levels of two proteins, one of which was identified by sequence analysis as GRP78. GRP78 accumulation started within one day and progressed with time of exposure. Studies *in vitro* showed further that GRP78 bound tightly to affinity columns with Pb^{2+} as the affinity ligand, and weakly when either Zn^{2+} or Ni^{2+} replaced the Pb^{2+}. GSH and BSA did not compete with GRP78 to chelate Pb^{2+}. However, the heavy metal binding domain (HMB) of Menkes protein competed with GRP78 for chelating Pb^{2+}. The data provide evidence that GRP78 may be a component of the Pb tolerance mechanism through its direct interaction with Pb^{2+}. Its increased synthesis could be part of the adaptive response to Pb exposure.

392 LEAD EXPOSURE ALTERS THE DEVELOPMENTAL EXPRESSION OF GALACTOLIPIDS AND CNPASE IN OLIGODENDROCYTES.

W. Deng and R. D. Poretz. *Rutgers University, New Brunswick, NJ.* Sponsor: D. Eaton.

Lead is known to cause myelin defects, although the mechanism is unclear. Our *in vivo* experiments indicate that lead exposure results in reduced cellular levels of enzymes in both the biosynthesis and degradation pathways of myelin-specific glycolipids in the developing rat brain. Myelin in the central nervous system is formed by oligodendrocytes, making these cells a possible target for lead. Development of the oligodendrocytes proceeds with a regulated temporal expression of specific glycolipids and glycoproteins which serve as markers of myelinogenesis. In the present study, rat oligodendroglial progenitor O2A cells and a CG-4-like cell line were used to examine the effect of lead on myelin-specific glycolipids and enzymes during developmental progression to mature oligodendrocytes. Lead acetate (5 μM) treatment, given prior to and during cell differentiation, altered the expression profile of cellular galactolipids during differentiation as detected by metabolic radiolabeling with 3-H-galactose. This exposure caused a decrease of approximately 50% in the ratio of galactocerebrosides/sulfatides in both undifferentiated and differentiated CG-4 cells. Lead treated CG-4 cells, as compared to appropriate controls, were found to possess 30% less activity for the oligodendrocyte marker, 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNPase), in both the undifferentiated and differentiated stages. Surprisingly, undifferentiated CG-4 cells expressed high levels of this enzyme activity which, however, remained unchanged during cellular differentiation. This contrasts with O2A primary cells which express significant CNPase activity only after cell differentiation. It appears that the CG-4-like cell line differs from O2A primary cultures in regard to the degree of expression of some markers of differentiation, but may still serve as an appropriate experimental model for the examination of the effects of lead on oligodendrocyte development. (Supported in part by grants from NIEHS, R01-ES08373, and the March of Dimes Foundation.)

393 INHIBITORY EFFECT OF LEAD (PB) ON THE UPTAKE OF THYROXINE BY THE PERFUSED OVINE CHOROID PLEXUS (CP).

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Lead exposure hinders brain development in children by a mechanism which remains unknown. Recent evidence shows that sequestration of Pb in the CP represses the production and secretion of transthyretin (TTR), a thyroxine transport protein, from the CP to CSF. This study was undertaken to determine if Pb treatment altered the unidirectional transport of T4 to the CSF in the isolated perfused CP. Sheep were injected subcutaneously with Pb acetate (6 mg Pb.kg-1; n=3) or Na acetate (control, n=3) every 48 hr for a period of 12 days. The CP was perfused with a modified Ringer via the internal carotid arteries.

The [125I]T4 uptake was determined by the paired-tracer method using the perfusate containing 0.5 Ci [125]T4 and 20 Ci [3H]mannitol at various concentrations of unlabeled T4 (trace to 15 M). While there was an increase in the [125I]T4 influx into the CP as the concentration of unlabelled T4 increased for both control and Pb treated animals, the actual influx was much reduced in the latter group. For example, the [125I]T4 influx into the CP at 15 M was 98.9 nmol.min-1.g-1+23.6SEM (n=7) and the mean value for the Pb-treated animals was 9.2 nmol.min-1.g-1+6.1, a reduction of 90% ($p<0.001$, by two-way ANOVA). The rate of vascular flow was also decreased by 67% from 2.6+0.13 to 0.87+0.08 ml.min-1.g-1 of CP (mean+SEM, $p<0.005$). The results suggest that Pb may reduce the uptake of T4 partially by decreasing the rate of vascular flow of the CP, an important factor in determining transport. (Supported by NIEHS RO1 ES08146.)

394 COMPARISON OF CYTOTOXICITIES INDUCED BY Mn(II) OR Mn(III) *IN VITRO*.

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The relationship between manganese (Mn) intoxication and Parkinsonism has long been established. However, the question as to what biological species of Mn is responsible for Mn-induced neurotoxicity remains unknown. This laboratory has previously demonstrated that Mn(II) inhibits aconitase, an enzyme possessing a [4Fe-4S] cubane cluster and regulating the cellular Fe homeostasis (Zheng, et al., Brain Res. 799:334, 1998). In the current study, we used cultured neuronal PC12 cells and purified mitochondrial aconitase to compare the *in vitro* cytotoxic effect of Mn(II) with Mn(III). When the aconitase was incubated with either Mn(II) as $MnCl_2$ (1 mM-2.5 mM) or Mn(III) as Mn acetate (125-700 μM), both Mn species decreased the rate of reaction catalyzed by aconitase. However, Mn(III) was evidently more effective than Mn(II) in enzyme inhibition. The ED_{10} (10% inhibition of aconitase activity) was 124 μM and 1164 μM for Mn(III) and Mn(II), respectively. Following exposure of cultured PC12 cells with 25-50 μM of either Mn(II) or Mn(III) in culture medium, both Mn species significantly retarded the cell growth compared to the controls by two-way ANOVA ($p<0.0001$). There was a significant statistical difference in the cell growth curves between Mn(II)- and Mn(III)-treated groups. For example, at day 5 after Mn treatment, the total viable cells in 50-μM Mn(II) group were 6.6% of the control, while it was 2.8% of the control values in 50-μM Mn(III) group. These *in vitro* results suggest that Mn(III) species seemed likely to be more cytotoxic than Mn(II) species. Whether the similar scenario also happens *in vivo* in animal models deserves further investigation.

395 COMPARATIVE TOXICOKINETICS OF MANGANESE CHLORIDE ($MnCl_2$) AND METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT) IN MALE SPRAGUE-DAWLEY RATS.

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The toxicokinetics of Mn was investigated in male and female rats either following a single intravenous (iv) or oral dose of $MnCl_2$ (6.0 mg Mn/kg), or following a single oral dose of MMT (20 mg MMT/kg or 5.6 mg Mn/kg). The plasma concentrations of Mn were quantified by AAS. Upon iv dose of $MnCl_2$, Mn rapidly disappeared from blood with a terminal elimination $t_{1/2}$ of 1.83 h and CL_s of 0.43 L/h/kg. The plasma concentration-time profiles of Mn could be described by C=41.9e-4.24t + 2.1e-0.44t. Following oral dose of $MnCl_2$, Mn rapidly entered the systemic circulation (T_{max}=0.25 h). The absolute oral bioavailability was 13%. Oral dose of MMT resulted in a delayed T_{max} (7.6 h), elevated C_{max} (0.93 μg/ml), and prolonged terminal $t_{1/2}$ (55.1 h). The rats receiving MMT had an apparent clearance (CL/F=0.09 L/hr.kg) about 37 fold less than did those who were dosed with $MnCl_2$. Accordingly, the AUC of Mn in MMT-treated rats was about 37 fold greater than that in $MnCl_2$-treated rats. A gender-dependent difference in toxicokinetic profiles of plasma Mn was also observed. Female rats displayed a greater AUC than that of male rats. Although the apparent Vd of Mn was similar in both sexes, the apparent CLs in males was about twice that observed in females. The results indicated that after oral dose, the MMT-derived Mn displayed a higher and more prolonged plasma concentration-time profile than $MnCl_2$-derived Mn. Thus, MMT-derived Mn appeared likely to accumulate in the body following repeated exposure.

396 MEDIA COMPOSITION AFFECTS MTT UPTAKE BY PRIMARY RAT ASTROCYTES IN RESPONSE TO MN EXPOSURE.

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The primary culture of glial cells from neonatal rats has proven to be a valuable model for studying neurotoxicity *in vitro*. Astrocytes perform supportive functions for neurons *in vivo*; therefore, effects of manganese (Mn) on astrocytes might contribute to its neurotoxicity. Primary rat astrocytes were obtained by shaking mixed glial cell cultures from various regions of brains of one day old pups and plated in 24-well poly-L-lysine coated plates. Cultures were maintained in DMEM/10%FBS until 48 hour exposure to varying concentrations of MnCl2 in either DMEM/10%FBS or OPTI-MEM/0.1%BSA. Manganese was found to have relatively little effect on astrocytes in DMEM/10%FBS media: uptake of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; as measured by absorbance at 570 nm) was consistently >50% of control cultures after 48 hours exposure to Mn concentrations up to 2 mM. There was little effect of brain region (cortical, striatal, mesencephalic) on the MTT uptake response to Mn exposure. In contrast, when astrocytes were exposed to Mn in OPTI-MEM/0.1%BSA, marked toxicity occurred at concentrations >250μM. We conclude that differences in nutrient and hormonal composition of the two media formulations affect astrocyte Mn toxicity. A systematic investigation of the factors affecting astrocyte Mn toxicity will yield valuable information about the mechanism of Mn neurotoxicity. (Supported by PHS/NIH NS10235 [EAM].)

397 OLFACTORY TRANSPORT OF INHALED ^{54}MANGANESE CHLORIDE TO THE BRAIN IN MALE CD RATS.

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Inhalation exposure of humans to high concentrations of manganese (Mn) is associated with elevated striatal Mn levels and a Parkinson's-like motor disorder. Olfactory transport of Mn to the brain has been demonstrated in the rat following nasal instillation of $^{54}MnCl_2$; however, the significance of this route of delivery following inhalation is unknown. Our purpose was to determine if inhaled Mn could undergo olfactory transport to the brain in rats and the relative contributions of the direct olfactory and blood-borne routes to brain Mn levels. Male, 8-wk old, CD rats underwent a single, 90 minute, nose-only exposure to 0.55 mg Mn/m^3 in the form of aerosolized $^{54}MnCl_2$ (MMAD ~2.2 μm). Thirty rats had their right nostril occluded, preventing deposition of $^{54}MnCl_2$ in the right side of the nose and theoretically olfactory transport to the right side of the brain. Thirty positive control rats were exposed to $^{54}MnCl_2$ with both nostrils open to evaluate the symmetry of delivery. The left and right sides of the nose and brain including the olfactory bulb, tract/tubercle, and striatum were sampled from 6 rats/group (control and occluded) at 0, 1, 2, 4, and 8 days post-exposure. The mean sample activity/gram tissue wet weight for the left and right sides was compared to determine the relative contribution of olfactory uptake to brain Mn levels. High levels of activity in the olfactory bulb and tract/tubercle were observed following ^{54}Mn inhalation on the side with an open nostril within 1-2 days post-exposure. Brain and nose samples on the occluded side had negligible levels of activity. Control rats had equivalent ^{54}Mn concentrations on both sides of the nose and brain. These results demonstrated olfactory transport of inhaled ^{54}Mn to the olfactory pathway of the brain but not the striatum in the male CD rat. Furthermore, the olfactory route contributed the majority (up to 90%) of the Mn found in the olfactory pathway of the brain up to 8 days following a single inhalation exposure.

398 INFLUENCE OF SUBCHRONIC MANGANESE SULFATE ON THE NEUROBEHAVIOR OF MALE RATS.

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The potential of manganese sulfate to produce neurotoxicity following ninety days of daily gavage was studied in male rats. The rats were gavaged with suspensions containing 0.1, 1.0, 10, 30, or 100, mg/kg manganese or the vehicle each day. The functional observational battery, motor activity measurements, and the step-down task were used in an initial screen for neurotoxicity. In the functional observational battery, manganese sulfate only significantly affected weight gain. Male rats given 100 mg/kg manganese gained less weight

than those gavaged with 0.1 or 1.0 mg/kg manganese, or the vehicle. The locomotor activity of the rats was measured for 1 hour in a figure 8 maze. The rats gavaged with 30 mg/kg manganese had more motor activity during the hour in the maze, relative to rats gavaged with the vehicle. In addition, the rats gavaged with 30 mg/kg manganese had more motor activity in the final 20 min. in the maze, relative to rats gavaged with 100 mg/kg manganese. The gavaged doses of the trace metal salt did not significantly alter the performance of the rats on the step-down task. The behavioral effects produced by ninety days of daily gavage indicate that the higher doses of manganese sulfate may be toxic in male rats. (Support by ATSDR grant U50/ATU398948).

399 THE NEUROTOXIC EFFECTS OF MANGANESE IN A RAT MODEL OF PRE-PARKINSONISM.

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Elevated exposures to manganese (Mn) can induce neurologic impairments similar to those observed in Parkinson's disease (PD). Little is known about the toxic effects of low level chronic Mn exposure, particularly on sensitive sub-populations such as the aged. Here, a rat model of pre-parkinsonism was utilized to evaluate the hypothesis that Mn exposure can trigger the onset of parkinsonism. Different degrees of pre-parkinsonism were generated via unilateral intra-striatal injections of 6-hydroxydopamine (6-OHDA). In this model, dopamine (DA) depletion is considered to represent the extent of unilateral loss of substantia nigra dopaminergic neurons innervating the striatum. Treatment groups included: (T_1) saline intra-cranially (ic) + saline ip; (T_2) saline ic + $MnCl_2$ ip; (T_3) 3 µg 6-OHDA ic + saline ip; (T_4) 3 µg 6-OHDA ic + $MnCl_2$ ip; (T_5) 5.5 µg 6-OHDA ic + saline ip; and (T_6) 5.5 µg 6-OHDA ic + $MnCl_2$ ip. Manganese exposure (4.8 mg Mn/Kg body weight, 3 inj./wk X 5 weeks) started 4 weeks after performing the striatal lesions. Measured outcomes were striatal dopamine (DA) and its metabolites 3,4-dyhydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Results demonstrate predictable degrees of DA depletion in the three 6-OHDA treatment groups (0%, 50%, 70%, respectively) that are consistent with sub-threshold pre-parkinsonism. The DA/metabolite ratios varied across 6-OHDA treatments, indicating compensation for DA depletion through an increase in the rate of DA turnover. Notably, Mn exposure had no measurable effect on DA depletion, but did produce neuromotor impairments that were more severe in animals with existing high sub-threshold DA depletion (see Witholt et al., this volume). This indicates that the Mn exposures used here did not induce dopaminergic cell death, but did affect the nigro-stratial pathway in an as yet undetermined manner. This pre-parkinsonism rat model could prove highly useful to investigate the effects of environmental agents on the onset of Parkinsonism.

400 THE NEUROBIOLOGICAL EFFECTS OF MANGANESE EXPOSURE IN A RODENT MODEL OF PRE-PARKINSONISM.

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Recent evidence has suggested that elevated exposures to manganese (Mn) and other metals is associated with the increased incidence of adult onset parkinsonism. Chronic elevated Mn exposure is known to cause neurotoxicity to the basal ganglia of mammals, resulting in neuromotor symptoms similar to those observed in parkinsonism. Here we utilized a rodent model of pre-parkinsonism (see Gwiazda et al., this volume) to investigate the effects of Mn exposure on neurotoxicity and the exacerbation of parkinsonism. A pre-parkinsonism state was induced using unilateral intra-striatal injection of 5.5µg 6-hydroxydopamine (6-OHDA). Mn exposure (4.8 mg $MnCl_2$/kg, i.p. 3 injections/week) was started four weeks after surgery and lasted five weeks. Female Sprague-Dawley rats (n=44) were divided among the following treatments (n=11/treatment): (A) Control, saline/vehicle; (B) Mn only; (C) 6-OHDA only; and (D) 6-OHDA + Mn. Neurobehavioral function was assessed following Mn exposure using a functional observational battery (FOB). Striatal dopamine concentrations and depletion (lesioned right vs. non-lesioned left striatum) were measured at sacrifice. Results indicated that the 6-OHDA lesion produced significant ($p<0.001$, ANOVA) depletion (60 - 70%) in striatal dopamine, but no measurable alteration of neurobehavioral function, thereby substantiating this pre-parkinsonism (i.e., sub-threshold) model. However, chronic Mn exposure resulted in significant ($p<0.05$) impairment of neurobehavioral function for eight of the ten FOB tests. No effects of Mn exposure on striatal dopamine depletion were detected. Notably, Mn exposure in the presence of a pre-parkinsonism state significantly exacerbated neurobehavioral impairment in the *reactivity to removal* and *hopping-left rear limb* (contralateral to lesion) FOB tests. These latter results suggest that chronic elevated Mn exposure may increase the risk of neurologic impairment in sub-populations that are in a pre-parkinsonism state.

401 INFLUENCE OF SUBACUTE MANGANESE SULFATE ON THE CHOICE ESCAPE LEARNING TASK IN MALE RATS.

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The choice escape learning task was used in rats to measure the effects of manganese sulfate on performance and on the acquisition of new information. Male Sprague-Dawley rats were given two weeks of daily gavage with suspensions containing 1.0, 10, 30, or 100, mg/kg manganese or vehicle each day. The vehicle contained 40% cornstarch, 10% sucrose, and 12% dextrinized cornstarch. After two weeks of daily gavage, each rat was given 100 Y-maze trials. The latency of escape (seconds) and errors (number of incorrect entries before the entry to correct arm) were analyzed. Rats gavaged with 1.0 or 100 mg/kg manganese, or the vehicle learned the correct choice for escape at a faster rate than rats given 30 mg manganese/kg ($p<.05$). Rats gavaged with 1.0 mg manganese/kg had a shorter latency of escape relative to rats given the vehicle or 30 mg manganese/kg ($p<.05$). In summary, the ability of manganese at 30 mg to impair the acquisition of new information, but not decrease the speed of responding, indicates that the trace metal at 30 mg/kg can decrease the learning processes of rats on the choice escape learning task. (Support by ATSDR grant U50/ATU398948.)

402 ASSESSMENT OF BIOACCUMULATION, HISTOPATHOLOGY AND NEURO-BEHAVIORAL DAMAGE IN PORTACAVAL ANASTOMOSIS RATS EXPOSED TO MANGANESE PHOSPHATE DUST: A PILOT STUDY.

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The addition of methylcyclopentadienyl manganese tricarbonyl (MMT) in unleaded gasoline, has resulted in increased attention of the effects of manganese (Mn). Hypothetically, people with chronic liver disease may be more sensitive to the adverse neurotoxic effects of Mn. Bioaccumulation of Mn in different organs/tissues, as well as histopathology and the neurobehavioral damage in end-to-side portacaval anastomosis (PCA) rats exposed to Mn phosphate via inhalation were investigated. The week before the PCA operation and four weeks after the PCA operation, the rats were investigated for a neurological evaluation (day-night activities). This neurological test was performed using a computerized autotrack system. Then a group of 8 PCA rats (E+) was exposed to 3000 mg m-3 (Mn phosphate) for 8 hr/day, 5 days/week for 13 consecutive weeks and compared to a control group (E-, n=8) of PCA rats exposed to the background level concentration of 0.03 mg m-3. At the end of the exposure period to Mn, another neurological evaluation was carried out. The rats were then sacrificed and Mn content in tissues and organs were determined by neutron activation analysis. The neuronal cell loss was assessed by neuronal cell counts, and the degree of Alzheimer type II astrocytosis was scored using a semiquantitative immunohistochemical procedure. Results show significant difference between E+ and E- rats. The results contribute to our understanding of the potential adverse effects of Mn in sensitive populations.

403 BIOACCUMULATION AND NEUROBEHAVIORAL EFFECTS OF INHALED MANGANESE DUST IN RATS.

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The concern over environmental manganese (Mn) contamination has increased since the introduction of methylcyclopentadienyl manganese tricarbonyl (MMT) in unleaded gasoline. Many neurodegenerative disorders similar to Parkinson's disease have been related to chronic Mn exposure. The primary goal of this study was to determine the effects of Mn exposure via inhalation. More precisely, the bioaccumulation of Mn in different organs and tissues and the locomotor activity were assessed. A total of 26 rats (E+) were

exposed to 3709 mg/m3 Mn dust for 6 hr/day, 5 days/week for 13 consecutive weeks and compared to a control group of 12 rats (E-). After exposure, a neurological evaluation was carried out for 36 hours (a night-day-night cycle) using a computerised autotrack system. Rats were then sacrificed by exsanguination and Mn content in organs and tissues were determined by neutron activation analysis. Mn concentrations in lung, kidney and cerebellum were significantly higher in E+ than in E- (0.30 vs. 0.18, 1.15 vs. 0.96, 0.73 vs. 0.54 ppm; $p<0.01$) as well as for frontal and for the globus pallidus (1.69 vs. 0.86, 1.9 vs. 0.81 ppm; $p<0.05$). Locomotor activity indicate higher distance covered in the first 12 hour period for E+ ($p<0.05$) and lower resting time in the last 12 hour period for E+ ($p<0.05$). The results of this study contribute to an improved understanding of the health concerns associated with inhalation exposure to Mn.

404 UPTAKE OF SOLUBLE MANGANESE CHLORIDE VIA NOSE-ONLY INHALATION THROUGH THE OLFACTORY BULB.

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Proton Induced X-ray Emission (PIXE) has been used to study the uptake of soluble manganese chloride via nose-only inhalation through the olfactory bulb. Six Fisher 344 rats were exposed to manganese chloride, (0.53±.1 mg/m^3 of Mn, mass median aerodynamic diameter was 2.1±0.5 mm). The OSHA ceiling Threshold Limit Value for Mn is 5mg/mm^3. A vehicle control group of six rats was concurrently exposed to filtered air. Exposures were for 8 hours/day for 14 days. The brain and the olfactory bulbs were removed from euthanized rats, frozen in isopentane and cryosectioned into 10 micron thick sagittal sections. To date we have completed PIXE analysis of anterior portions of freeze dried sections (two per animal) obtained from three Mn dosed and two control animals. Two Mn dosed animals show uptake of Mn in both tissue sections while the third Mn dosed animal shows no evidence of Mn uptake from the exposure in either section and measured Mn contents are similar to levels measured in controls. As with uptake of soluble and insoluble forms of aluminum; nickel-63; and americium-241, the uptake of Mn occurs in only a subset of animals. In such animals Mn uptake occurs in the first mm of the olfactory bulb to levels of approximately 30mg/kg and subsequent deposition appears to occur at about 4mm. Pathology of nasal tissue and comparison of serial stained sections has not been completed and so we have not yet identified in what cell layer or nucleus the increase in Mn at 4mm occurs. However, the data indicates that Mn, once entering the bulb is moving transsynaptically beyond the olfactory glomeruli.

405 PROCONVULSANT EFFECT OF CHRONIC ARSENIC IN MICE.

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Arsenic (As) is a common pollutant of food and water. Human exposure to this element causes toxic effects in several organs. In humans (CNS) it produces convulsions, mental confusion, encephalopathy and coma. Studies in animals have shown that sodium arsenite ($NaAsO_2$) increases brain lipid peroxidation and alterates neurotransmitter levels. The present study was undertaken to investigate if arsenic has a proconvulsivant effect in mice chronically exposed to $NaAsO_2$ and to determine the brain lipid peroxidation in the same animals. Male BALB/c mice, weighing 25-30 gr were randomly allocated to each of groups (n = 6 - 10). Two independent groups of animals were intragastrically administered with either 2.5, 5.0 or 10 mg/kg $NaAsO_2$, or deionized water (Controls) daily during 30 days. At the end of treatments, all animals were injected with 3-mercaptopropionic acid (3-MPO) i.p., a known inhibitor of GAD (glutamate decarboxylase) in order to study the proconvulsant effect; another group of animals were used to quantify the lipid peroxidation in tissue homogenates from frontal cortex, corpus striatum and hippocampus. All the animals intoxicated with $NaAsO_2$ showed myoclonus and tonic-clonic convulsions generalized 2 minutes after injection of 3-MPO; whereas the animals of control group, presented myoclonus and tonic-clonic convulsions 4 minutes after administration of 3-MPO. Lipid peroxidation levels were higher ($p < 0.05$) in the frontal cortex (at doses 2.5 y 5.0 mg/kg) and corpus estriatum (at dose 2.5 mg/kg) in groups exposed to $NaAsO_2$. Sodium arsenite induced proconvulsivant effect in the animals exposed and it also increased the lipid peroxidation levels. Further studies are needed to elucidate the mechanisms for this As proconvulsivant effect in the brain.

406 MORPHOLOGICAL ALTERATIONS AND ENHANCED LIPID PEROXIDATION IN BRAIN REGIONS OF RAT AFTER PERINATAL COMBINED EXPOSURE TO CADMIUM AND DEXAMETHASONE.

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Industrial and environmental exposure to cadmium (Cd) is well known to produce multiorgan toxicity in humans. Cd has been reported damage endothelial cells in several organs both adult or developing brain. On the other hand, metallothionein (MT) is an intracellular ligand for Cd. MT has been shown to protect against Cd-induced toxicity in many organs. We studied microscopical and lipid peroxidation changes in parietal cortex, striatum, hippocampus and cerebellum of rats following perinatal exposure to both Cd and dexamethasone (Dx), a drug known to induced MT synthesis in brain. Wistar rats of 13 days of age were treated for 5 days as follows: 1) saline solution, 2) $CdCl_2$ 1mg/kg/day, 3) Dx 2mg/kg/day 4)$CdCl_2$+Dx2mg/kg/day. Rats were killed on either 18 or 28 days of age. Their brains were included in paraffin and stained by Hematoxilin-Eosine and silver techniques. No lesions were observed in the brain of control rats. Rats treated with Dx at 28 days of age showed interstitial oedema in the four regions. Cd-treated rats at 28 days of age showed pyknotic cells, interstitial oedema in parietal cortex. Striatum shown initial congestion and oedema between nervous fibers. Hippocampus shown few pyknotic cells. Purkinje cells showed pyknosis and nuclear damage.The molecular and granular layers showed interstitial oedema. Lipid peroxidation increased in striatum of rats treated with Cd at 18 days of age. Rats treated with Cd+Dx showed decreased levels of lipid peroxidation in four regions at 18 days of age with respects to cd-treated rats. In general data suggest that Dx treatment attenuated cadmium-induced lesions.

407 THE EFFECTS OF ADRENALECTOMY ON TRIMETHYLTIN-INDUCED HIPPOCAMPAL INJURY IN RATS.

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Sponsor: Y. Aoki.

Trimethyltin (TMT), an organic compound, is known to cause the hippocampal damage and also to show an transient increase of plasma corticosterone (CORT) level on day 3 and 4 after treatment, raising a question regarding the possible effects of CORT on the hippocampal damage. In our recent study, TMT was shown also to induce apoptotic cell death in hippocampal neurons. In the present study, therefore, the effects of adrenalectomy (ADX) on the TMT-induced hippocampal apoptotic cell death were investigated. Male adult Sprague-Dawley rats (aged 6 weeks) were assigned to the following treatment groups: sham+water (control), sham+TMT (9mg/kg, p.o.), ADX+water, and ADX+TMT. TMT or water vehicle were administered 7 days after ADX or sham operation. On 7 days after TMT (or water vehicle) treatment, rats were sacrificed and brain samples were collected. Brain samples were processed by TUNEL for detection and counting apoptotic cells. A higher number of apoptotic cell were found in the infrapyramidal blade, as compared with the suprapyramidal blade of the dentate gyrus in sham+TMT rats. In ADX+water rats, on the contrary, suprapyramidal blade exhibited a greater number of apoptotic cells then the infrapyramidal blade. TMT treatment to ADX rats increased apoptotic cells both in suprapyramidal blade and infrapyramidal blade. The present results may indicate that the topographical pattern of the granule cell degeneration induced by TMT treatment is different from that induced by ADX, and that the interaction of TMT treatment and ADX is greater than the sum of the individual effects.

408 TRIMETHYLTIN AND LPS INDUCE DISTINCT PATTERNS OF CYTOKINE AND CHEMOKINE EXPRESSION IN RAT HIPPOCAMPUS: LACK OF AN ASSOCIATION WITH INJURY-INDUCED GLIOSIS.

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Cytokines are proposed to play a role in the central nervous system (CNS) responses to injury and disease. One such response is neuronal damage-induced glial activation (gliosis). Here we examined the *in vivo* expression of cytokines and chemokines linked to CNS damage and gliosis. We used the

trimethyltin (TMT) model of neuronal injury (8mg/kg, i.p.) because the time-course of neuronal loss and glial activation have been characterized. Peripheral administration of lipopolysaccharide (LPS) (0.5 mg/kg), i.p.) was used as a positive control because it is known to elevate cytokines in the periphery and the CNS but does not itself induce neural damage. Using semi-quantitative RT-PCR, we found that cytokines (TNF-alpha, IL-1, TGF-beta) and chemokines (MIP-1, RANTES, and IFN-gamma) were elevated in liver, blood, and hippocampus following peripheral LPS at 2, 5, and/or 8 hours after administration, however, glial fibrillary acidic protein (GFAP) (a marker of gliosis) was not altered at any time point. In contrast, TMT-treated rats showed GFAP and GFAP mRNA increases in hippocampus by day 5 (300%). Analysis by Atlas Array of the most affected time point (day 5) corroborated RT-PCR results. This glial response was not accompanied by a significant elevation in TNF-alpha or IL-1alpha or beta. These results suggest that 1) pro-inflammatory mediators may be elevated within the CNS in the absence of neuronal damage and glial activation and 2) toxicant-induced neuronal damage and glial activation do not require upregulation of proinflammatory cytokines.

409 ORAL TREATMENT OF MICE WITH SODIUM SELENITE BUT NOT SELENOMETHIONINE INCREASES DOPAMINE METABOLITES IN THE STRIATUM.

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Selenium (Se) is an essential as well as a toxic trace element. The exposure to Se causes blind staggers in cattle, poliomyelomalacia in pigs, and nervous system disorders in people. In this study, groups of 5 male BALB/c mice each were administered sodium selenite or selenomethionine in drinking water *ad libitum* at 0, 1, 3, and 9 ppm as Se for 14 days. At the end of treatment, their brains were removed, dissected, and concentrations of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were determined in different brain regions. Food and water intake, and body weight gain were decreased in the group treated with the highest concentration of sodium selenite. In mice administered sodium selenite at 3 and 9 ppm, DOPAC was significantly higher in the striatum than in the control group. The striatal HVA was also increased in the group treated with 3 ppm Se; the DA showed a similar pattern but the increase was not statistically significant. No alterations of NE, 5-HT, and 5-HIAA levels were detected in any brain region of mice treated with sodium selenite. No significant differences in any parameter among the groups treated with selenomethionine were observed. The alterations of DA metabolites by inorganic Se in DA-rich striatum suggested a Se-specific increased neural activity of dopaminergic pathways. Results may be useful in elucidation of neurotoxicity of Se and in establishing a safe level of Se intake.

410 A *PRIORI* PREDICTION OF TISSUE:PLASMA PARTITION COEFFICIENTS OF DRUGS FOR PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS.

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The tissue:plasma ($P_{t:p}$) partition coefficients (PCs) are drug-specific input parameters in PBPK models. Until now the use of PBPK models in early stages of the drug discovery process was not possible, since the estimation of $P_{t:p}$ of new drug candidates by using conventional *in vitro*, *in vivo* and/or quantitative structure-property relationships (QSPR) methods is too time and cost intensive. The objectives of this study was to develop and validate two mechanistic equations for predicting *a priori* the rabbit, rat and mouse $P_{t:p}$ of eight non-fatty and non-excretory tissues for 67 structurally unrelated drugs. The first equation predicts $P_{t:p}$ at steady state, assuming a homogenous distribution and passive diffusion of drugs into tissues, from a ratio of solubility and macromolecular binding between tissues and plasma. The ratio of solubility was estimated from log vegetable oil:water PCs of drugs as well as lipid and water levels in tissues and plasma, whereas the ratio of macromolecular binding for drugs was estimated from tissue interstitial fluid-to-plasma concentration ratios of albumin, globulins and lipoproteins. The second equation predicts $P_{t:p}$ of drugs residing predominantly in the interstitial space of tissues. Therefore, the fractional volume content of interstitial space in each tissue replaced drug solubilities in the first equation. The average ratio of predicted-to-experimental $P_{t:p}$ values was 1.26 (S.D. = 1.40, r = 0.90, n = 269), and 85% of the 269 predicted values were within a factor of three of the corresponding literature values obtained under *in vivo* (most of the cases) and *in vitro* conditions. The major discrepancy observed between the predicted and experimental $P_{t:p}$, is for lung in the case of basic lipophilic drugs. It has been reported in the literature that the partitioning of these drugs into lung is principally governed by an lipophilic-ionic lysosomal trapping, which it has not been taken into account for predicting the $P_{t:p}$ of lung in the present study. The results indicate that our methodology for predicting $P_{t:p}$ is a valuable tool to aid the pharmacokinetic screening with PBPK models in early stages of drug discovery.

411 USING MUSCLE:PLASMA PARTITION COEFFICIENTS (PCS) AS SURROGATES FOR PCS OF OTHER TISSUES.

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The muscle is the most important organ for drug distribution on the basis of body mass (> 50% for mammalian species). Furthermore, for several mammalian species, the muscle contains similar levels of lipids, water and binding macromolecules (albumin, globulins, lipoproteins) as other tissues. Consequently, the muscle can potentially be used as a reference/surrogate organ for predicting the tissue:plasma partition coefficients ($P_{t:p}$) as drug-specific input parameters for physiologically-based pharmacokinetic (PBPK) models. The objective of this present study is to evaluate the adequacy of using $P_{t:p}$ of muscle as a surrogate for $P_{t:p}$ of other tissues for several structurally unrelated drugs. The method consists of performing regression analyses between $P_{t:p}$ of muscle and those of other non-fatty and non-excretory tissues (bone, brain, heart, intestine, lung, muscle, skin, spleen) to verify whether linear relationships can be obtained. Regression analyses were performed for experimental as well as predicted rabbit and rat $P_{t:p}$ of these above tissues. The experimental $P_{t:p}$ correspond to literature values determined under *in vivo* conditions, whereas those predicted correspond to optimized values obtained from PBPK modelling exercises, and calculated values presented in a companion poster. For predicted and experimental $P_{t:p}$, linear relationships ($r > 0.9$) were observed between muscle and other tissues, suggesting that $P_{t:p}$ of muscle is a good predictor for the $P_{t:p}$ of other tissues. This can be rationalized by the fact that the lipid, water and major binding macromolecule levels are not so different between these tissues. However, the correlation between $P_{t:p}$ of muscle and $P_{t:p}$ of brain, is less evident as for the other tissues. Therefore, it might possible that a permeability-limited process is present in brain under *in vivo* conditions in the case of some drugs, by contrast to the other tissues. The present study suggests that $P_{t:p}$ of muscle can be a good predictor for $P_{t:p}$ of non-fatty and non-excretory tissues for drugs distributing homogeneously or non-homogeneously into tissues predominantly by a passive process.

412 PREDICTION OF ADIPOSE TISSUE: PLASMA PARTITION COEFFICIENTS OF LIPOPHILIC IONIZED DRUGS.

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Adipose tissue is an important organ into which the lipophilic drugs can be distributed. The adipose tissue:plasma ($P_{at:p}$) partition coefficients (PCs) may be over-predicted by a factor more than three from *n*-octanol:water PCs ($P_{o:w}$) for lipophilic ionized drugs (LIDs). During the determination of $P_{o:w}$ with the conventional shake-vial method, the water phase may be partly solubilized in the *n*-octanol phase, which is also polar because of the presence of an hydroxyl group in molecules of *n*-octanol. Consequently, $P_{o:w}$ would increase if the drugs are soluble or substantially ionized in the water phase (which is the case for LIDs). The fact that $P_{o:w}$ are probably over-estimated in the experimental studies may explain why $P_{at:p}$ are over-predicted from $P_{o:w}$ for LIDs. Alternatively, vegetable oil (constituted of neutral lipids; triglycerides) has been recently suggested as a better surrogate than *n*-octanol for biotic neutral lipids in adipose tissue (constituted of 90% triglycerides and free fatty acids). The water phase is insoluble in the oil phase. Therefore, experimental vegetable oil:water PCs ($K_{vo:w}$) are observed to be lower than $P_{o:w}$ for LIDs. Hypothetically, $K_{vo:w}$ would better predict $P_{at:p}$ than $P_{o:w}$ do. The objective of the present study was to compare the predictability of rat and rabbit $P_{at:p}$ from $K_{vo:w}$ and $P_{o:w}$ for eighteen structurally unrelated LIDs. The $P_{at:p}$ were predicted using experimental $K_{vo:w}$ or $P_{o:w}$, and the unbound fraction in plasma as the only drug-specific input parameters in a tissue composition-based equation. The values of $P_{at:p}$ predicted with $K_{vo:w}$ are within a factor of two of their corresponding experimental $P_{at:p}$ values, whereas the values of $P_{at:p}$ predicted with $P_{o:w}$ are in most cases over a factor of three of their corresponding experimental $P_{at:p}$ values. The present study demonstrates that $K_{vo:w}$ is a better predictor of $P_{at:p}$ than $P_{o:w}$ for LIDs.

413 *IN VITRO-IN VIVO* EXTRAPOLATION OF TISSUE: PLASMA PARTITION COEFFICIENTS OF DRUGS.

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The *in vitro* and *in vivo* tissue:plasma ($P_{t:p}$) partition coefficients (PCs) are used in physiologically-based pharmacokinetic (PBPK) models to estimate tissue distribution of drugs. Ideally, a value close to one is expected for the ratio of *in vitro* to *in vivo* $P_{t:p}$ ($P_{t:p\ in\ vitro/in\ vivo}$). However, this is not the case for several lipophilic ionized bases. The *in vitro-in vivo* extrapolation of $P_{t:p}$ is not well understood at the present time. *In vivo*, it is assumed that only the non-ionized form can cross the plasmatic membrane to be distributed into tissues. However, *in vitro* it is considered that the non-ionized and ionized forms can cross the artificial membrane used in the dialysis cells to separate the plasma and tissue homogenates. Consequently, the lipid:water PC in tissues, at steady state, would be different *in vivo* and *in vitro* especially for ionized drugs. This may principally explain the quantitative differences between the *in vitro* and *in vivo* $P_{t:p}$. The objective of this present study was to develop and validate a mechanistic method for predicting $P_{t:p\ in\ vitro/in\ vivo}$ for six non-fatty tissues and adipose tissue in rats. First, this is done for eleven drugs for which the distribution in a tissue is assumed to be homogeneous. Their $P_{t:p\ in\ vitro/in\ vivo}$ were predicted with a tissue composition-based equation as equal to the ratio between the *in vitro* and *in vivo* lipid:water PCs estimated for tissues. The predicted values of $P_{t:p\ in\ vitro/in\ vivo}$ differed to the experimental values obtained form the literature by an average factor of 1.07 (s.d. = 0.44, r = 0.90, n = 28). Specifically, for lipophilic ionized bases with a $pK_a > 8.1$, it is clearly demonstrated that values of $P_{t:p\ in\ vitro/in\ vivo}$ are lower than 0.5, whereas those for hydrophilic ionized bases with a $pK_a < 8.1$, acids with a pK_a between 7 and 8 as well as neutral compounds are close to one. For two drugs residing in interstitial space of tissues, their experimental $P_{t:p\ in\ vitro/in\ vivo}$ over than two were assumed to be principally governed by a difference in the tissue distribution between *in vitro* (homogenates) and *in vivo* (intact tissues). For these drugs, the predicted values of $P_{t:p\ in\ vitro/in\ vivo}$ differed to the experimental values by an average factor of 1.62 (s.d. = 0.85, r = 0.41, n = 9). The practical aim of this study is the *in vitro-in vivo* $P_{t:p}$ extrapolation.

414 ESTIMATING RAT BLOOD:AIR PARTITION COEFFICIENTS (PCs) OF VOLATILE ORGANIC CHEMICALS (VOCs) USING RECONSTITUTED MIXTURES OF *n*-OCTANOL, WATER, AND HEMOGLOBIN.

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The rat blood:air PCs ($P_{b:a}$) of lipophilic VOCs cannot be predicted only with the consideration of their solubility in blood water and lipids, suggesting an important role of protein binding. Experimental evaluations of the contribution of hemoglobin (Hb) binding to the $P_{b:a}$ of VOCs, its possible dose-dependency and the quantitative nature of VOC-binding to hemoglobin [i.e., association constant (K_a), number of binding sites (n)] have not been performed. The objectives of this study were to: i) assess the contribution of hemoglobin to the $P_{b:a}$ of VOCs, and ii) estimate K_a and n for VOCs. *In vitro* vial equilibration studies were conducted using whole blood, or an equivalent mixture of water and *n*-octanol (with or without Hb) that were exposed to single (300 ppm) or varying concentrations of VOCs [bromoform (BF, 120-12725 ppm), chlorobenzene (CB, 125-27325 ppm), chloroform (CF, 125-207211 ppm), and ethylbenzene (EB, 125-22610 ppm)]. The PCs of VOCs determined using whole blood were comparable to those obtained using a reconstituted mixture of *n*-octanol, water and Hb (mean±SE, n=3-4) for BF (154±1.5), CB (55±6), CF (15±0.87), and EB (30±1.5). Also, the $P_{b:a}$ of all four VOCs decreased significantly at higher exposure concentrations, with no significant change in their octanol/water mixture:air PC. For each exposure concentration, the concentration of free chemical (C_{free}) in rat blood was calculated with the PC for the *n*-octanol/water mixture, whereas the concentration of bound plus free chemical (C_{tot}) was calculated from knowledge of the PC determined experimentally with whole blood. The values of K_a and n estimated by linear regression of a plot of C_{tot}/C_{free} vs $1/C_{free}$ were: BF (0.8, 4), CB (2.8, 1.4), CF (1.8, 1.2), and EB (2, 1.4). The results of this study confirm that a mixture of *n*-octanol, water and hemoglobin can adequatly predict the $P_{b:a}$, and that the dose-dependent nature of $P_{b:a}$ need not be considered for modeling inhalation exposures to VOCs for up to 2000-5000 ppm.

415 A QSAR-TYPE PBPK MODEL FOR INHALED CHLOROETHANES.

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The chemical-specific parameters required for PBPK models are either determined experimentally or predicted from the use of theoretical algorithms. The parameter values of related compounds may be useful for developing quantitative relationships that consistently describe the change in parameter values as a function of molecular structure. Such quantitative relationships between molecular structure information and V_{max}, K_m or partition coefficients (PCs) have not yet been developed. The purpose of this study was (i) to develop quantitative structure-activity relationship (QSAR)-type equations for associating the molecular structure of chloroethanes with the numerical values of parameters required for PBPK modeling (i.e., blood:air PC (P_b), tissue:blood PCs (P_t), V_{maxc} and K_m), in rats and humans, and (ii) to incorporate these QSAR-type equations within PBPK models such that molecular structure can be used as the sole input to simulate the pharmacokinetics of various chloroethanes in rats and humans. Experimental values of the parameters for monochloro-, 1,1- and 1,2-dichloro-, 1,1,2-trichloro, 1,1,2,2- and 1,1,1,2-tetrachloro, pentachloro- and hexachloro-ethanes were taken from the literature. Each molecule was described as the composite of a basic structure (C-C) and carbon substituent group (H_3, H_2Cl, HCl_2 or Cl_3). The quantitative relationships between structural features and the values of the parameters (P_i) were described by a Free-Wilson additive model: $P_i=A_i+\Sigma(f_s*C_{si})$ where A_i = contribution of the basic structure to the parameter P_i, f_s = frequency of occurrence of each substituent S and C_{si} = contribution of each S to P_i. The average ±SD (range) ratio of estimated/experimental values were 1.08±0.38 (0.64-1.57), 1.17±0.70 (0.5-4.18), 1.10±0.46 (0.6-2.12), 1.11±0.31 (0.79-1.8) for rat PCs, human PCs, V_{maxc} and K_m, respectively. The ratio of Ca_{ss} simulated using the QSAR-type PBPK models and the conventional PBPK models ranged from 0.78 to 1.21 (1.03 ±0.13) and from 0.76 to 1.55 (1.06±0.26), for rats and humans, respectively. The QSAR-type PBPK model as developed in the present study should be useful for examining the influence of change in number and nature of substituents on the kinetics of related chemicals.

416 PBPK MODELING OF ESTRADIOL DURING ESTROUS CYCLE AND ITS RELATIONSHIP TO UTEROTROPHIC EFFECTS IN RATS.

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A physiologically based pharmacokinetic (PBPK) model for 17β-estradiol (E2) has been developed by Plowchalk (Toxicologist, 42: 1180). This model simulates the kinetics of E2 without considering E2 variations during the normal estrous cycle. E2 concentrations in blood during the estrous cycle varies by about a factor of seven. At the present time, there are no published models that simulate the blood concentration profiles of E2 by accounting for both the endogenous cycle dependent production of E2 and exogenous E2. Further, only limited efforts have been made to evaluate linkage between tissue doses of E2 and health effects. The objectives of the present work were 1) to extend the Plowchalk PBPK model for E2 to include the cyclical hormone levels in the rat, and 2) to use this PBPK model to study the relationship between exogenous E2 exposure and uterotrophic effects. The PBPK model for estradiol contained seven compartments (blood, richly perfused tissues, slowly perfused tissues, pituitary, uterus, ovaries and liver). Data on blood E2 during estrous cycle, obtained from the literature, were used to develop a table function to incorporate into the rat E2 model written in ACSL®. Subsequently, the model simulated the blood concentration profiles of E2 following oral dosing, including the time-dependent changes in endogenous E2 levels. Dose-response data for E2-induced uterotrophic effects were obtained from the literature and their relationship to internal dose surrogates of E_2 examined. A non-linear relationship was observed between AUC for E2 concentration in uterus and uterus weight. The internal E2 dose response relationship established in rat may be useful as a basis for predicting the consequences of exposure to xenoestrogens that would cause specific changes in the circulating E2 levels.

417 PHYSIOLOGICAL MODELING OF HUMAN EXPOSURE TO CONTAMINANTS IN AMBIENT ENVIRONMENT AND FOOD CHAIN.

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Human exposure to environmental pollutants is evaluated with the knowledge of their concentration in the exposure media, consumption rate, and body weight. The concentration in food materials is sometimes considered equal to the average concentration and frequently calculated using a bioconcentration or bioaccumulation factor. The conventional approach also does not always account for the bioavailability and the kinetics of absorption of the chemicals in food materials. The objective of this study was to develop a physiologically-based modeling framework for evaluating human exposure to contaminants in the ambient environment and the food chain. The approach consisted of developing a set of physiologically-based pharmacokinetic (PBPK) models for humans and key animal species representative of the food chain such as fish, chicken and cow, and interconnecting them. All species are exposed to the same ambient environment or different environments. The output of the three animal models (such as tissue concentrations), along with the consumption patterns, served as input (via the gastro-intestinal tract) to the human PBPK model, which then simulated the internal dose of the specific chemicals modeled. The individual components of the PBPK model framework facilitate the simulation of the kinetics of chemical concentrations in tissues or other relevant compartments (e.g., egg, milk, muscle) in each of the species in the food chain. The three animal PBPK models have been validated by comparing the predictions with previously published experimental data on the concentrations of several PCBs following oral administration. The modeling framework developed in this study represents a biologically-based tool useful for improving our ability to assess accurately human exposure to contaminants present in the ambient environment and food chain.

418 PBPK MODELING AND EXTRAPOLATION OF PHARMACOKINETIC INTERACTIONS FROM SIMPLE TO COMPLEX CHEMICAL MIXTURES.

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Methodologies for the health risk assessment of single chemicals are well developed but it is not the case with chemical mixtures. This situation is a result of our inability to predict the consequences of pharmacokinetic and pharmacodynamic interactions potentially occurring between mixture components. PBPK modeling provides a unique framework that can account for the higher order pharmacokinetic interactions in mixtures solely based on information obtained with binary mixtures. The ability to extrapolate pharmacokinetic interactions from simple to complex mixtures using PBPK models needs to be proven with increasingly complex chemical mixtures. The objective of the present study was to validate the PBPK modeling approach for extrapolation of pharmacokinetic interactions from binary to more complex mixtures of benzene (B), toluene (T), ethylbenzene (E) and *m*-xylene (X) and dichloromethane (D). The methodology consisted of initially obtaining and linking the single chemical PBPK model of each of these components at the level of the liver compartment on the basis of interaction mechanisms. Analysis of blood kinetic data from exposure to all binary combinations suggested that competitive metabolic inhibition was the most plausible interaction mechanism amongst all combinations. The metabolic inhibition constant (K_i) for each of these binary combinations was quantified and incorporated within the complex mixture PBPK model. The binary interaction-based mixture model was then used to predict the consequences of interactions occurring in more complex mixtures (4 or 5 components). The mixture PBPK model predicted adequately the blood kinetics of T, E, X, B and/or D present in mixtures of BTEX, DTEX and DBTEX. The results of the present study demonstrate that the pharmacokinetics of the components of complex mixtures can be described/predicted solely from mechanistic information on binary level interactions.

419 DERIVATION OF CHEMICAL-SPECIFIC ADULT-CHILDREN SAFETY FACTORS USING A PHYSIOLOGICAL MODELING APPROACH.

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The adult-children differences in physiological and biochemical factors are thought to be responsible, in part, for the potential differences in pharmacokinetics and tissue dose of chemicals. The objective of this study was to derive chemical-specific safety factors for conducting adult-to- children extrapolation of safe exposure concentrations. Using a physiologically-based algebraic expression, the ratios of steady-state arterial blood concentrations (Cass) for several volatile organic chemicals (VOCs) (dichloromethane, toluene, trichloroethylene, chloroform, carbon tetrachloride, styrene, and m-xylene) in adults and 4-yr old children were obtained. This algebraic expression, derived from PBPK models, was used to calculate Cass as a function of inhaled concentration, alveolar ventilation rate (Qp), cardiac output (Qc), blood:air partition coefficient (Pb), fraction of cardiac blood flow to liver (Qlc) and hepatic extraction ratio (E). Based on literature data on adult-children differences in physiological parameters, it was found that the Cass slightly varied between adults and children of various age groups. The results indicate that the adult exposure concentration of VOCs should be divided by a factor ranging from 1.02 - 1.84 to obtain an equivalent Cass in 4-yr old children. For children of other age groups (5 - 17 years), the factor is even lower and closer to 1. The magnitude of difference in Cass between children and adults is a result of differences in uptake and metabolic clearance. The difference in uptake and the dose absorbed when adults and children are exposed to the same external concentration results from differences in Qp, Qc, Qlc, Pb and E. The factor accounting for differences in uptake between 4-yr old children and adults for dichloromethane, for example, is 1.2. The magnitude of difference in metabolic clearance, as determined by differences in body weight and liver blood flow rate, between 4 year old children and adults is in the order of 1.4. For older age groups (5-17 years), this factor is lower and closer to 1. This safety factor represents the default, which can be modified by the ratio of E in children and adults. The present study represents the first attempt of deriving a safety factor based on the differences in mechanistic determinants of pharmacokinetics between adults and children.

420 A SIMPLE METHOD FOR UNCERTAINTY ANALYSIS IN PBPK MODELS.

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Methodologies for conducting uncertainty analysis in PBPK models have been developed by several authors. These methodologies in general use Monte Carlo method for sampling input parameters used in PBPK models written in programming or simulation languages. It is possible to solve differential equations and conduct PBPK simulations using spreadsheet programs such as Microsoft EXCEL®. EXCEL® also permits the use of add-in program such as Crystal Ball which facilitates Monte Carlo simulation. The objective of this study was to develop a simple method for conducting uncertainty analysis in PBPK models. The approach consisted of writing PBPK model equations in EXCEL® spreadsheets, and describing certain input parameters (physiological, biochemical) with a distribution function (normal, lognormal, uniform). Whereas the model equations were written and solved in EXCEL® spreadsheets, the input parameters were provided by Crystal Ball® based on the distribution and available parameter statistics. During the conduct of simulations, the investigator should specify the cell corresponding to the output for which the distribution should be graphically displayed. The distribution of the output is the result of the use of Monte Carlo method by Crystal Ball® to randomly generate parameter values. The PBPK model framework developed in Excel®/Crystall Ball® was used to conduct uncertainty analysis in simulations of pharmacokinetics of chloroform, methyl chloroform, trichloroethylene, benzene, carbon tetrachloride and methylene chloride in workers exposed for 8-hr to threshold limit values (TLVs) of these substances. Probability distributions of blood and tissue concentrations of these chemicals were generated for each sampling time. The results of the uncertainty analysis obtained using the simple method developed in the present study compared well with those obtained previously using simulation softwares such as Simusolv®.

421 AN ALGEBRAIC APPROACH FOR CONDUCTING SENSITIVITY AND VARIABILITY ANALYSES IN HUMAN PBPK MODELS.

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Sensitivity analysis in PBPK models facilitates the evaluation of the relative sensitivity of pharmacokinetic (PK) endpoints to changes in input parameters. Analysis of the impact of the variability of input parameters facilitates an evaluation of the possible magnitude of interindividual differences in pharmacokinetics. Health risk assessment approaches typically consider chronic exposure to contaminants, during which steady-state is attained. The steady-

state concentrations of chemicals in blood and tissues can be adequately determined using algebraic equations that use fewer parameters than full-blown PBPK models. The purpose of this study was to develop a simple algebraic approach for conducting sensitivity and variability analyses in human PBPK models of volatile organic chemicals (VOCs). Since the human models are used to simulate lifetime exposures, algebraic equations instead of full-blown PBPK models were used to obtain essentially the same results. Using the algebraic equation, the sensitivity of steady-state arterial blood concentration [$Ca_{ss} = 1/(1/P_b+Q_{lc}*E)$ where P_b = blood:air partition coefficient, Q_{lc} = fraction of blood flow to the liver and E = hepatic extraction ratio] of 17 volatile organic chemicals (VOCs) was determined for unit change in the numerical values of E, P_b and Q_{lc}. The percent change in Ca_{ss} for 1% change in P_b, E or Q_{lc} ranged from 0.04 to 0.99, 0.01 to 0.97, and 0.01 to 0.97, respectively. Given a 15 % and 22 % variability of P_b and Q_{lc} in a population, Ca_{ss} would vary by ±0.5-14.8% and ±0.27-26%, respectively. A 50% increase in E would lead to 0.6-29% decrease of Ca_{ss}, while a 50% decrease in E resulted in 0.6-92% increase in Ca_{ss}. This algebraic approach can provide the extreme values of PK endpoints (e.g., Ca_{ss}) for a given range of variability of input parameters in a population. Distributions of parameter values, if available, can also be used with this algorithm for assessing the probabilistic nature of the outcome.

422 IMPROVING THE RELIABILITY OF PARAMETERS IN PBTK MODELS USING MARKOV CHAIN MONTE CARLO SIMULATIONS.

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The kinetics of volatile substances can be described with the aid of physiologically based toxicokinetic (PBTK) models. However, such modeling is often performed without assessing the reliability of the parameters and their variability in the population. The aim of this study was to address these issues within a population PBTK framework using Markov chain Monte Carlo (MCMC) simulations. The MCMC method creates parameter estimates in the shape of distributions, incorporating existing data on the parameter distributions in the population. In a previous study (Carlsson, Scand J Work Environ Health. 8 (1982), 43-55), 6 male volunteers were exposed to toluene vapor for two hours at 80 ppm during various levels of exercise. Extensive physiological and kinetic data, such as toluene in arterial blood, subcutaneous adipose tissue and end-exhaled air, were collected up to one week postexposure. Thus, the Carlsson data were deemed sutiable for this study. The kinetic data were fit to a published PBTK model for toluene (Pierce et al., Toxicol Appl Pharmacol 139 (1995), 49-61). The results suggest that a separate compartment for perirenal fat should be added to the the model from Pierce et al. in order to describe the concentration-time profile in subcutaneous fat correctly. Furthermore, by using the information supplied by the experimental data it is possible to improve the reliability of the distributions of the fat-related physiological parameters. (The study was financially supported by the Swedish Council for Work Life Research.)

423 PROBABILISTIC DISTRIBUTIONS FOR PBPK MODEL PARAMETERS.

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The most recent attempt by the regulatory agencies to use physiologically-based pharmacokinetic (PBPK) modeling for rulemaking (OSHA, 1997) has involved the use of probabilistic techniques to assess variability in population pharmacokinetics/toxicokinetics and to assess uncertainty in model predictions. Incorporation of probabilistic techniques into PBPK modeling is a significant technical challenge, and several key issues have been identified as a result of these efforts: 1.) How should model parameter distributions be defined? 2.) How should correlations between parameter distributions be defined (e.g., tissue blood flow should be from the upper end of the distribution when tissue volume is from the upper end of its distribution) and which parameters should be correlated? 3.) How should distributions be updated with new data or information? The application of probabilistic methods to PBPK modeling allows a more scientific representation of the uncertainty and variability in the model predictions of internal dose (kinetics) and response (dynamics). Fitting distributions and correlations to model parameters is the process of removing the uncertainty about - and capturing the variability inherent in - these physical systems. This work is the extension to the efforts by Brown et al. (1997) to provide distributions of model parameters. Specifically, distributions for most animal-specific PBPK model parameters are presented. Probabilistic bounds are also incorporated to assure the assumptions of the distribution are consistent with realistic bounds found in the data sets. In the same way, correlations between the parameters were estimated. The effect of these correlation estimates on the model output are compared to bounds on the output derived by alternative methods.

424 AN ADVANCED PBPK MODEL INPUT SYSTEM AND DATA REPOSITORY FOR COMPLEX MODELS WITH EXPORT FOR THE ACSL MODEL ENGINE.

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As Physiologically Based Pharmacokinetic (PBPK) Models grow in complexity preparing inputs becomes more tedious and prone to mistakes. This comes after extensive research and literature searches to accumulate the required physiological inputs. In addition the user must often define very complex exposure scenarios. Often, even the most careful organization of inputs starts to break down under the pressures of preparing large numbers of inputs. An advanced input system has been developed for the Dose Estimating Exposure Model (DEEM) that incorporates a database of user generated data sets which can be exported to the specified model engine (currently ACSLTOX). The input screens are presented to demonstrate the scope of input choices. The user defines a new Model Data Set including units and model version. The subsystems and compartments active for the simulation are chosen. Then the exposure chemicals and their metabolites are specified with the type of metabolism and inhibitor if required. Inputs are then specified for each chemical. The compartments active for a chemical are specified. The binding, elimination and metabolism constants are input. The exposures are defined for one or more chemicals and one or more scenarios for each of eight exposure routes. Exposure time histories can be input for Inhalation, Rate Ingestion, and Skin Surface Water Exposures. NOTICE: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA. Mention of tradenames or commercial products does not constitute endorsement or recommendation for use.

425 APPLICATION AND USE OF DOSE ESTIMATING EXPOSURE MODEL (DEEM) FOR ROUTE TO ROUTE DOSE COMPARISONS AFTER EXPOSURE TO TRICHLOROETHYLENE (TCE).

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Route to route extrapolations are a crucial step in many risk assessments. Often the doses which result in toxicological end-points in one route must be compared with doses resulting from typical environmental exposures by another route. In this case we used EPA's Dose Estimating Exposure Model (DEEM) to examine the route comparisons of different measures of internal dose after exposure to TCE. DEEM is a physiologically based model architecture for estimating internal tissue doses resulting from actual or simulated exposures. Because of different kinetic rates in the body not each possible measure of dose has the same quantitative relationship with exposure. Modeling shows that for different choices of internal dose the "equivalent" exposures are different. For example, we first chose the dose of interest to be the area under the curve (AUC) of the metabolite, trichloroacetic acid (TCA). In this case and with this model set-up an 8-hour 30 ppm exposure via inhalation was equivalent to drinking water intake of 50mg per day. For other measures of dose the point of equivalent exposure is far different. Thus, information about the mode of action and selection of internal dose is crucial before route to route extrapolations can be rationally made. (NOTICE: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA.)

426 APPLICATION AND USE OF DOSE ESTIMATING EXPOSURE MODEL (DEEM) FOR DOSE COMPARISONS AFTER EXPOSURE TO TRICHLOROETHYLENE (TCE).

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DEEM, developed over the last few years, is a model architecture for estimating internal tissue doses resulting from actual or simulated exposures. Exposure profiles are inputted into DEEM through scenario-based modules or through time-histories of exposure concentration. The relationship of exposure to dose is complex and depends on many factors and is different for each choice of dose. In this example DEEM was specifically configured to simulate TCE pharmacokinetics in the human. The model was first tested against data taken from human volunteers (Fisher, et al.) Next we looked at the impact of different exposure regimens on various internal measures of dose for TCE. Our "base" exposure was 30 ppm for eight hours repeated for five days. The results of this exposure were compared to several profiles whose time-weighted average was 30 ppm over the 8-hour period. The profile concentrations ranged anywhere from 0 to 131 ppm to give the time-weighted 30 ppm average. The peak height of TCE concentration in the blood varied considerably upon exposure concentration, duration, and frequency as well as physiologic factors. In contrast, concentrations of the metabolites of TCE, trichloroacetic acid (TCA) and trichloroethanol (TCOH), were virtually the same regardless of which exposure profile was simulated. In some circumstances, for these measures of internal doses, an 8-hour average exposure suffices for their estimation. We present several different simulations to examine the impact of exposure on internal dose. Findings such as these could have impact on future exposure study designs. (NOTICE: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA.)

427 UNCERTAINTY ANALYSIS OF TCE USING THE DOSE ESTIMATING EXPOSURE MODEL (DEEM) IN ACSL.

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The ACSL based Dose Exposure Estimating Model (DEEM) under development by EPA is used to perform an uncertainty analysis of a physiologically based pharmacokinetic (PBPK) model of trichloroethylene (TCE). This model involves several circulating metabolites such as trichloroacetic acid (TCA) and trichloroethanol (TCOH) whose exact clearance parameter values are not always well known. A combined sensitivity and Monte Carlo analysis is presented. Some base model parameter values and associated experimental measurements were supplied by Fisher et. al. For imprecisely known parameters, sensitivity analyses use reasonable assumptions about parameter central tendency and natural variation. The natural variation is used in model runs to identify the most sensitive model parameters under various linear and nonlinear model concentration conditions. Using various sets of sensitive parameters, mixed normal and non-normal statistical distributional assumptions are used in Monte Carlo analyses under the assumption of independently varying parameters. Adjustments are made for correlated parameters. Central tendency and upper and lower percentile bounds are provided for time-dependent concentration curves of TCE, TCA, and TCOH, along with similar results for summary dose measures such as steady-state concentrations or area under the concentration curves. Experimental concentration measurements are plotted against predicted model variability. (NOTICE: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.)

428 DOES CHLOROFORM CONFORM TO HABER'S INHALATION RULE FOR DIFFERENT RAT AGES? A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBNK) MODELING APPROACH.

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Chloroform ($CHCl_3$) is a disinfection by-product of water chlorination, for which there is a well established PBPK model. The objective of this study was to use this PBPK model for $CHCl_3$ to test Haber's rule, and evaluate how the predictions compared for young (2 month) and old (2 years) versus adult (3 month) rats. The default method for estimating inhalation toxicity makes use of Haber's rule, an empirical law that states that the inhalation concentration (C) times the exposure length (T) is a constant toxicological effect (k). For example, a 200 ppm, 2 hr length exposure would be theoretically toxicologically equivalent to a 400 ppm, 1 hr exposure, since their product is the same.Two sets of CxT scenarios were chosen to study higher concentrations with short durations and lower concentrations with longer durations. In the first scenario, C varied from 0.1 to 1600 ppm and length varied from 1600 to 0.1 hours. In the second scenario, C ranged from 0.1 to 48 ppm with exposures durating from 48 to 0.1 hours. The maximum rate of metabolism (rAM) in the liver and kidney were assumed to be directly related to toxicity for all simulations. When rAM for each organ was plotted versus concentration, these preliminary results were not a constant line, as would be expected from Haber's rule, but nonlinear results were observed. In addition, percent difference calculations using the adult values as the basis for comparison, showed the young rats to have 30% lower rAM, and the aged rats to have 27% higher rAM when compared to the adults for both types of scenarios tested. In summary, PBPK modeling was used as a tool to test the applicability of Haber's rule for inhalation exposures that vary in length and time. PBPK modeling suggested that $CHCl_3$ toxicity may not be described by Haber's rule. (This abstract does not refect EPA policy).

429 INCORPORATION OF STRAIN- AND AGE-SPECIFIC INPUT PARAMETERS INTO A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR CHLOROFORM ($CHCl_3$).

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$CHCl_3$ is a trihalomethane (THM) present in drinking water disinfected either by chlorination or by ozonation processes that involve post-treatment with either chlorine or chloramine. PBPK models are available for $CHCl_3$, including one developed in our laboratory based on closed-chamber uptake curves for ~60-day-old male F-344 rats and Sprague-Dawley physiological input parameters (Yang et al., 1995). The objective of the present work was to determine the appropriateness of the 60-day-old $CHCl_3$ model for 90-day-old rats. This was prompted by the recent finding that a relatively small increase in age (2 months) resulted in an increase in the toxicity of another volatile organic solvent, carbon tetrachloride (Schoeffner et al., 1999). The existing $CHCl_3$ model was updated with physiological input parameters for F-344 rats (Delp et al., 1998) that have recently become available. Simple linear interpolation (between 60 and 180 days) was used to adjust the following: total cardiac output; fractional blood flow to liver, kidney, fat and muscle; and, fractional volume of the liver and kidney. Optimization (Simusolv®, Version 3.0, 1993) of the closed-chamber data obtained in ~60-day-old F-344 rats with input parameters specific for this age and strain resulted in a Vmaxc of 4.7 mg/kg/hr (compared to a Vmaxc of 4.1 mg/hr/kg in our previous model, with Km held constant). To evaluate the ability of the updated model to extrapolate across a small age range, ~90-day-old male F-344 rats were exposed to initial concentrations of 500 or 1000 ppm $CHCl_3$ for 6 hr by closed-chamber techniques. The uptake curves predicted by use of physiological input parameters for ~90-day-old rats closely matched the experimentally derived uptake curves. These results indicate that age extrapolation of physiological input parameters was successful in predicting uptake and metabolism of $CHCl_3$. The newly updated strain- and age-appropriate CHCl3 PBPK model is now available to investigate the pharmacokinetic interaction between the two most prevalent THMs, $CHCl_3$ and bromodichloromethane, using closed-chamber data being collected in ~90-day-old male F-344 rats. (This abstract may not reflect EPA policy. JR Morin supported by CT-826513.)

430 AN APPROACH TO OPTIMIZE GAS UPTAKE EXPERIMENTS TO IMPROVE PARAMETER ESTIMATION.

J. H. Overton and M. V. Evans. *USEPA, Research Triangle Park, NC.*

A non-invasive method of estimating metabolism parameters involves the use of the closed gas uptake chamber technique, PBPK models, and parameter estimation. This presentation deals theoretically with two questions: (1) What are some gas uptake chamber experimental designs that may reduce the uncertainty in estimated parameters? (2) Is there a method for choosing one design over another? In response to (1), three types of design are considered: (A) different initial chamber concentrations, (B) chamber volumes, and (C) time spans of pre exposure to a constant concentration prior to closing the chamber. For estimation of a rat metabolic rate, V_{max}, a PBPK model is used to sim-

ulate chamber concentrations (CI) and calculate sensitivity coefficients (SC, of CI to V_{max}) for all possible combinations of designs. Our approach to (2) is to assume that CI is approximately linear in V_{max} and treat the optimization as a linear regression in which SC is the independent variable. With assumptions about the error term and using all of the simulated "observations" for each design, the standard deviation (SD) of V_{max} for each design is estimated. The SDs represent the relative uncertainty of each experimental design and can be used to discriminate among the designs. For a "large" V_{max}, results generally indicate that the smaller the pre exposure times and the smaller the volumes the smaller the SD; but SD mainly decreases with increasing initial chamber concentration. With a V_{max} 1/50-th of the large V_{max}, SD behaves the same way as for the large V_{max} in relation to pre exposure time and chamber volume. In contrast to the large V_{max} results, the small V_{max} SD increases with increasing initial concentration. In conclusion, this method of discriminating among designs is an approach to developing more efficient experiments that reduce uncertainty in estimated parameters, and thus, should lead to improved risk assessments. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

431 COMPARATIVE ANALYSIS OF SOFTWARE FOR PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING: SIMULATION, OPTIMIZATION, AND SENSITIVITY ANALYSIS.

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SimuSolv® and the related ACSL® software packages have been widely used for development and application of physiologically based pharmacokinetic (PBPK) models. Due to the widespread use of PBPK modeling in toxicology and risk assessment and the cessation of further development and releases of SimuSolv®, evaluation of other packages with comparable capabilities is essential. A previously developed PBPK model for the environmental contaminant trichloroethylene (TCE) was used to compare the two software packages: SimuSolv® and Matlab®. The TCE model provides a framework for assessing common PBPK modeling activities in Matlab®, with an emphasis on comparisons to SimuSolv®. The model is flow-limited with 6 compartments (brain, liver, slowly perfused, fat tissue, kidney, and rapidly perfused). Matlab® provided a convenient framework for coding the model. Very similar results for model simulations were achieved using initial chamber concentrations from 10 to 1000 ppm. Differences between the two packages for predicted chamber and tissue concentrations were on the order of 10-4. Differences of these small magnitudes may be attributed to the different numerical methods used in simulating the models. Estimates of the Michaelis-Menten parameters for TCE metabolism in the liver (Vmaxc and Km) differed slightly due to the use of different optimization routines. SimuSolv® uses maximum likelihood and estimated Vmaxc as 4.4 while Matlab® uses least squares and had Vmaxc equal to 4.5. Both methods do yield parameter estimates that provide a good visual fit to the data. Sensitivity coefficients were calculated in SimuSolv® and code was written in Matlab® to calculate the sensitivity coefficients. Very similar results for sensitivity coefficients were achieved when using the same values of Vmaxc and Km in each package. In summary, Matlab® is an adequate replacement for SimuSolv® in this PBPK application. However Matlab® requires more programming by the user and it does not provide built in commands for parameter estimation or sensitivity analysis like SimuSolv®. (This is an abstract of a proposed presentation and does not reflect EPA policy.)

432 A FAMILY APPROACH FOR ESTIMATING REFERENCE DOSES/CONCENTRATIONS FOR SERIES OF RELATED ORGANIC CHEMICALS.

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We developed pharmacokinetic (PK) methods to streamline development of Reference Concentrations (RfCs) and Reference Doses (RfDs) for compounds that are sequential metabolites of common precursor compounds. This approach is illustrated for the butyl series of chemicals: butyl acetate (BuAc), butanol (BuOH), butyraldehyde (BuCHO), and butyric acid (BuCOOH). These compounds are high volume industrial chemicals. Their major systemic effects are nonspecific. Administration of butyl acetate to test animals produces internal doses of the acetate and each of the subsequent metabolites - BuOH, BuCHO, and BuCOOH. It is possible to extrapolate from BuAc systemic toxicity studies to estimate potentially toxic levels of each of the metabolites. Using internal dose metrics simulated with a physiologically-based pharmacokinetic (PBPK) model, we developed methods to predict minimally toxic levels of each of the metabolites. The PBPK model combines submodels for each of the metabolites. The model describes intravenous, inhalation, and oral exposures. Using the PBPK analysis, we estimated the BuOH, BuCHO, and BuCOOH exposures that would result in the same blood AUC for these compounds as observed in the BuAC studies. The RfC or RfD of the metabolite can then be estimated from the study of the parent compound toxicity and the estimated tissue exposures of the various metabolites. This approach streamlines toxicity testing, and makes better use of the toxicity data and fiscal resources for risk and hazard characterization and risk assessment. This work was sponsored by the CMA Oxo Process Panel.

433 QUANTITATIVE EVALUATION OF THE PHARMACOKINETIC INTERACTIONS BETWEEN TRICHLOROETHYLENE (TCE), TETRACHLOROETHYLENE (PERC), AND 1,1,1-TRICHLOROETHANE (MC) USING GAS UPTAKE STUDIES AND PBPK MODELING.

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Volatile organic solvents are widely distributed environmental pollutants and common contaminants of many chemical waste sites. TCE, PERC, and MC are among the top ten 1997 ATSDR CERCLA high priority chemicals with 'Completed Exposure Pathway' and are frequently found in combination. To investigate the pharmacokinetic interactions among TCE, PERC and MC, gas uptake experiments were performed using a closed chamber exposure system. In each experiment, two rats (Fischer 344, male, 8-9 weeks old) were exposed to different initial concentration of TCE, PERC, and MC, applied singly or as a mixture, and their concentration in the gas phase of the chamber was monitored over a period of 6 h. A PBPK model was developed to test multiple mechanisms of inhibitory interactions, i.e. competitive, noncompetitive, or uncompetitive. The mixture exposure data were described by a system of equations in which a PBPK model was provided for each chemical and each was regarded as an inhibitor of the others' metabolism. At initial concentrations higher than 300 ppm, PERC and MC inhibit the metabolism of TCE. TCE kinetics were not visibly affected by co-exposure to PERC and MC at initial concentrations lower than 300 ppm. Model simulations indicated that among these three chemicals the inhibition was competitive. The metabolic rates of the three chemicals follow the order of TCE>PERC>MC. Results with binary combinations (TCE+PERC, TCE+MC, and PERC+MC) permit confirmation of the competitive interactions in the ternary mixture. Such interactive PBPK models will be of value in risk assessment of occupational and environmental exposure to solvent mixtures. (This study was supported by ATSDR [Cooperative Agreement U61/ATU881475], and NIEHS Superfund Basic Research Program [P42 ES05949].)

434 *IN-VITRO* TO *IN-VIVO* EXTRAPOLATION OF BENZENE (BZ) METABOLISM FOR PBPK MODELING OF BZ AND ITS METABOLITES IN MICE.

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High-level BZ exposure to humans over extended periods has been shown to cause leukemia. There is considerable uncertainty in the risks of low-level BZ exposure from sources such as cigarette smoke, gasoline vapors, and automobile emissions. Conversion of BZ in the liver to benzene oxide (BO), phenol (PH), and/or hydroquinone (HQ) is thought to be an activating process for BZ-induced hematotoxicity. The higher sensitivity of mice than rats to BZ-induced hemato- and myelotoxicity corresponds to faster rates of BZ metabolism in mice relative to rats. Presuming that these endpoints are indicators of leukemogenic potential, the risk of BZ-induced leukemia in humans is expected to correlate with the levels of key BZ metabolites in human bone marrow. Biologically based, mathematical models describing BZ metabolism in rodent and human liver fractions have been previously developed. In order to predict dosimetry of BZ metabolites in humans, a PBPK model based on these *in vitro* models is now being developed. However, BZ dosimetry data in humans that could be used to validate this model are limited. Therefore, we have first developed such a model in mice, where more dosimetry data are available. The model tracks circulating levels of BZ, BO, PH, and HQ, as well as rates of conjugation and excretion of BO, PH, and HQ, and the rate of conversion of BO to muconic acid (MA). But even for mice, not all of the kinetic data needed for an extrapolation is available. The rate of conversion of BO to MA,

in particular, must be fit to *in vivo* data due to a lack of *in vitro* kinetic data. Likewise the rate of irreversible binding to macromolecules must be estimated from *in vivo* data in order to obtain reasonably good fits. Thus, it appears that complete *in vitro* to *in vivo* extrapolation of BZ metabolism is not possible with the existing set of *in vitro* kinetic data.

435 PRELIMINARY PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING FOR GENISTEIN DOSIMETRY IN RATS AND HUMANS.

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A significant portion of total human exposure to endocrine-active compounds is through the diet. Phytoestrogens, such as genistein, found in soy-derived foods are a primary component of this dietary exposure. The National Toxicology Program (NTP) has initiated a multigeneration toxicity study on genistein in rats, and additional studies are underway to complement this work. In order to quantify and extrapolate any exposure-response relationships that may be observed in these studies, we seek to develop PBPK models that will estimate internal dosimetry of genistein in rats and humans. Existing pharmacokinetic data for genistein is not sufficient to accurately estimate all of the parameters of a PBPK model. Therefore we developed "preliminary" PBPK models that will serve to identify key data gaps and can be used in experimental design to fill these gaps. We initially developed PBPK models with a simple structure and few unknown parameters. After estimating those parameters we augmented the models to match existing data. The data include biliary excretion during a portal vein infusion of genistein (Sfakianos et al. 1997), biliary excretion during and after a duodenal infusion of genistein (Barnes et al. 1996), plasma concentration with urinary and fecal elimination after oral doses of genistein (King et al. 1996), and biliary and urinary excretion after oral doses of genistein (Yasuda et al. 1996). Augmentation of the models included compartments for GI tissue and bladder, and delayed variables to account for delays in both biliary and fecal elimination. Physiological parameters such as blood flows and tissue volumes were taken from the literature. The Mathworks' software package MATLAB was used to fit the unknown genistein-specific parameters to the published data. Given an infusion or initial oral dose, the preliminary PBPK models are now able to reasonably predict total concentrations of genistein and its metabolites in the blood and amounts in urine, bile and feces. For example, model predictions indicate that after an oral dose of 60 mg/kg genistein in the rat, the majority of genistein in circulation may be unconjugated for over 7 hours and biliary excretion rates are comparable to urinary excretion rates.

436 PHYSIOLOGICALLY-BASED PHARMACOKENETIC AND TWO STAGE CLONAL GROWTH MODELING OF CHLOROBENZENE-INDUCED PRENEOPLASTIC FOCI WITHIN THE ITO MEDIUM-TERM BIOASSAY.

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ITO assay is a medium-term (8-week) liver bioassay involving sequential administration of initiating agent, test compound, and mitogenic stimulus. Comparative results from the ITO and two-year chronic bioassay show good correspondence. We describe here a quantitative description of glutathione-S-transferase π foci formation within the ITO framework, using PBPK and clonal growth model for tetra-, penta-, and hexachlorobenzene. To estimate parameters for the PBPK model, a single gavage study (0.1 mmol/kg) was conducted, and tissue concentrations at 7 time points over a 5-day period were collected. Two PBPK models were constructed to analyze the experimental data. The first model, which incorporated α-2μ binding in the kidney and diffusion-limited tissue uptake in the fat compartment, accurately described chlorobenzene levels in kidney and liver, but underestimated blood concentrations. The second model with protein binding in blood adequately described blood and tissue concentrations. To estimate parameters for the clonal growth model, several cell and biochemical parameters were collected, including 5-bromo-2'-deoxyuridine labeling index for analysis of cell division rate, and kinetic changes in foci size and number. The model successfully described the time-dependent changes in foci number, while underestimated the volume fraction of foci in the liver. To further improve model performance, observed time-dependent changes in hepatocyte density following penta- and hexachlorobenzene exposure were incorporated into the model. The application of PBPK and clonal growth modeling presented here provides a quantitative framework for the comparison of carcinogenic potential among chlorobenzene isomers. The approach may also serve as an example of biologically-based dose response modeling for other tumor promoters. (Supported by NIEHS P42 ES05949.)

437 PHYSIOLOGICAL PHARMACOKINETIC MODEL REDUCTION APPLIED TO HUMAN RISK ASSESSMENT.

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Physiological pharmacokinetic models provide quantitative information between the exposure level and the biophase concentration of the xenobiotic based on physiological realities. However, it is difficult to apply physiological models directly to humans due to the limitation of data from humans. Similar problems occur in drug development where there is no information to validate a mechanistic model for humans. Therefore, a nonlinear mixed effect (NLME) pharmacokinetic (PK) approach is widely accepted in clinical trial data analysis. The NLME PK approach introduces covariates to the classical PK models to explain the interindividual variability that the classical PK models alone are unable to address. However, the NLME method is a purely statistical approach, where the covariates are determined through residual analysis. The present study combined both physiological modeling and the NLME approach to analyze human population data. This was done in three stages. First, a PBPK model was developed based on information from experimental animals. Second, model reduction was conducted and the physiological realities were expressed in the simplified model through well defined covariates. At stage three, a NLME modeling was conducted to establish the population PK model as well as individual PK models using sparse data. TCDD was used as an example since both a PBPK model for animals and sparse data from humans are available. This example demonstrates the potential use of this new approach for species extrapolation in human health risk assessments. (This study does not represent USEPA policy.)

438 INCORPORATING A VALIDATED PBPK PREGNANT RAT MODEL INTO A BBDR MODEL FOR THE EMBRYOTOXICITY OF 5-FLUOROURACIL.

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5-Fluorouracil (5-FU) is a widely used antitumor agent that is embryotoxic in rats at maternal therapeutic levels. A biologically based dose-response model (BBDR) was developed that relates a single dose of 5-FU on gestation day 14 in rats to cell cycle effects in developing fetuses, specifically the inhibition of thymidylate synthetase (TS; due to formation of a FdUMP/TS/folate complex) with subsequent reduction in thymidylate (dTMP) levels, thymidine-5'-triphosphate (dTTP; a DNA precursor) and, ultimately, disruption of DNA synthesis. The initial modeling of the pharmacokinetic component of the BBDR model was based upon a two-compartment PBPK model (Collins et al., 1980) for the maternal kinetics with an added compartment for the fetal kinetics of 5-FU. This BBDR model described well the 5-FU concentration data from gestation day 19, but fetal compartment concentrations on day 14 and the time course of the inhibition were modeled empirically rather than mechanistically. To incorporate more physiological information, a pregnant rat PBPK model developed by O'Flaherty et al. (TAP 112:245-256, 1992) for the weak acid dimethloxazolidine-2,4-dione (DMO) was adapted for use in the 5-FU BBDR model. The O'Flaherty model adjusts for changes in the dam and fetal body weights, blood flows, and organ volumes as the gestation progresses. Metabolism of 5-FU and tissue partitioning was also added to the model. Sensitivity analysis and use of different data sets as the basis for the parameter estimates in this more detailed PBPK component provided insights into the importance of 5-FU pharmacokinetics to the resulting changes in thymidylate synthesis compared with other potential pharmacodynamic interactions. (This abstract does not necessarily reflect EPA policy.)

439 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ANTHRAQUINONE IN MALE AND FEMALE RATS AFTER SINGLE INTRAVENOUS AND ORAL DOSES AND CHRONIC DOSING IN FEED.

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Anthraquinone (AQ) is an industrial chemical widely used in the production of dyes and paper products. It is a direct-acting mutagen in *Salmonella*. Toxicity and carcinogenicity tests performed by the National Toxicology Program (NTP) have shown AQ, when added to feed, to be carcinogenic to the female rat kidney and the male and female mouse liver. A physiologically based pharmacokinetic (PBPK) model for AQ was developed to characterize tissue concentrations of AQ in male and female rats resulting from intravenous and oral exposure. Plasma concentration and biliary secretion time-course data used to create the anthraquinone PBPK model were obtained from single administration intravenous and oral gavage toxicokinetic studies done as part of the NTP study, as well as from plasma samplings done at five intervals (8 days, and 13, 26, 52, and 78 weeks) during the chronic study. This PBPK model describes AQ as slowly and incompletely absorbed from the lower intestine into the blood via the lymph. AQ is represented as distributed slowly to tissues by a diffusion-limited transport process, stored in fatty tissues, and metabolized by both hepatic and extra-hepatic pathways. AQ secreted into the bile is reabsorbed from the intestine. The model reproduces plasma concentrations, which peak more than 8 hr after oral administration, and biliary secretion rates for anthraquinone as parent chemical after single intravenous and oral gavage exposures. The model was then used to simulate plasma concentrations for anthraquinone after chronic exposure in feed.

440 MODELING OF THE DISPOSITION OF GLYCYRRHIZIC ACID, A COMPOUND SUBJECT TO PRESYSTEMIC METABOLISM AND ENTEROHEPATIC CYCLING, IN HEALTHY VOLUNTEERS.

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Glycyrrhizic acid (GD) is currently of clinical interest for treatment of chronic hepatitis. It is also applied as a sweetener in food products and chewing tobacco. In some highly exposed subgroups of the population, serious side effects like hypertension and electrolyte disturbances have been reported. For the health risk evaluation of GD exposure, a detailed insight into the kinetics of glycyrrhizic acid and its metabolites was obtained by the development of a kinetic model. Rat data show that after oral intake GD is hydrolyzed in the gastrointestinal tract by commensal bacteria and absorbed as its aglycon glycyrrhetic acid (GA). This compound is predominantly cleared from plasma by the liver after glucuronidation. Following biliary excretion commensal bacteria reconvert this GA-glucuronide to GA , which is reabsorbed by the gut. These kinetic processes were modeled in a biological motivated manner. Using the results of a clinical study, 5 of the 18 model parameters, having the most influence on the GA plasma concentration forecast, were successfully fitted to the data. In a 4-way cross over design, all volunteers consumed 4 different forms of liquorice. The model, parameterized with the mean of the fitted parameter values, successfully forecasted the GA plasma concentrations of another clinical study. The distribution of the individual parameters within the clinical study population was used to simulate the GA disposition within the general population by a Monte Carlo simulation. This simulation shows that especially in people having a prolonged gastrointestinal residence time, GA accumulates after repeated liquorice consumption, specifically increasing the health risk of this subgroup of individuals.

441 COMPARISON OF *IN VITRO* AND *IN VIVO* KINETICS OF TETRACHLOROBENZYLTOLUENES (UGILEC 141) AND PCBs.

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Tetrachlorobenzyltoluenes (TCBTs) were introduced in Europe as a replacement for polychlorobiphenyl mixtures in hydraulic devices in the early 1980s. Since 1994 their use is prohibited. *In vitro* studies demonstrated that metabolism of TCBTs in mammalian species is relatively rapid compared to PCBs. However, *in vivo* studies showed that TCBT elimination was determined by their flow-limited release from the adipose tissues. It is therefore relevant to study the quantitative role of metabolism in the whole body elimination of TCBTs relative to that of PCBs. For this purpose, we determined *in vitro* metabolic rate constants for three TCBTs and seven PCBs using rat hepatic microsomes. In addition, we conducted an *in vivo* kinetic study in the rat. Rats were either fed with a mixture of TCBTs and PCBs or intravenously injected with the same mixture. Analysis of these *in vivo* results with a physiologically-based pharmacokinetic model confirms that the metabolism of TCBTs is limited by the perfusion of, and diffusion to, the adipose tissues.

441A ORGAN-SPECIFIC DIFFERENCES IN BASE-EXCISION REPAIR FOLLOWING ACUTE TREATMENT WITH BENZO[A]PYRENE.

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Benzo[a]pyrene (B[a]P), a known carcinogen, is widely distributed in the environment. Studies suggest that the carcinogenic effect of B[a]P results from the formation of promutagenic DNA lesions, specifically 8-oxoguanosine and etheno-adducts. The lung is a target organ for B[a]P toxicity. Previous studies have demonstrated the presence of oxidatively modified bases in the lung compared with other tissues, following B[a]P treatment. The objective of this study was to determine the capacity of different organs to repair 8-oxoguanosine and etheno-adducts after treatment with B[a]P. Male Sprague-Dawley rats were treated with 20 mg/kg B[a]P i.p., 2 times/day for 5 days. At 24, 72, and 120 hours after the last treatment, lung, liver, and kidney were harvested. The capacity to repair 8-oxoguanosine and 3-methyladenosine were determined. Tissue extracts were incubated with a 32P-labelled oligonucleotide containing either 8-oxoguanosine or ethenoadenosine. Excision repair activity was determined by comparing cut and uncut DNA. The lung had a significant decrease in capacity to cleave 8-oxoguanosine at 72 hours following treatment, but recovered to above control values at 120 hours. The activities for liver and kidney remained at baseline for all times studied. 3-methyladenosine activity remained constant for all organs. This study suggests that organ-specific differences exist in base-excision repair activity in response to acute treatment with B[a]P. (Supported in part by NIOSH grant 416005-01 and VA Merit Review grant.)

442 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ETHYLENE GLYCOL AND ITS MAJOR METABOLITE, GLYCOLIC ACID.

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Ethylene Glycol (EG) is a major industrial chemical. An extensive database has been amassed on EG's toxicity and modes of action. At high oral bolus doses (≥500 mg/kg/day), EG causes renal and developmental toxicity. EG's toxicity has been primarily attributed to its major metabolite, glycolic acid (GA), which shows a high degree of dose-, route- and species-dependency. A physiologically based pharmacokinetic (PBPK) model was developed to describe the disposition of EG and GA in female rats, including pregnancy. Metabolic rate constants for EG and GA were estimated from liver slice kinetic studies. Partition coefficients for EG and GA were determined by vial equilibration and ultrafiltration methods. The PBPK model included inhalation, oral, dermal, intravenous and subcutaneous routes of administration. Metabolism of EG and GA were described in the liver with elimination via the kidneys. Several rat metabolism studies were simulated. Pregnancy had no effect on maternal EG and GA kinetics over a broad dose range. Simulations were consistent with studies indicating that metabolism of EG to GA was essentially first-order (linear) up to 2500 mg/kg/day while the metabolism of GA saturated between 200 and 1000 mg/kg/day. This resulted in non-linear increases in blood GA concentrations which correlate with the toxicity of EG. (Sponsored by the Ethylene Glycol Panel of the Chemical Manufacturers Association).

443 BIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF UPTAKE AND INTRACELLULAR ACIDIFICATION OF ORAL EPITHELIAL CELLS INDUCED BY VINYL ACETATE EXPOSURE FROM DRINKING WATER.

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Recent two year drinking water studies with vinyl acetate (VA) in rats and mice have shown statistically significant increases in tumor incidences in several sites along the oral cavity, esophagus and fore-stomach at high doses (10000ppm) at terminal sacrifice, and not at lower doses (400, 2000 ppm). VA is extensively hydrolyzed by carboxylesterase (CE) to acetic acid (AA) and acetaldehyde (AAld). AAld is further rapidly oxidized to AA by a saturable aldehyde dehydrogenase (ADH). AAld is a potential clastogen and recent evidence suggests that an acidic intracellular pH is also clastogenic. It is proposed that intracellular accumulation (of VA and metabolism to) AA causes intracellular acidification resulting in cytotoxicity followed by reparative mucosal hyperplasia. At high doses, cell proliferation under conditions including high AAld concentration and lowered nonphysiological pH may lead to mutation, cellular transformation, and increased tumors. This same mode of action was used successfully to develop a biologically-based model for inhalation exposures in rats. A GI tract model was constructed to describe regional uptake and metabolism using information collected on (1) the temporal drinking water patterns in rodents, (2) the mucosal surface area and tissue thickness in different regions of the GI tract, (3) enzyme histochemical localization within the GI tract, and (4) regional enzyme kinetics for carboxylesterase in the GI tract of rodents. The resulting model describes diffusion-limited uptake of VA and intracellular pH changes in the epithelial tissue layer. Initial simulations of 24 hour exposure to 1000, 2000, 5000, and 10,000 ppm VA in drinking water yielded estimates of steady-state epithelial pH of 6.97, 6.88, 6.78, and 6.7, respectively. This model will be used for interspecies dose-response assessment in a harmonized approach to cancer and noncancer toxicity of oral VA exposures.

444 PRELIMINARY DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR PERCHLORATE IN ADULT HUMANS.

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Perchlorate-contaminated groundwater presents a potential environmental health issue. Perchlorate is not metabolized and is almost completely excreted; however while in the body, it competitively inhibits iodide uptake in the thyroid and gut. Hence, there's concern that chronic exposure to low levels in drinking water could lead to hypothyroidism. A human physiologically-based pharmacokinetic (PBPK) model to predict the inhibition of thyroid iodine uptake and effect on thyroid hormones is under construction. The preliminary model, presented here, describes systemic clearance of perchlorate and simultaneous concentrations in the blood, lungs, slowly perfused tissue, rapidly perfused tissue, gut and thyroid. The gut and thyroid compartments are comprised of two compartments to describe non-linear uptake of perchlorate. Tissue/blood partition coefficient values were derived from published animal studies. Systemic clearance of perchlorate was established by fitting limited published human urinary excretion data. Urinary elimination rate constants ranged from 4 to 6 hr^{-1}- kg BW. The model adequately simulated the urinary excretion of perchlorate. Human studies are ongoing, which will provide data for further model development.

445 DEVELOPMENT OF A PHYSIOLOGICAL MODEL FOR PERCHLORATE INDUCED INHIBITION OF THYROIDAL UPTAKE OF IODIDE IN THE RAT.

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NASA and DoD use ammonium perchlorate (NH_4ClO_4) salt as an oxidizer in solid rocket propellants. NH_4ClO_4 is highly soluble in water and completely dissociates to NH_4^+ and ClO_4^-. The ClO_4^- ion, because of its similarity in ionic size to I^- competes with I^- for uptake into the thyroid gland by the Na^+/I^- symporter. Consequently, the availability of I^- for thyroid hormone synthesis (thyroxine (T_4) and, 3,5,3'-triiodothyronine (T_3)) may be depleted leading to a state of hypothyroidism. A series of pharmacokinetic laboratory experiments were carried out using cold and radiolabeled perchlorate and ^{125}I in male Sprague-Dawley rats for development of a physiologically based pharmacokinetic (PBPK) model for perchlorate and ^{125}I in the rat. Subsequent pharmacokinetic experiments were conducted to examine the dose-response characteristics of perchlorate induced inhibition of uptake of ^{125}I in the thyroid. Perchlorate is excreted in urine (> 80% over 24 hrs). While in the body of naïve rats, perchlorate inhibits uptake of radiolabeled iodide. For perchlorate doses ranging from 0.01 to 3 mg/kg uptake of iodide in the thyroid gland was inhibited from about 20 to 80%. A rat PBPK model for perchlorate induced inhibition of uptake of iodide in the thyroid is presented.

446 TRICHLOROACETATE TISSUE DOSIMETRY AND PPARα-MEDIATED LIVER CANCER INDUCTION BY TRICHLOROETHYLENE AND PERCHLOROETHYLENE.

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A biologically-motivated dose-response analysis was undertaken for liver cancer induction in mice by trichloroethylene (TCE) and perchloroethylene (PERC). The mechanistic hypothesis evaluated was whether tumors resulted from a peroxisome proliferator-activated receptor-alpha (PPARα)-mediated process initiated by formation of trichloroacetate (TCA) from the two volatile organics. Available mechanistic data for TCE, PERC, and TCA were compared with those for prototypical PPARα-ligands. Several early and late events were consistent: increased liver to body weight ratio (LW/BW) due to induction of peroxisomes, hypertrophy, and cell proliferation; the phenotype of induced foci; and reversibility of tumor response with cessation of exposure. One difference was hepatic accumulation of lipid with PERC, but not the other compounds. Use of precursor events for analyzing cancer dose response was evaluated; increased LW/BW was suggested as a useful indicator of the pleiotropic response necessary for PPARα-mediated liver carcinogenesis. Physiologically based pharmacokinetic models provided estimates of internal dose metrics for TCA. Oral and inhalation studies for LW/BW and cancer were evaluated to obtain points of departure. Low dose extrapolation used a margin of exposure approach for TCE; TCA and PERC were not evaluated. While there are difficulties in analyzing the hypothesis due to the variety of exposure protocols used in the studies with TCE, PERC, and TCA, this analysis indicated substantial consistencies in the database supporting a PPARα-mediated process for TCE- and TCA-induced liver carcinogenesis as a major causative process. (Abstract does not reflect EPA policy.)

447 DIALLYL SULFIDE PROTECTS AGAINST ALCOHOL ENHANCED ACETAMINOPHEN HEPATOTOXICITY IN *Cyp2e1(-/-)* MICE.

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One approach to determine the role of a particular form of CYP in drug toxicity involving bioactivation is to investigate whether treatment with a specific inhibitor of that CYP protects animals from the toxicity. A significant problem with this approach is that inhibitors administered *in vivo* may prove less specific than when used *in vitro*, due to the very high concentrations often achieved in the liver of the intact animal. Diallylsulfide is a chemical reported to specifically inhibit CYP2E. Protection against ethanol-mediated increases in acetaminophen (APAP) hepatotoxicity by diallyl sulfide is taken as evidence for a major role of CYP2E1. However, diallyl sulfone, a metabolite of diallyl sulfide, has been shown to also inhibit CYP3A and CYP1A2. Here we show that short-term treatment with diallyl sulfide protects *Cyp2e(-/-)* mice from APAP hepatotoxicity in animals pretreated with ethanol and isopentanol. Liver damage was assessed histochemically and by the elevation in serum levels of alanine aminotranferase. The absence of CYP2E1 in these mice was confirmed by immunochemical analyses of microsomes from all animals in the study. The results show that diallyl sulfide is not specific for CYP2E1 *in vivo*. (Supported by the Department of Veterans Affairs.)

448 HETEROLOGOUS EXPRESSION STRATEGIES OF THE CYTOCHROME P450 2F FAMILY ENZYMES.

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Cytochrome P450 enzymes 2F1 (human) and 2F3 (goat) have been shown to be selectively expressed in pulmonary tissues. Both of these enzymes are believed to participate in the biotransformation of pneumotoxic compounds to their respective reactive intermediates. 3-Methylindole is a pneumotoxin that requires P450-mediated dehydrogenation to elicit its toxic effects. Cytochrome P450 2F1 and 2F3 exclusively catalyze dehydrogenation of this substrate. To elucidate the biochemical parameters of this unique P450-mediated reaction, a system for the production of large amounts of pure enzyme is required. Bacteria remain the most preferred organism for the heterologous expression of mammalian P450 enzymes due to their lack of endogenous membrane-bound P450 enzymes and their ability to produce large quantities of the protein. Several strategies are currently being investigated for the expression of both 2F1 and 2F3 in a bacterial system. Cytochrome P450 2F3 has been expressed in a lac based expression system that co-expressed cytochrome P450-NADPH reductase. The maximal level of P450 expression was 24 nmols/liter of culture. Expression of cytochrome P450 2F1 has been attempted in this system, a lac based system without the reductase and a T7-based expression system. Heterologous expression of 2F1 in bacteria was not successful using these vectors, despite the greater than 85% sequence identity to 2F3. A chimeric construct of the cDNA encoding the N-terminal region of cytochrome P450 2F3 and C-terminal region of cytochrome P450 2F1 has recently been produced. Studies with this construct are currently being investigated. (Supported by USPHS Grant HL16345.)

449 DOES TCDD CAUSE PERINATAL IMPRINTING OF CYP4501A2 ACTIVITY IN THE HUMAN?

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This study addresses the question of whether high body burdens of TCDD during development may imprint the Ah receptor-Cytochrome P4501a2 system complex resulting in alteration of the adult's expression and /or inducibility of P450 1a2 activity. Study subjects were recruited during 1992 to 1995 from individuals who were exposed to very high levels of TCDD in 1976 following an industrial accident that released the TCDD into a residential environment. Thirty three females and 33 males were recruited into the study who were a few months to 54 years of age in 1976 when the exposure occurred. All subjects were healthy, none smokers, had a TCDD measure in serum in 1976 after the accident, and in 1992-1995. The [13]Caffeine breath test (CBT), to monitor P4501a2 activity, was conducted between 1992 and 1995. According to the subject's age at the time of the exposure, the subjects were divided into an Early Childhood Exposure Group (a few month to 5 years of age), a Late Childhood and Adolescence Exposure Group (6 to 16 years of age), and a Adult Only Exposure Group (16 years old and older). Comparison of CBTs between exposure groups was conducted using the Wilcoxon rank test. TCDD levels for the females ranged between 30 and 56,000 ppt in 1976, and 12 and 800 ppt in 1992;those for the males were between 50 to 11,000 ppt in 1976 and 1 to 700 ppt in 1992. The CBT, expressed as per cent of carbon thirteen label administered which is exhaled over two hours after the caffeine was taken, was significantly lower in the Early Childhood Exposure Group (median 3.8, range 1.3-4.8) as compared to the other two group (median 6, range 3.2-10; median 6.5, range 3.0-15 respectively, $p < 0.01$). This was observed even though the TCDD serum levels of all the groups were not different in 1976 or in 1992. The molecular mechanism by which apparent imprinting occured and the relative importance of the expression of P450s and their imprinting to the overall susceptibility of the human to the PAHs, and to normal development remains to be identified. The cooperation and understanding of the study subjects of Seveso are greatly appreciated. (Support: STAR grant from the USEPA and of Region Lombardia, Milano.)

450 NADPH- AND HYDROPEROXIDE-SUPPORTED 17BETA-ESTRADIOL (E2) HYDROXYLATION CATALYZED BY A VARIANT FORM (432L, 453S) OF HUMAN CYTOCHROME P450 1B1 (CYP1B1).

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Human CYP1B1 catalyzes the hydroxylation of E2 at C-4 with a lesser activity at C-2. The E2 4-hydroxylase activity of human CYP1B1 was first identified in MCF-7 breast cancer cells. Sequencing of polymerase chain reaction products revealed that CYP1B1 expressed in MCF-7 cells is not the previously characterized enzyme but a polymorphic form with leucine substituted for valine at position 432 and serine substituted for asparagine at position 453. To investigate the NADPH- and organic hydroperoxide-supported E2 hydroxylase activities of the 432L, 453S form of human CYP1B1, the MCF-7 CYP1B1 cDNA was cloned and the enzyme was expressed in Sf9 cells. In microsomal assays supplemented with human NADPH:cytochrome P450 oxidoreductase, the expressed 432L, 453S form catalyzed NADPH-supported E2 hydroxylation with a similar preference for 4-hydroxylation as the 432V, 453N form, with maximal rates of 1.97 and 0.37 nmol/min per nmol P450 for 4- and 2-hydroxylation. Cumene hydroperoxide efficiently supported E2 hydroxylation by both the 432V, 453N and 432L, 453S forms at several-fold higher rates than the NADPH-supported activities and lesser preference for E2 4- versus 2-hydroxylation (2:1). The hydroperoxide-supported activities of both forms were potently inhibited by the CYP1B1 inhibitor, 3,3',4,4',5,5'-hexachlorobiphenyl. These results indicate that the 432V, 453N and 432L, 453S forms of CYP1B1 have similar catalytic properties for E2 hydroxylation, and that human CYP1B1 is very efficient in catalyzing the hydroperoxide-dependent formation of catechol estrogens. (Supported by NIH Grants ES04913, ES03561 and DC02640.)

451 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN AND DI-INDOLYLMETHANES ON CYTOCHROME P450 1A1, 1B1 AND 19 IN H295R HUMAN ADRENOCORTICAL CARCINOMA CELLS.

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Di-indolylmethane (DIM) is a condensation product of indole-3-carbinol, a consituent of cruciferous vegetables, and is formed in the stomach. DIM has been shown to have antiestrogenic properties; it alters estrogen metabolism and appears to antagonize breast tumor promotion. DIM is a weak agonist for the aryl hydrocarbon (Ah) receptor. DIM and four synthetic analogs (5,5'-dimethyl-, 5,5'-dimethoxy-, 5,5'-dibromo- and 1,11'-dimethoxy-C-methoxyphenyl-DIM) were examined in H295R cells for effects on three cytochrome P-450 (CYP) enzymes involved in estrogen synthesis and/or metabolism: CYP1A1, CYP1B1 and CYP19 (aromatase). Aromatase activity was measured by the conversion of [1β-^{3}H]-androstenedione to estrone and ^3H_2O. H295R cells were exposed to the test chemicals dissolved in dimethyl sulfoxide for 24 h prior to analyses. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (0-30 nM) and DIM (0-10 μM) induced ethoxyresorufin O-deethylase (EROD) activity, as a measure of CYP1A1 and 1B1 activity, concentration-dependently with EC_{50} values of about 0.3 nM and 3 μM, respectively. DIM, but not TCDD, induced aromatase activity concentration-dependently to an apparent maximum of two-fold at 10 μM; greater concentrations of DIM and analogs were cytotoxic. TCDD (30 nM) increased levels of mRNA for CYP1A1 and 1B1 about 2 to 2.5 fold, but had no effect on mRNA for CYP19. DIM (3 μM) increased mRNA levels for all three CYPs between 1.8 and 2.5 fold. The DIM analogs (3 μM) induced aromatase and/or EROD activity, together with the corresponding mRNA levels. DIM and certain DIM analogs appear to induce CYPs via multiple pathways, including the Ah receptor-mediated pathway for CYP1A1 and 1B1 induction, and the protein kinase A-mediated pathway for the induction of aromatase.

452 DEVELOPMENTAL REGULATION OF ADIPOGENESIS, CYP1B1, AND THE ARYL-HYDROCARBON RECEPTOR IN MOUSE EMBRYO FIBROBLASTS.

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Mouse embryo fibroblasts (MEFs) are pluripotent cells that can be stimulated to differentiate into adipocytes, osteocytes, chondrocytes, or myocytes. We show that primary MEFs obtained from gestational day (gd) 14 are not responsive to hormonally-stimulated adipocyte differentiation, whereas gd16 MEFs are highly responsive. Primary MEFs derived from these days differ in the basal and induced expression of the peroxisome proliferator activated receptor γ (PPARγ), a key regulator of adipocyte differentiation, which has low expression in gd14 MEFs compared to gd 16 MEFs. Pretreatment with the DNA-hypomethylating agent 5-azacytidine for the two days preceding hormonal stimulation of adipocyte differentiation improved PPARγ expression in gd14 MEFs by threefold and allowed for differentiation to proceed in these cells. Adipocyte differentiation can be blocked by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) via activation of the aryl-hydrocarbon recep-

tor (AhR). We have shown that this corresponds to a prolongation of MAP kinase activation during the initial 24 hours following hormonal stimulation. The AhR controls constitutive and TCDD induced levels of CYP1B1 in gd16 MEFs. We have shown that gd16 MEFs, compared to gd14 MEFs, show ten-fold higher expression of cytochrome P450 (CYP) 1B1, the predominant P450 in mesenchymal cells. AhR protein expression and down regulation by TCDD were similar in gd14 and gd16 MEFs. In both gd14 and gd16 cells activation of AhR-responsive constructs was similar. CYP1B1 and AhR levels were not affected by 5-azacytidine treatment. Combined, these results suggest that: 1) the developmental pattern of CYP1B1 expression in MEFs does not stem from AhR levels or activity; 2) AhR and CYP1B1 do not control the developmental differences in adipogenic potential; 3) instead, these differences are due to PPARγ suppression in gd14 MEFs.

453 PAH/METAL MIXTURES: EFFECT ON PAH INDUCTION OF CYP1A2 IN CULTURED HUMAN HEPATOCYTES.

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The carcinogenicity of PAHs is potentiated by their CYP1-catalyzed bioactivation, and induction of the bioactivating enzymes by PAH facilitates this process. Metals (frequently environmental co- contaminants with PAHs) could affect PAH carcinogenicity by modifying PAH induction of CYP1A. The four most hazardous environmental metals (EPA and ATSR) were used to test this hypothesis in human hepatocyte cultures grown on collagen matrix. Studies were conducted in 96-well plates of hepatocyte cultures and extents of CYP1A2 induction by 10 μM benzo[a]pyrene (BAP), benzo[b]fluoranthene (BBF), dibenzo[a,h]anthracene (DBAHA), benzo[a]anthracene (BBA), or benzo[k]fluoranthene (BKF) were probed by ethoxyresorufin-O-deethylase activity. Cultures showed large interindividual variations and means of data from several individuals are reported. Induction efficiency (relative to dimethyl sulfoxide controls) was in the order of BKF (8±8-fold) > DBAHA (6±4-fold) > BAP (5±5-fold) > BAA (4±2-fold) > BBF (3±3-fold) (n=5-6), and was approximately 20% of that achieved by BKF or DBAHA and approximately the same for the other PAHs for CYP1A1 induction in HepG2 cells. At 1 μM concentrations of the metals, which did not produce cytotoxicity, CYP1A2 induction by the PAHs was diminished in a metal- and PAH-dependent manner: with BAP as inducer the inhibition was As (50±10%), Cd (24±11%), Pb (18±12%), and Hg (15±18%), n=3. HepG2 cells yielded similar data for CYP1A1 induction. Thus the metals in PAH/metal mixtures could diminish PAH carcinogenicity by decreasing induction of their bioactivation by CYP1A2. (Funded by EPA grant R827180010.)

454 IDENTIFICATION OF CYP2C9 AS THE MAJOR HUMAN LIVER MICROSOMAL LINOLEIC ACID EPOXYGENASE.

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Leukotoxin (9,10-epoxy-12-octadecanoate) and isoleukotoxin (12,13-epoxy-9-octadecenoate) are monoepoxides of linoleic acid, synthesized by a cytochrome P450 monooxygenase and possibly by an oxidative burst of inflammatory cells. Recent experiments in this laboratory have indicated that the toxicity of leukotoxin and isoleukotoxin are not due to these epoxides, but to the 9,10- and 12,13-diol metabolites. These lipids may be involved in inflammation. This study used a combination of experimental approaches to identify the major cytochrome P450 enzyme in human liver involved in linoleic acid epoxidation. The kinetic paramenters were determined; the Km of linoleic acid epoxidation by pooled human liver microsomes was 170 μM and the Vmax was 58 pmol/mg/min. Correlation analysis was performed using individual samples of human liver microsomes, and the best correlation of linoleic acid epoxidation activity was with tolbutamide hydroxylase activity, CYP2C9. Experiments with inhibitory anti-cytochrome P450 antibodies also suggested that the major enzyme in linoleic acid epoxidation was a CYP2C enzyme. Incubations with chemical inhibitors had similar results; no chemical except tolbutamide, a competing substrate for CYP2C9, and sulfaphenazole, an inhibitor of CYP2C9, caused more than 20% inhibition of linoleic acid epoxidation. Both tolbutamide and sulfaphenazole caused greater than 80% inhibition. Finally, although many of the recombinant cytochrome P450 enzymes metabolized linoleic acid, only CYP2C9 had significant epoxidation activity. These results suggest that CYP2C9 is the predominant cytochrome P450 enzyme responsible for the epoxidation of linoleic acid in the human liver. This enzyme, therefore, may serve as a therapeutic target in the treatment of inflammation in order to reduce the amount of circulating leukotoxin and isoleukotoxin and their corresponding diols. (Supported by NIEHS postdoctoral fellowship ES05808, Amgen-American Liver Foundation Postdoctoral Fellowship, ES02710, ES05707 and ES04699.)

455 INDUCTION OF HEPATIC MICROSOMAL CYP1A ACTIVITY BY CYHALOTHRIN.

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The effects of the pyrethroid type II cyhalothrin on hepatic cytochrome P450 (CYP) activities were investigated in male Wistar rats. Animals were divided into 3 groups of six animals each and received orally cyhalothrin dissolved in 0.5 ml corn oil at two dose leves of 4 and 8 mg/kg for 6 days. Control group rats received 0.5 ml corn oil orally. All animals were killed 24 h after last dosing, liver were removed and microsomal pellets prepared for enzyme determinations. Treatment with cyhalothrin enhanced the O-deethylation of ethoxyresorufin (EROD), an activity exclusively catalysed by CYP1A subfamily, and particularly CYP1A1 isoenzyme. Similar changes were observed in the O-demethylation of methoxyresorufin (MROD), an activity also associated with the CYP1A subfamily and particularly the CYP1A2 isoenzyme. Because of CYP1A subfamily has been implicated in the metabolic activation and toxicity of many chemicals, the induction of CYP1A1/2 by cyhalothrin raises the possibility that these enzymes may metabolically convert cyhalothrin to a 'reactive electrophile(s)' with toxicological consequences. (Work supported by Projects No. PB97-1236 (DGICYT), No. 08.8/0002/98 (CAM) and 99/0936 (FIS), Spain.)

456 CYP6L1 IS SPECIFICALLY EXPRESSED IN THE TESTIS AND ACCESSORY GLAND OF GERMAN COCKROACHES.

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Cytochrome P450s are a very important enzymatic system involved in the metabolism of endogenous compounds and xenobiotics. The multiplicity of P450 isoforms in every organism, the broad substrate spectra of some isoforms, and the difficulty in purification of isoforms make it challenging to study individual P450s. Using 3′ and 5′ RACE (rapid amplification of cDNA end) techniques, we have recently cloned full length cDNA of a new P450 gene, *CYP6L1*, from German cockroach, *Blattella germanica*. Using Northern Blot analysis, we found that CYP6L1 is only expressed in the abdomen of adult male German cockroaches. Further studies showed that it is specifically expressed in the testis and accessory gland of male adults. This is the first sex specifically expressed insect P450. Insect P450s are involved in the synthesis and degradation of insect hormones, including juvenile hormone and molting hormone, which are important for insect development and reproduction. The specific expression of CYP6L1 in the reproductive system suggests its possible involvement in the reproduction of German cockroaches. Understanding the role(s) of CYP6L1 will offer new insights into insect reproduction and may offer novel targets for the development of insect control agents.

457 EXPRESSION AND STEROID HYDROXYLATION ACTIVITY OF A CYP3A-LIKE PROTEIN IN CHANNEL CATFISH PROXIMAL AND DISTAL INTESTINE.

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An important cause of incomplete bioavailability is prehepatic metabolism in the GI tract, mainly by the CYP3A subfamily of enzymes. Our work examines the expression and activity of constitutive CYP3A proteins along the intestine of channel catfish, Ictalurus punctatus, fed commercial chow or semi-purified diets. Polyclonal antibodies (IgG) generated against trout CYP3A27 reacted strongly with catfish intestinal microsomes, showing a band with MW of 59 kDa. In catfish fed with chow, the expression of this protein was much higher in the proximal segment (0.101±0.031 nmole/mg protein) than in the distal part (0.032±0.023 nmole/mg protein). Gentest Supersomes human CYP3A4 was the CYP3A standard. Testosterone 6-beta-hydroxylation activity was monitored as the catalytic indicator of CYP3A. Washed catfish microsomes give 3 major metabolites, one unknown metabolite with relative Rf value 0.83, 6-beta-hydroxylated testosterone and androstenedione. Human CYP3A4 formed only 6-beta-hydroxy product under the same reaction conditions. Testosterone 6-beta-hydroxylation activity was higher in proximal than distal catfish intestine (263±80.3 and 88.6±15.6 pmol/min/mg protein for proximal and distal). The 6-beta-hydroxylation activities in the two segments correlated with the amount of CYP3A.

CYP3A amount and steroid-6beta-hydroxylation activities were lower in both segments of intestine from fish fed purified diet compared with commercial chow, but with the same trend along intestine. These results demonstrated that the expression of a CYP3A protein was higher in proximal than distal intestine. Testosterone 6-beta-hydroxylation was a good marker of CYP3A. (Supported by ES 05781 and 07375.)

458 PAH/METAL MIXTURES: EFFECT ON PAH INDUCTION OF HUMAN CYP1A1 IN HEPG2 CELLS.

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Environmental PAHs and metals coexist and such mixtures could affect the carcinogenicity of PAHs. One possible mechanism involves modification of PAH induction of the PAH-bioactivating CYP1A. The effect on PAH-mediated CYP1A induction of As, Pb, Hg, or Cd (ranked as the most hazardous environmental metals by EPA and ATSDR) has thus been investigated. Human, hepatic HepG2 tumor cells were selected as the model because of poor availability of human hepatocytes. Induction of CYP1A1 by 10 μM benzo[a]pyrene (BAP), benzo[b]fluoranthene (BBF), dibenzo[a,h]anthracene (DBAHA), benzo[a]anthracene (BBA), or benzo[k]fluoranthene (BKF) was probed by ethoxyresorufin-O-deethylase activity (EROD) in 96-well plates of HepG2 cells. Cells rapidly took up PAHs from medium; by 24 hr only 14% remained in the medium, and no detectable PAH bound to well walls. Induction efficiency (relative to dimethyl sulfoxide controls) was in the order DBAHA (29-fold) > BKF (19- fold) > BAA (7-fold) > BAP (6-fold) > BBF (1-fold), all at 10μM PAH. The metals did not affect cell viability up to concentrations of As, 5μM; Pb, 250μM; Hg, 5μM; and Cd, 2.5μM. At 1μM, all of the metals decreased levels of PAH-induced CYP1A1 activities (inhibition of EROD activity was excluded) by variable extents and in a PAH-dependent manner. With BAP as inducer decreases in induction were: As, 33%; Cd, 29%; Hg, 19%; and Pb 13%. Future studies will resolve the mechanism of metal-mediated decreases in PAH induction. We conclude that these metals in environmental PAH mixtures could diminish PAH carcinogenic potential by decreasing PAH-mediated induction of their bioactivation by CYP1A1. (Funded by EPA grant R827180010).

459 COMPARISON OF CYTOCHROMES P450 1B1, 1A1, AND 1A2 PROTEIN EXPRESSION IN LIVER AND LUNG MICROSOMES FROM SMOKERS AND NONSMOKERS.

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To investigate the inducibility of CYP1B1 by cigarette smoke, we determined the expression of this protein in lung and liver microsomes from smokers and nonsmokers. For comparison, we also measured CYP1A1 and CYP1A2 protein expression in the same samples. Anti-CYP1B1 antibody, which was raised in rabbits against a synthetic peptide corresponding to amino acids 280-295 of human CYP1B1, was specific for this CYP as assessed by immunoblot analysis with a panel of human recombinant CYP and purified rat CYP proteins. The levels of human CYP1A1 and CYP1A2, which can be resolved by SDS-PAGE as distinct bands, were determined using anti-rat CYP1A2 sera that reacted with both proteins. As expected, CYP1A2 was expressed constitutively in liver microsomes. In the same samples, CYP1A1 and CYP1B1 were also expressed, albeit at lower levels. The mean CYP1A1 and CYP1A2 levels were greater in liver microsomes from smokers, although a large inter-individual variability existed. In contrast, no apparent difference in CYP1B1 protein levels was found in liver microsomes from smokers and nonsmokers. In human lung microsomes, only CYP1A1 was detected reliably and the expression of this CYP appeared to be greater in smokers, but there were large inter-individual differences in CYP1A1 levels. In conclusion, based on our panel of human liver and lung microsomes, smoking does not appear to influence CYP1B1 protein expression, whereas it increases lung CYP1A1 and hepatic CYP1A1 and CYP1A2 levels.

460 SUPERIORITY OF MIDAZOLAM AS A PROBE FOR ASSESSING CYP 3A4 ACTIVITY IN THE HUMAN ENDOTOXIN MODEL.

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Critically ill septic patients receive many xenobiotics that require metabolism by P450s. The extent to which CYP3A4 activity in humans is altered during inflammation may have important clinical relevance. We compared results of midazolam pharmacokinetics (MDZ), erythromycin breath test (ERMBT) and urinary 6β-OH cortisol/cortisol ratio (6βC/C) as probes for CYP 3A4 in a human endotoxin model of sepsis. Ten healthy non-smoking male volunteers were studied twice receiving either two consecutive daily IV doses of LPS (4ng/kg) or saline in a balanced crossover design. ^{14}C-erythromycin (3μCi) and MDZ (20 μg/kg) were given IV after saline or the second dose of LPS. 6β-cortisol & cortisol were assayed in 24hr urines after saline and each dose of LPS. Pharmacokinetics of MDZ showed no statistically significant changes between control and LPS in clearance, volume of distribution, half-life or 30min serum 1-OH-MDZ/MDZ ratio. ERMBT, in contrast, showed a significant 19% decrease in the dose of $^{14}CO_2$ exhaled/h. However, CO_2 production after LPS is known to increase by 14% after LPS. Urinary 6βC/C ratio dropped 66% after the first LPS dose, but total urinary cortisol was markedly increased due to the adrenocortical response to LPS. Although ERMBT and urinary 6βC/C ratio did decrease after LPS, these changes are suspect because of the physiologic responses to inflammation. The lack of change in MDZ suggests CYP3A4 is not decreased in this model and MDZ is a more suitable probe.

461 INDUCTION OF CYP1A1 AND CYP1A2 IN THE HUMAN LUNG.

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We examined the presence and inducibility of CYP1A1 and CYP1A2 in the human lung, using a tissue explant culture system and freshly resected lung specimens obtained from a population of patients undergoing therapeutic lobectomy. CYP1A1 and CYP1A2 were detected in the lung specimens at the level of protein (by western blot analysis), mRNA (by RT-PCR analysis), and activities (as measured by the (i) formation of adducts in pulmonary DNA by added benzo(a)pyrene (B[a]P) and (ii) pulmonary S9-catalyzed bioactivation of B[a]P and 4-aminobiphenyl to mutagens in S. typhimurium TA100 and TA98, respectively). The constitutive levels of CYP1A1 and CYP1A2 exhibited extensive inter-individual variability. The enzymes were induced to varying extents by TCDD, pyridine, and nicotine, with the induction exhibiting extensive inter-individual variability as well. Pyridine was a more effective inducer than TCDD in some subjects, and vice versa, whereas both compounds were effective inducers in other subjects. B[a]P and Omeprazole were also inducers of the enzymes. The results show that CYP1A2 is present and inducible in the human lung, and indicate the heterogeneity in individual sensitivity to pulmonary CYP1A induction, as well as in the compounds capable of effecting the induction. (Supported by ES06414 and ASPET SURF Program.)

462 PROTECTIVE ROLE OF EPOXIDE HYDROLASE AGAINST OLFACTORY TOXICITY OF COUMARIN AND 2,6 DICHLOROBENZONITRILE.

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Coumarin and 2,6-dichlorobenzonitrile (DCBN) cause tissue-selective cytotoxicity and consequent depletion of P450 in the olfactory mucosa (OM). The reactive intermediates of DCBN and coumarin are epoxides, therefore, microsomal epoxide hydrolase (mEH) may play an important role in their detoxification. Since mEH is expressed only in specific zones in the OM while expression of OM P450s are not zonal, we speculated that cells expressing mEH would be selectively protected and mEH levels in OM would not be decreased, or not to the same extent as do the P450s following DCBN or coumarin treatment. We found that one or five weeks after a single ip injection of DCBN in C57BL/6 mice at 15 mg/kg, the levels of several P450s, such as CYP1A, 2A, and 2G1, but not of mEH, were significantly decreased in OM

microsomes. With a DCBN dose of 25 mg/kg, both P450 and mEH protein levels were decreased at 1 week following injection, but the extent of decrease was lower for mEH than for P450s. In Wistar rats, a single ip injection of DCBN at 25 mg/kg caused a modest decrease in the level of CYP2A/2G and an injection of coumarin at 50 mg/kg caused a dramatic depletion of P450 at 1 week following treatment, with no decrease in mEH level. These results support a protective function of mEH in the OM against DCBN and coumarin toxicity and call for further studies on the cell-specific regulation of mEH in rodent and human nasal mucosa. (Supported in part by NIH grant ES07462.)

463 DIFFERENTIAL INDUCTION OF MOUSE CYP2A5 AND CYP2J BY PYRAZOLE.

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We have recently shown that rat CYP2J4 is inducible by pyrazole in liver, intestine, and olfactory mucosa (Zhang et al., Drug Metab. Dispos., 1999, in press). The aim of the present study was to determine whether mouse CYP2J isoforms are also inducible by pyrazole, which was previously found to induce CYP2A5 in mouse liver but not the olfactory mucosa (Su et al., Drug Metab. Dispos. 26:822-824, 1998). CYP2J proteins were detected in mouse liver, lung, kidney, heart, eye, olfactory mucosa, and small intestine by immunoblot analysis with an anti-CYP2J4 antibody. Induction of CYP2J proteins was observed in liver, lung, kidney, olfactory mucosa, and small intestine following daily i.p. injection of pyrazole at 120 or 200 mg/kg for three consecutive days. CYP2J proteins were induced similarly in C57BL/6 and DBA-2 mice. In the same experiments, CYP2A5 was detected in the small intestine in addition to the liver and olfactory mucosa; however, treatment with pyrazole induced CYP2A5 in the liver, but not in the olfactory mucosa or the small intestine. Induction of CYP2J mRNAs was also observed by Northern blot analysis with a CYP2J4 cDNA probe. RNA-PCR analysis with isoform-specific primers showed that CYP2J5 was expressed in kidney and liver, but not in the other tissues examined, while CYP2J6 was detected in all tissues examined. These results indicate that CYP2Js are inducible by pyrazole in multiple mouse tissues at both protein and mRNA levels. However, the different tissue selectivities in CYP2A5 and CYP2J induction by pyrazole suggest involvement of different regulatory mechanisms. (Supported in part by NIH grants ES06256 and ES07462.)

464 IDENTIFICATION OF THE HEPATIC CYTOCHROMES P450 THAT CATALYZE COUMARIN 3,4-EPOXIDATION AND 3-HYDROXYLATION.

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Coumarin is a rat liver and mouse lung toxicant. Coumarin-mediated injury is metabolism- and species-dependent, resulting from the formation of coumarin 3,4-epoxide (CE) and its rearrangement product, o-hydroxyphenylacetaldehyde (o-HPA). o-HPA formation is greatest in mouse >> rat > man. 3-Hydroxycoumarin (3-HC) is a non-toxic product formed via a metabolic pathway distinct from that of CE. The goals of the current study were to identify the P450s involved in the formation of CE and 3-HC, and determine whether CE formation differs qualitatively in hepatic microsomes from mice, rats and man. Of a panel of 10 recombinant human P450 forms, 1A1, 1A2, and 2E1 catalyzed o-HPA formation. Rat recombinant 1A1 and 1A2 likewise formed o-HPA, but 1A2 produced o-HPA at a rate 11-times faster than the human form. In contrast, 3-HC formation was formed predominately by recombinant 3A4. Antibodies inhibitory to P450s 1A1/2 and 2E1 together blocked up to 87% of o-HPA activity in pooled F344 rat and individual human liver microsomes (n=3). In contrast, o-HPA formation in mouse liver was inhibited by only 42%, a result that suggests that additional enzymes may play an important role in mouse o-HPA production. Inhibition studies using naphthalene and 5-phenyl-1-pentyne suggest that this form may be P450 2F2. Thus, species differences in rat and human o-HPA production may result largely from differences in enzyme affinity, whereas high rates of CE formation in mouse liver may be attributed to a species-specific expression of additional high-capacity enzymes.

465 INHIBITION OF CYTOCHROME P450 6D1 BY ALKYNYLARENES, METHYLENE DIOXYARENES AND OTHER SUBSTITUTED AROMATICS.

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We evaluated 33 compounds, comprising 5 different structural groups (alkynylpyrenes, alkynylphenanthrenes, methylenedioxyarenes, flavones and miscellaneous), as inhibitors of house fly P450 6D1 (CYP6D1). In general, alkynylpyrenes were the most potent group of inhibitors, with maximum effectiveness noted when the substituent was in the 4 position. Substituted phenanthrenes were reasonable CYP6D1 inhibitors with the lowest IC50 observed for the analogue with the methylenedioxy substituent between the 9 and 10 positions. The ten methylenedioxyarenes varied considerably in their ability to inhibit CYP6D1. Piperonyl butoxide was the most potent inhibitor with increasing length of the alkyl substituent resulting in decreasing inhibition. Karinjin had the lowest IC50 of the four flavones that were tested. Overall, the most potent CYP6D1 inhibitors were large planar compounds. Four inhibitors were evaluated as permethrin synergists in the LPR strain of house fly (permethrin is detoxified via CYP6D1 in this strain) and they increased permethrin toxicity by 16- to 83-fold. The effect of structure on inhibition potency, as well as the mechanism of inhibition for seven representative inhibitors is discussed.

466 INSULIN DESTABILIZES CYP2E1, BUT NOT CYP2B OR CYP3A, MRNA IN PRIMARY CULTURED RAT HEPATOCYTES.

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The expression of CYP2E1 mRNA and protein is enhanced several-fold by experimental diabetes. CYP2B and CYP3A expression is also enhanced slightly under these conditions. We have previously demonstrated that insulin decreases CYP2E1 mRNA and protein levels in primary cultured rat hepatocytes, while having little effect on CYP2B or 3A expression. To examine whether insulin affects the stability of CYP2E1 mRNA, RNA degradation experiments were performed in primary cultured rat hepatocytes. Hepatocytes were cultured on Vitrogen in modified Chee's medium in the absence of insulin for the first 48 h of culture. Cells were then cultured in the presence or absence of 10 nM insulin for 2 h, followed by addition of 10 μg/ml Actinomycin D. Cells were harvested at 10, 13, 16, and 22 h after Actinomycin D addition, and CYP2E1, 2B, and 3A mRNA levels were monitored by Northern blot. The half-life of CYP2E1 mRNA was decreased from ~21 h in the absence of insulin to ~13 h in the presence of 10 nM insulin. In contrast, the half-life of CYP3A mRNA (~15 h) was unaffected by insulin treatment, while that of CYP2B mRNA appeared to be increased, from ~15 h to ~30 h, in the presence of insulin. In order to determine a possible mechanism for the destabilization of CYP2E1 mRNA by insulin, gel shift analysis for binding of cytosolic protein(s) to CYP2E1 mRNA was performed. A construct representing the 3'-portion of CYP2E1 mRNA (including the 3'UTR) formed two complexes following incubation with cytosolic extracts from hepatocytes cultured in the absence of insulin, or in the presence of 0.1-100 nM insulin for 24 h. The intensity of the CYP2E1 RNA-protein complexes decreased as insulin concentration increased. These data suggest that insulin decreases CYP2E1 mRNA levels in primary cultured rat hepatocytes by enhancing its degradation, possibly through reduced interaction with protective RNA binding protein(s). (Supported by NIH Grant ES03656 and NIEHS Center Grant P30 ES06639.)

467 CHARACTERIZATION OF THE RAT CYP1B1 GENE, INTERACTION BETWEEN CAMP AND AHR-RESPONSIVE SEQUENCES.

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CYP1B1 is appreciably expressed constitutively in multipotential mouse embryo fibroblasts and in steroidogenic cells, but is poorly expressed in hepatocytes even with TCDD induction. TCDD induces expression preferentially in mouse embryo fibroblasts while cAMP is more effective in steroidogenic cells. To understand the molecular mechanisms underlying the cAMP or TCDD regulated expressions, a 16kb rat genomic DNA was isolated and characterized, which contains DNA corresponding to the 7kb of 5'-flanking

sequence and the 9kb of three exons, two introns and 3'-flanking sequences. DNA sequence analysis revealed that rat, mouse and human CYP1B1 genes share similar gene structural organization and function motifs in the proximal promoter(PP) and a TCDD-responsive enhancer centered 1kb from the transcription start site. We used rat CYP1B1-luciferase reporter constructs (7kb of 5'-flanking and deletions) in mouse embryo fibroblast C3H10T1/2 cells, testicular Leydig MA10 cells and adrenal Y-1 cells. Luciferase activity responded in a cell selective manner in C3H10T1/2 (TCDD>>cAMP), MA10 (cAMP>>TCDD) and Y-1 (only cAMP). 1.2kb of 5'-flanking sequence allowed induction by cAMP only in MA10 cells but this was completely removed by a single mutation in the key XRE of the TCDD-responsive enhancer. This supports the involvement of this motif in the basal activity and cAMP-induction as well as TCDD-induction. We have also identified a sequence in the PP that is essential to all transcription. Deletion of a further PP sequence removed cAMP-induction but not TCDD-induction. The sequence only confers cAMP-inducibility in the presence of the upstream enhancer and apparently like basal transcription requires basal AhR-Arnt activity. cAMP inductions in both C3H10T1/2 and Y-1 cell lines are totally dependent on the second enhancer located within the region -5428/-5206. cAMP induction in MA10 cells also was potentiated two folds by inclusion of this region indicating regulation through both mechanisms. Several potential binding sites for transcription factors including STAT, CREB and RORA were identified in this region, which may be responsible for the cAMP regulation. These data demonstrate that multiple cAMP response sequences exist in CYP1B1 gene, which selectively function through AhR-dependent or independent pathways in different cells.

468 *IN VIVO* IH636 GRAPE SEED PROANTHOCYANIDIN EXTRACT (GSPE) EXPOSURE INHIBITS MOUSE LIVER MICROSOMAL CYP4502E1-DEPENDENT ANILINE HYDROXYLATION *IN VITRO*.

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Previous studies in our laboratories have demonstrated the protective abilities of a novel IH636 GSPE against oxygen free radicals, tobacco-induced programmed cell death in human oral keratinocytes, myocardial ischemia-reperfusion injury and infarction, and drug and chemically-induced multiorgan toxicity. This study compared the *in vitro* aniline hydroxylation patterns of *in vivo* GSPE pre-exposed hepatic microsomes with induced (1% acetone in drinking water for 3 days) and uninduced rat liver microsomes. Male B6C3F1 mice were fed GSPE containing diet (ADI 100 mg/kg body wt.) for 4 weeks. Liver microsomes were isolated from both control and GSPE-exposed mice, and aniline hydroxylation was assessed as a specific marker of CYP450-2E1 activity. Interestingly, GSPE supplementation for 4 weeks inhibited aniline hydroxylation by 40%, as compared to the control animals. Similar to *in vivo* GSPE preexposed mouse liver microsomes, rat liver microsomes when incubated with various concentrations of GSPE (100 and 250 μg/ml), inhibited aniline hydroxylation to various degrees (Uninduced: 40% and 60% and Induced: 25% and 50%, respectively). These data were compared with hydroxylation pattern of another hepatoprotective agent, such as 4-aminobenzamide (4-AB), which shows that 4-AB did not alter aniline hydroxylation at all. Collectively, these results may suggest that the ability of GSPE to inhibit CYP4502E1 is an additional cytoprotective attribute, in conjunction with its novel antioxidant efficacy.

469 INDUCTION OF CYTOCHROME P4501A1 IN HUMAN HEPATOMA HEPG2 CELLS BY 6-NITROCHRYSENE.

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The effects of 6-nitrochrysene (6-NC) on human cytochrome P450 (P450)-dependent monooxygenases were determined using human hepatoma HepG2 cells treated with the nitrated polycyclic aromatic hydrocarbon. Treatment with 6-NC caused concentration- and time-dependent increases of monooxygenase activity in HepG2 cells. Treatment with 100 μM 6-NC for 24 hr markedly increased benzo(a)pyrene hydroxylation, 7-ethoxycoumarin and 7-ethoxyresorufin O-deethylations activities in cell microsomes. The S9 fraction derived from 6-NC-treated HepG2 cells was more effective than the fraction from untreated cells in metabolic activation of 6-NC in the Salmonella typhimurium mutagenicity assay. Immunoblot analysis of microsomal proteins using mouse monoclonal antibody 1-12-3 to rat P450 1A1 revealed that 6-NC induced a P450 1A1-immunorelated protein in the hepatoma cells. RNA blot analysis of cellular total RNA using a human P450 1A1 3'-end cDNA probe showed that 6-NC increased the level of P450 1A1 mRNA. Nuclear transcription assay using the human P450 1A1 cDNA probe demonstrated that 6-NC increased the transcription rate of CYP1A1 gene in HepG2 cells. Similar to 6-NC, chrysene and 6-aminochrysene treatments increased P450 1A1 protein and mRNA in the hepatoma cells. Treatment of HepG2 cells with 6-NC increased cytosolic glutathione S-transferase and N-acetyltransferase activities. Treatment of lung carcinoma NCI-H322 cells with 6-NC resulted in induction of P450 1A1, similar to the inductive effects observed with the hepatoma cells. The present study demonstrates that 6-NC has the ability to induce catalytic activity, protein, and mRNA of human P450 1A1 in HepG2 cells and the induction mechanism involves a transcriptional event. Induction of the liver and lung P450 1A1 may be an important factor to consider in the assessment of 6-NC metabolism and toxicity in humans. (Supported by grant NSC88-2314-B002-047.)

470 TRANSIENT INDUCTION OF CYP1A1/CYP1B1- CATALYZED ESTROGEN METABOLISM IN T-47D CELLS BY BENZO[A]PYRENE IS DIMINISHED BY ARSINITE.

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Polycyclic aromatic hydrocarbons (PAH) and metals are often co-contaminants in the environment, yet there have been relatively few studies of the combined effects of PAHs and metals on cytochrome P450 induction. In this study we examined the combined effects of metals and benzo[a]pyrene (BAP) on estrogen metabolism in T-47D human breast cancer cells. Exposure to BAP caused elevated rates of both the 2- and 4-hydroxylation pathways of estrogen metabolism, indicating induction of both CYP1A1 and CYP1B1. Induction of both pathways was transient, with metabolite formation peaking at 12 h after exposure and returning to near basal levels at 48 h. Dose-response studies showed maximal induction of both the 2- and 4- hydroxylation pathways at 3 mM BAP; higher levels of the PAH caused reduced rates of metabolism due to inhibition of CYP1A1 and CYP1B1. Experiments in which T-47D cultures were co-treated with 3 mM BAP and varying concentrations of Pb, Cd, Hg or As showed varying degrees of decreases in the induction of CYP1A1 and CYP1B1 by the metals. Arsenic in the form of arsinite caused the most pronounced decrease in the induction of CYP1A1 and CYP1B1 by BAP, with IC50s of approximately 3 mM for the 2- and 4-hydroxylation pathways. Hg and Pb were much less effective, causing only 50% decreases in induced metabolism at 30 and 300 mM, respectively. These effects could not be attributed to cytotoxicity as determined in cell viability assays. These results indicate a specific inhibitory effect of arsinite on the expression of CYP1A1 and CYP1B1 in T-47D human breast cancer cells. (Funded by EPA Grant R827180010 and NIH Grant ES03819.)

471 REAL-TIME FLUOROGENIC 5' NUCLEASE BASED ASSAYS FOR THE QUANTITATION OF RAT CYTOCHROME P450 MRNA.

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Accurate and sensitive quantitation of mRNA expression profiles is becoming increasingly important in molecular toxicological studies. To address these issues, we developed real-time PCR 5' nuclease quantitation assays for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1, CYP3A1, CYP3A2, and albumin mRNA using the ABI Prism 7700 system and dual-labeled fluorogenic sequence specific probes. This real-time PCR technique eliminates the reliance on endpoint measurements and also the need for a competitor to be co-amplified along with the target. Rat CYP450 mRNA was reverse transcribed from 1μg total RNA which yielded enough cDNA for the quantitation of more than 15 different mRNA targets. Measurement of these CYP450 mRNAs showed highly reproducible values for triplicate reads (Coeff. of variation <0.05) and very precise standard curve values (R2 >0.995). Additionally, results were verified by comparison to previous data from both competitive RT-PCR (using a precisely constructed co-amplified competitor RNA molecule) and RNA slot blot analyses. This non-radioactive, non-intercalator based PCR product detection method is a substantial improvement over the previously established RNA analysis techniques and it allows for faster, more sensitive, and more accurate quantitation of very small amounts of mRNA. (Supported by NIEHS Center ES-07033 and ES-04696.)

472 DEVELOPMENT OF FLUORESCENT 5'-NUCLEASE ASSAYS FOR THE ALLELIC DISCRIMINATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN BIOTRANSFORMATION ENZYME GENES.

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Detection of single nucleotide polymorphisms (SNPs) provides the basis for most current molecular epidemiology studies. As studies have become more complex, however, cost-efficient, high-throughput methods for genotyping these polymorphisms have become a necessity. We have developed fluorescent 5'-nuclease assays to genotype specific SNPs in several biotransformation enzyme systems including cytochrome P450 (CYP) 1A1, CYP 2D6, CYP 2E1, catechol-o-methyltransferase, microsomal epoxide hydrolase, methyltetrahydrofolate reductase, N-acetyl transferase, and superoxide dismutase as well as those present in transforming growth factors, tumor necrosis factors and vitamin D receptors. The assay takes place in a single PCR reaction and utilizes the 5'-nuclease activity of Taq DNA polymerase to cleave allele-specific probes. These probes are labeled with two fluorescent dyes - a reporter and a quencher - and are added to the standard PCR assay. When both dyes are attached to the probe, the fluorescence of the reporter is quenched. During each extension cycle, however, the polymerase cleaves the reporter dye from the probe, thus separating it from the quencher, and the fluorescence is emitted. An ABI Prism® 7700 was used to collect and analyze the data. This procedure, combining the simplicity of PCR with the discrimination of allele-specific fluorescence, provides accurate results in an efficient, cost-effective format. (Supported by NIEHS Center ES-07033 and Superfund ES-04696.)

473 SEX DIFFERENCES IN HEPATIC CYP4A INDUCTION AND BIPHENYL METABOLISM IN BIPHENYL- ADMINISTERED BDF1 MICE.

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In order to examine sex differences in the effects of biphenyl (BiP), we investigated effects of BiP on hepatic beta-oxidase activity, drug-metabolizing enzyme activities and BiP metabolism using in female and male BDF1 mice. BiP was orally administered at the dose of 1.3, 2.6 and 5.2 mmol/kg at 72, 48 and 12 hrs before sacrifice. In female mice, BiP significantly increased hepatic KCN-insensitive palmitoyl CoA (PCoA) oxidation (2.6 and 5.2 mmol/kg; 1.5-1.9 fold) and lauric acid (LA) 12-hydroxylation (1.3-5.2 mmol/kg; 2.1-3.8 fold). Furthermore, BiP markedly enhanced Cyp4a apoproteins in a dose-dependent manner. In male mice, BiP showed no significant increase of KCN-insensitive PCoA oxidation and LA 12-hydroxylation. In both the sexes, 7-pentoxyresorufin O-depentylation and level of Cyp2b apoprotein were elevated by BiP at the dose of 5.2 mmol/kg. On the other hand, the administration of BiP (5.2 mmol/kg) caused the increase of 4-hydroxybiphenyl, 4,4'- and 2,5-dihydroxybiphenyl production from BiP in the liver microsomes of female and male BDF1 mice. The increase in the rate of 4,4'-dihydroxybiphenyl production in females was 2-fold higher than that in males. These effects of the administration of BiP on BiP metabolism were qualitatively similar to that of phenobarbital but not clofibrate. These results indicated the possibility of sex differences in the toxicity of BiP.

474 MODULATION OF DRUG METABOLIZING ENZYMES FOLLOWING ADMINISTRATION OF BILIRUBIN IN RAT.

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Induction of stress proteins in liver, such as heme oxygenase 1 (HO-1), as a result of pathobiological stress, leads to the conversion of heme to its endogenous metabolite, bilirubin. Modulation of the cytochrome P450 (CYP) monooxygenase system (P450) by bilirubin was investigated using microsomes prepared from liver of male, adult Sprague-Dawley rats treated intraperitoneally (i.p.) with a single daily dose (0, 1, 2.5, 10, or 100 mg/kg) of bilirubin for 3 consecutive days. Animals were sacrificed 24 h after receiving the last dose of bilirubin. Hepatic total CYP content in microsomes decreased maximally to 50% of control values. P450-dependent 7-ethoxyresorufin O-deethylation (EROD) activities and 7-pentoxyresorufin O-deethylation (PROD) (CYP1A1/2 and 2B1/2, respectively) were minimally altered by bilirubin, decreasing to 70 and 80% of control values, respectively. Hepatic testosterone 2a-hydroxylase (T2aH), 6b-hydroxylase (T6bH) and 7a-hydroxylase (T7aH) activities (CYP2C11, 3A1/2 and 2A1 - dependent activities, respectively) were affected in a dose dependent manner. T2aH and T7aH activities were maximally reduced to 25 and 20% of control values and T6bH activity to 60%. A dose dependent decrease in total plasma bilirubin levels (~20 fold) occurred. Hepatic HO activity significantly increased to 240% of control values, which corresponded with maximum decreases of CYP content and P450 catalytic activities. Microsomal NAD(P)H:quinone oxidoreductase activity increased 210% above control levels, in the liver. Hepatic total glutatione S-transferase activity did not change but GST-Ya-dependent activity decreased 55% below control levels. No differences were observed in malondialdehdye (MDA) formation in either liver microsomes or serum, indicating little or no lipid peroxidation had occurred. The present findings demonstrate that bilirubin directly or indirectly is involved in the modulation of drug metabolizing enzymes in an isozyme selective manner.

475 CYTOCHROME P450 INHIBITION ASSAYS — THE CHALLENGES IN INTERPRETING DATA FOR CYP3A4.

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We have developed microtiter plate-based, direct, fluorometric assays for the activities of the five principal human drug metabolizing enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. For individual CYP1, and CYP2 enzymes, the potency of enzyme inhibition (IC50) is generally maintained regardless of probe substrate or assay method employed. In contrast, CYP3A4 inhibition for a specific inhibitor shows significant differences in potency depending on the probe substrate being used. We have investigated these differences through the use of several structurally distinct fluorescent substrates for CYP3A4 and several classical substrate probes (for example, testosterone, nifedipine, and midazolam), with a panel of 20 chemicals about half were clinically significant CYP3A4 inhibitors. Under highly controlled test conditions the concordance among all 4 fluorometric probe substrates was only 70%. Several chemicals were found to inhibit the metabolism of some substrates while activating the metabolism of others. The mean range of nominal IC50 values was 27-fold [the range varied from 4.5-fold (ethynylestradiol) to 122-fold (cyclosporin A). Some of the differences can be rationalized with a more detailed interpretation of the inhibition curves. A prudent approach appears to be the use of multiple probe substrates to characterize the inhibition potential of xenobiotics for CYP3A4. However, even with this approach, there will always be uncertainty in predicting CYP3A4 inhibition.

476 SQUALESTATIN 1-INDUCIBLE CYP2B1 TRANSCRIPTION IS NOT MEDIATED THROUGH STEROL RESPONSIVE ELEMENT BINDING PROTEIN (SREBP).

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We previously demonstrated that squalestatin 1, an inhibitor of squalene synthase and sterol biosynthesis, is a potent and selective inducer of CYP2B in rat liver and primary cultured rat hepatocytes. Because cellular sterol depletion is known to regulate gene expression through the proteolytic activation of the SREBP class of transcription factors, we examined the abilities of transiently transfected SREBP-1a and SREBP-2 transcription factors to activate reporter gene expression from a 3-hydroxy-3-methylglutaryl coenzyme A synthase-luciferase construct (SYNSRE-Luc) or from a CYP2B1 5'-luciferase construct (CYP2B1-Luc), containing the phenobarbital-responsive unit, in primary cultured rat hepatocytes. Treatment of hepatocyte cultures with 10^{-7} M squalestatin 1 activated luciferase expression from SYNSRE-Luc and CYP2B1-Luc by ~4.5-fold and 7.3-fold, respectively, relative to untreated control cultures. As expected, co-transfection of hepatocytes with 1 or 2 ng of a plasmid expressing either the active SREBP-1a or SREBP-2 transcription factor increased luciferase expression from SYNSRE-Luc by 25- to 42-fold. However, co-transfection of hepatocytes with either of the SREBP expression plasmids had no effect on CYP2B1-Luc expression. These findings demonstrate that squalestatin 1-inducible CYP2B expression is not attributable to SREBP activation. We propose that squalestatin 1 inhibits the synthesis of an endogenous sterol that represses CYP2B1 transcription through an SREBP-independent mechanism. (Supported by NIH grant HL50710 and NIEHS Center grant ES06639.)

477 ESTRONE (E1) HYDROXYLATION CATALYZED BY HUMAN CYTOCHROMES P450 1A1 (CYP1A1) AND 1B1 (CYP1B1).

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Human CYP1A1 and CYP1B1 are expressed or are inducible in a number of extrahepatic, estrogen-responsive tissues including breast and uterus. Both enzymes catalyze the hydroxylation of 17beta-estradiol (E2); CYP1A1 is an E2 2-, 6alpha, and 15alpha-hydroxylase, whereas CYP1B1 is predominantly an E2 4-hydroxylase with a lesser activity at C-2. Metabolism of E1 by CYP1A1 and CYP1B1 is not well characterized, in part because of the difficulty in measuring E1 hydroxylase activity at low substrate concentrations. In this study we used gas chromatography/mass spectrometry (GC/MS) for the analysis of hydroxy-E1 metabolites produced in microsomal reactions with cDNA-expressed enzymes. Metabolites were recovered from microsomal reactions by solid-phase extraction and subjected to a conventional two-step derivatization procedure involving conversion of the 17-keto functions to methyl oximes and conversion of the hydroxyl groups to trimethylsilyl ethers. Analysis of these derivatives by GC/MS provided pmol sensitivity, appropriate for kinetic analyses of microsomal E1 metabolism. By using the GC/MS technique, human CYP1A1 expressed in Sf9 cells was found to catalyze 2- and 4-hydroxylation of E1 with Km values of 11.5 and 32.4 micromolar and Vmax values of 4.6 and 0.71 nmol/min per nmol P450, respectively. Human CYP1B1 catalyzed 2- and 4-hydroxylation with Km values of 6.8 and 4.0 micromolar and Vmax values of 0.30 and 0.53 nmol/min per nmol P450, respectively. Neither enzyme catalyzed detectable 16alpha-hydroxylation of E1. These results indicate that CYP1A1 and CYP1B1 catalyze E1 hydroxylation with significantly lower catalytic efficiencies with E1 as compared to E2 as substrate. (Supported by NIEHS Grant ES04913.)

478 RELATIVE ROLES OF CYP2E1 AND CYP1A2 IN MOUSE UROPORPHYRIA CAUSED BY ACETONE: IS THIS A MODEL OF PORPHYRIA CUTANEA TARDA.

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Porphyria cutanea tarda is a liver disease with skin lesions caused by hepatic overproduction of uroporphyrin (URO), and is associated with consumption of alcoholic beverages and moderate iron overload. Previously, we reported that acetone (AC), which, like ethanol, is an inducer of CYP2E1, stimulates the onset of hepatic URO accumulation in mice treated with iron dextran (Fe) and 5-aminolevulinic acid (ALA), the porphyrin precursor. Here we investigated whether CYP2E1 has an essential role in the AC stimulation using *Cyp2e1(-/-)* mice. Hepatic URO accumulation was stimulated by AC in *Cyp2e1(-/-)* mice to the same extent as in wild-type mice. In the absence of AC, there was less URO in the mutant mice treated with Fe and ALA than in the wild-type mice. URO accumulation caused by AC, Fe and ALA was completely prevented in *Cyp1a2(-/-)* mice. AC was not an inducer of CYP1A2 in the wild-type mice, as indicated by western blots of liver homogenates and microsomal methoxyresorufin demethylase activities. Increases in uroporphyrinogen oxidation activity were observed in hepatic microsomes from wild-type C57BL/6 mice treated with AC. We conclude that CYP2E1 is not required in AC stimulation of uroporphyria but CYP1A2 is required. CYP2E1 did contribute to uroporphyria in the absence of AC.

479 ALCOHOL CAUSES HEPATIC STEATOSIS AND ENHANCES ACETAMINOPHEN HEPATOTOXICITY IN *Cyp2e (-/-)* MICE.

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Mice genetically engineered to delete expression of a particular CYP are being used to identify the role of that CYP in a toxicological response. Untreated *Cyp2e1(-/-)* mice were reported to be more resistant to APAP hepatotoxicity than wild type mice. Here we found that treatment with ethanol in combination with isopentanol increased steatosis and acetaminophen (APAP) hepatotoxicity in *Cyp2e(-/-)* and wild type mice. Steatosis from alcohol alone was similar in both strains. With APAP, maximal toxicity was observed at 300 mg APAP/kg in wild type mice and 400 mg/kg in *Cyp2e(-/-)* mice. Alcohol increased CYP2E and CYP3A in wild type mice and CYP3A in *Cyp2e(-/-)* mice. Although these findings suggest that CYP2E contributes to alcohol-mediated increases in APAP hepatotoxicity in wild type mice, the alcohol treatment caused greater apparent increases in CYP3A in these mice compared to the null strain. CYP3A and APAP activation activity were higher in female mice compared to male mice, yet the alcohol pretreatment was required to enhance APAP hepatotoxicity in both genders. The findings suggest that the alcohols have additional effects on the liver, other than increasing CYP3A, that contribute to the increase in APAP hepatotoxicity. The findings also indicate that CYP2E1 is not essential for alcohol-mediated increases in steatosis and APAP hepatotoxicity. (Supported by the Dept of Veterans Affairs.)

480 STUDIES WITH TRANSGENIC MICE ON THE ROLE OF CYP2E1 IN 1,1-DICHLOROETHYLENE- AND 1,2 DICHLOROETHANE-INDUCED TOXICITY IN MICE.

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1,1-Dichloroethylene (1,1-DCE) and 1,2-dichloroethane (1,2-DCE) are of concern because of their demonstrated toxicity and carcinogenicity and their extensive use in the chemical industry. We investigated the role of CYP2E1 in 1,1-DCE- and 1,2-DCE-induced toxicity in CYP2E1-null and wild type mice. Male and female mice were given 1,1-DCE and 1,2-DCE (intragastrically, in corn oil) at 4-5 PM and then fasted overnight before sacrificing for analysis of toxicity. Knockout of CYP2E1 significantly prevented the 1,1-DCE (75 mg/kg)- and 1,2-DCE (400 mg/kg)-induced liver and kidney toxicity as reflected in the blood levels of GPT and urea nitrogen, respectively. No CYP2E1-null mice died, whereas 80% to 100% of wild type mice died after receiving 1,1-DCE at a dosage of 200 mg/kg or 1,2-DCE at a dosage of 600 mg/kg. 1,2-DCE-induced liver and kidney toxicity was completely prevented by knockout of CYP2E1 as indicated by biochemical and pathological analyses, suggesting that CYP2E1 is the enzyme responsible for 1,2-DCE-induced acute toxicity in mice. Nevertheless, liver and kidney toxicity was found in CYP2E1-null mice treated with 1,1-DCE, indicating that pathways other than CYP2E1 also contribute to 1,1-DCE-induced liver and kidney toxicity. The 1,1-DCE-induced toxicity was more severe in male mice than in female mice, possibly due to the higher CYP2E1 activity in male mouse kidney and the contribution of other male-specific P450 isozymes. (Supported by NIH grant ES03938 and facilities from the NIEHS Center Grant ES05022.)

481 PROTECTION AGAINST TCDD-INDUCED TOXICITY AND PORPHYRIA IN C57BL/6 CYP1A2 (-/-) KNOCKOUT MICE.

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We have previously shown that porphyria, characterised by hepatic accumulation of uroporphyrin, does not occur in C57BL/6 Cyp1a2 (-/-) knockout mice whereas it does occur in Cyp1a2 (+/+) wild type by combinations of iron, 5-aminolevulinic acid and the inducers of CYP1A2 3-methylcholanthrene and hexachlorobenzene. Here we have investigated the effects of TCDD the prototype AH receptor ligand and the most powerful CYP1A2 inducer and porphyrogenic agent, on both hepatic toxicity and porphyria. Mice received TCDD as an oral dose (75µg/kg) and were terminated after 5 weeks. Massive porphyria occurred in the wildtype but not in the -/- mice. Plasma ALT and AST levels were elevated 27- and 12- fold, respectively, in +/+ mice but elevations were <25% of that in the null mice. Histology of the liver confirmed considerably less toxicity (hepatocyte necrosis and biliary proliferation) in the -/- mice but there were no differences between genotypes in the degree of thymic atrophy caused by TCDD. CYP1A activities (EROD and MROD) and Western blotting confirmed absence of CYP1A2 induction but no depression of CYP1A1 expression. These results demonstrate that hepatic CYP1A2 expression has a profound influence on both porphyria and hepatic toxicity induced by TCDD supporting the proposal that the mechanisms are linked, probably by an oxidative mechanism.

482 EFFECTS OF HYPEROXIA ON LUNG INJURY AND CYTOCHROME P4501A1 EXPRESSION IN WILD TYPE AND AH RECEPTOR (AHR) KNOCKOUT MICE.

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We reported earlier that exposure of rats to 48 h of hyperoxia induces the activities and contents of hepatic CYP1A1 and 1A2, followed by a decline of induction at 60 h. In the present investigation, we tested the hypotheses that: (i) AHR (-/-) mice would be more susceptible to hyperoxic lung injury than wild type (C57BL/6J) mice; and (ii) hyperoxia would modulate CYP1A1 expression in lung by AHR-dependent mechanisms. AHR (+/+) and AHR (-/-) mice were maintained in room air (RA) or exposed to greater than 95% oxygen (hyperoxia) for 48 h, and lung weight/body weight ratios, pulmonary microsomal activities of ethoxyresorufin O-deethylase (EROD) (CYP1A1), and CYP1A1 apoprotein contents were determined. AHR (-/-) mice exposed to hyperoxia were more susceptible to hyperoxic lung injury, as indicated by significantly higher lung weight/body ratios than AHR (+/+) mice. Hyperoxia caused a 52% increase in pulmonary EROD activities in AHR (+/+) mice, and this was paralleled by enhancement in CYP1A1 apoprotein contents. However, hyperoxia altered neither EROD activities nor CYP1A1 apoprotein contents in AHR (-/-) mice, suggesting that AHR-dependent mechanisms contributed to CYP1A1 induction hy hyperoxia. In conclusion, our results showing increased susceptibility to hyperoxic lung injury of AHR (-/-) mice, which did not express lung CYP1A1, are consistent with the hypothesis that AHR-mediated pulmonary CYP1A1 induction by hyperoxia in the AHR (+/+) mice may play a protective role in hyperoxic lung injury. (Supported in part by NIH grant ES09132 and by ALA of Texas.)

483 CYTOCHROME P4501B1 MEDIATES INDUCTION OF BONE MARROW CYTOTOXICITY AND PRE-LEUKEMIA CELLS IN MICE TREATED WITH 7,12- DIMETHYLBENZ[A] ANTHRACENE.

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Humans are exposed to polycyclic aromatic hydrocarbons (PAH) through many environmental pollutants, especially the inhalation of cigarette smoke. These chemicals cause a variety of tumors and immunotoxic effects, as a consequence of bioactivation by P450 cytochromes to dihydrodiol epoxides. The recently identified cytochrome P4501B1 (CYP1B1) bioactivates PAHs, while CYP1B1 deficiency is linked to congenital human glaucoma. This investigation demonstrates that CYP1B1 null mice are almost completely protected from the acute bone marrow cytotoxic and preleukemic effects of the prototypic PAH 7,12-dimethylbenz[a]anthracene (DMBA). CYP1B1 null mice lacked the appreciable amounts of bone marrow DMBA-DNA adducts present in wild type mice, despite comparable hepatic levels of the predominant PAH-metabolizing cytochrome P450 CYP1A1. Wild type mice constitutively expressed CYP1B1 in bone marrow stromal cells, suggesting that local metabolism of PAHs initiates their cytotoxic and carcinogenic effects. Preliminary evidence suggests that benzo[a]pyrene, which exhibits greater binding to the AhR and induces a greater induction of hepatic CYP1A1 than DMBA, causes less bone marrow cytotoxicity than DMBA. This study substantiates the importance of local bioactivation of PAHs by CYP1B1 for bone marrow cytotoxicity. These findings, together with other recent reports, suggest that constitutive CYP1B1 expression may contribute to cancer susceptibility in specific tissues.

484 A THEORETICAL THERMODYNAMIC METHOD FOR IDENTIFYING POTENTIAL RATE-LIMITING STEPS IN CHEMICAL OXIDATIONS BY THE LIVER CYTOCHROME P4502E1.

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The rate-limiting steps determining the maximal velocity (V_{max}) of an enzymatic oxidation of low molecular weight volatile organic chemicals (LMVOCs) by the cytochrome (cyt) P-450, are hypothesized from the literature to be the formation of the monoatomic oxygen before the insertion into the substrate, and/or the insertion of the monoatomic oxygen into the substrate. Furthermore, for several structurally unrelated LMVOCs, for which it is assumed that their hepatic metabolism is predominantly governed by an oxidation involving the cyt P-450 2E1, similar values of V_{max} *in vivo* (in mole hr^{-1}) have been obtained in rats. This may be a consequence of rate-limiting steps determining chemical oxidations in rat livers under *in vivo* conditions. The objective of the present study was to develop a theoretical thermodynamic method for identifying potential rate limiting steps in LMVOC oxidations by the rat liver cyt P-450 2E1. The method consists of using a conventional thermodynamic equation of the transition state theory, to calculate the catalytic rate constant (K_{cat}) for the *in vitro* metabolism of LMVOCs in rat liver microsomes. This above theoretical exercise demonstrates that K_{cat} *in vitro* for LMVOCs are similar and well calculated, then, a rate-limiting step probably independent of the molecular structure could govern K_{cat} *in vitro* for chemical oxidations. The calculated K_{cat} *in vitro* was extrapolated to *in vivo* and used to predict a common value of V_{max} *in vivo* (= K_{cat} * [cyt P-450 $2E1_{liver}$] for twenty-fourth structurally unrelated LMVOCs. The experimentally determined V_{max} *in vivo* (avg.: $2.0x10^{-5}$ mole hr^{-1}, s.d. = $6.9x10^{-6}$, n = 24 LMVOCs) obtained from several experimental studies published in the literature, is in the range predicted for the common V_{max} *in vivo* (i.e., $1.5x10^{-5}$ to $9.5x10^{-5}$ mole hr^{-1} as a function of the concentration of cyt P-450 2E1 in rat livers). A practical aim of this present study is the use of the common V_{max} predicted for the *in vivo* situations in pharmacokinetic models instead of the experimentally determined V_{max} *in vitro* for first estimates of blood concentration-time profiles of each new/untested LMVOC.

485 ISOZYME-SELECTIVE INACTIVATION OF HEPATIC MICROSOMAL CYTOCHROME P450 BY REACTIVE OXYGEN SPECIES.

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The objective of this study was to test the hypothesis that cytochrome P450 (P450) isozymes vary in susceptibility to inactivation by reactive oxygen species (ROS), with the thinking that ROS-inactivated isozymes will be preferentially release heme substrate. Hepatic microsomes were incubated at 37°C for 15 min in the presence of hydrogen peroxide (H2O2, 0.1-1 M) or a FeCl3/H2O2/ascorbic acid free radical generating system (reagents 0.5-1.25 mM). CYP1A1 dependent ethoxyresorufin O-dealkylation (EROD), CYP1A2 dependent methoxyresorufin O-dealkylation (MROD) and CYP2B1 dependent pentoxyresorufin O-dealkylation (PROD) activities showed concentration-dependent, non isozyme-selective inactivation when treated with H2O2, culminating with total loss of activity at 0.5 M. CYP3A dependent erythromycin-N-demethylation activity showed considerable resistance to H2O2-induced inactivation and retained nearly 60% activity at 1 M H2O2. When incubated in the presence of FeCl3/H2O2/ascorbic acid, only PROD activity showed statistical decreases from control values at concentrations of 0.75 mM and 1.0 mM. Preliminary findings indicate that ROS selectively inactivate P450 isozymes which may preferentially release heme, the substrate for heme oxygenase-1 induced by elevated concentrations of ROS. (This work was supported by MRC grant #MT9972 to JRB.)

486 EPITOPE MAPPING OF CYP2B1 MONOCLONAL ANTIBODIES AND CONFIRMATION BY ELISA USING SYNTHETIC PEPTIDES.

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We have 12 monoclonal antibodies (MAbs) directed against rat CYP2B1 that recognize six spatially distinct epitopes. Our goal is to map the epitopes corresponding to these MAbs so that we can use this information to design antibodies for other P-450s. In our earlier studies we had made fourteen different constructs of CYP2B1, generated fusion proteins with glutathione S-transferase (GST), expressed them in E.coli and detected them by PAGE and Western blots. We narrowed down the epitopes for one group of inhibitory MAbs viz. BEA33, BE44, BE45 and BE28 to a region corresponding to amino acid sequence 250-261 in 2B1 protein. Other antibodies recognized amino acid sequences 262-272 in 2B1 (MAb 29) and 306-491 (BE46, B50 and BE49) in CYP2B1. Non-inhibitory MAbs BE26 and BE32 were mapped to a region 380-398 in 2B1 protein. However, BEA33 behaved differently from BE28, BE44 and BE45 in our earlier studies with inhibition. Our experiments with the fourteen constructs did not reflect a difference in the epitopes for these MAbs. We, therefore, made five new constructs covering nucleotide positions 463 to 801 of CYP2B1 to understand this further. Our results clearly indicate that there exists a subtle but noteworthy distinction between the

actual epitope positions of BEA33 and the others in this group viz. BE28, BE44 and BE45. The data collectively indicates that while the epitopes for BE28, BE44 and BE45 lie in the region corresponding to nucleotides 747-784 and BEF29 lies between nucleotides 784-801, that for BEA33 overlaps the junction of these two regions sharing a few amino acids that comprise the epitopes for the above mentioned MAbs. This difference may explain the data from the inhibition studies. The mapped epitopes for MAbs BE26/BE32 and MAb BEF29 have been confirmed by ELISA using synthetic peptides that were made in the same region. Identifying the sequence of amino acids composing these epitopes would aid in designing specific and inhibitory antibodies for this family and individual enzymes of other families of CYPs.

487 CLOTRIMAZOLE (CTMZ) AND KETOCONAZOLE (KTZ) AS POTENT AND SELECTIVE INHIBITORS OF RAT CYP 3A1/2 AND 2B1/2 ENZYMES.

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In this study, CTMZ and KTZ were tested to determine their inhibitory potential towards CYP3A-mediated reactions, i.e., metabolism of testosterone to 6 beta-hydroxy-testosterone (6 beta-OH T), in rat liver microsomes. The particular objectives were to compare the inhibitory potencies (IC50 and ~90% inhibitory effect) using liver microsomes from adult female rats treated with dexamethasone (F-DEX) and untreated adult male rats (M-CTR), which are known to contain high levels of CYP isozymes 3A1 (3A23) and 3A2, respectively. The results demonstrated that CTMZ is a very potent and selective inhibitor of 6 beta-OH T production in all metabolic systems tested. Additionally, it was found that CTMZ is a potent inhibitor of CYP2B1/2-mediated reactions in liver microsomes from adult female rats treated with phenobarbital (F-PB). Indeed, the IC50 value for 6 beta-OH T formation was computed as 9.6 nM in F-DEX, 6.7 nM in M-CTR, and the IC50 for 16 beta-OH T formation as 12.4 nM in F-PB. The *in vitro* inhibitory potency for CTMZ was found to exceed significantly the same parameters for KTZ, a well-established specific inhibitor of CYP3A-mediated reactions *in vitro* and *in vivo*. In agreement, it was found that the IC50 for KTZ for 6 beta-OH T formation as 70 nM in F-DEX and 784 nM in M-CTR, which is more than 10-fold and 100-fold higher, respectively, than that for CTMZ. However, the inhibitory potency of KTZ for 16 beta-OH T formation was nearly equal to that of CTMZ, with the IC50 as 13.0 nM in F-PB. CTMZ and KTZ, at the concentrations that inhibit 90% and more 3A1/2- and 2B1/2-mediated reactions, has less or no effect on the activities of other major rat liver CYP isozymes, such as 1A1, 1A2, 2C11, and 2E1. In summary, CTMZ is a more potent and selective inhibitor of 3A1/2-mediated reactions than that of KTZ. However, KTZ is 10-fold more potent at inhibiting 3A1- than 3A2-mediated reactions.

488 EFFECTS OF ALBENDAZOLE TREATMENT ON HEPATIC CYTOCHROME P450 (CYP450) EXPRESSION IN THE RAT.

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The anthelmintic drug albendazole (ABZ) is a benzimidazole highly efficient in the treatment of neurocysticercosis. Its formula, methyl(5-(propylthio)-1H-benzimidazol-2-yl)carbamate, has been reported to act as a 3-methylcolanthrene-like CYP inducer. We looked at the effects of ABZ treatment (i.p. and p.o. administration) on the expression of some hepatic CYPs involved in the metabolism of environmental mutagens and carcinogens. Two different strategies were used: a) Immunoblots to specifically identify P450 proteins 1A1/2, 2B1/2 and 2E1; b) Quantification of alkoxyresorufin O-dealkylases (AROD) and p-nitrophenol hydroxylase (pNPH) activities in microsomal samples. Intraperitoneal administration of albendazole (50 mg/kg body weight/day/3 days in corn oil) to rats, caused an induction of hepatic CYP1A1 and CYP1A2 as well as a reduction of CYP2E1 expression as determined by western blot; AROD activities were increased and a slight decrease in pNPH catalytic activity occurred. When the two main metabolites of ABZ were used, results indicated that CYP1A1 induction was produced by the sulfoxide but not by the sulfone metabolite. Oral administration of ABZ at therapeutic dose (20 mg/kg body weight/day/3 days) produced an increase in CYP1A1 protein content 24 h after the first intake. The high protein level was maintained during the treatment and 24 h after the last administration. CYP1A1 basal protein levels were achieved 24 h later. Our results confirm and extend previous data on the inducing properties of ABZ. This CYP1A induction should be taken in to consideration to avoid exposure to other drugs and environmental mutagens/carcinogens that are putative substrates of this CYP subfamily like cigarette smoke, charcoal cooked meats or nitrite containing foods.

489 KINETICS OF 3-METHYLINDOLE OXYGENATION/DEHYDROGENATION BY CYTOCHROME P450 ENZYMES.

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3-Methylindole (3MI) is a selective pulmonary toxicant, and cytochrome P450 bioactivation of 3-methylindole is a prerequisite for toxicity. There are at least 3 pathways for 3MI bioactivation: hydroxylation, epoxidation and dehydrogenation. In metabolic/ kinetic studies using CYP2F1 and CYP2F3, it was shown that dehydrogenation of 3MI to 3-methyleneindolenine was catalyzed exclusively (V/K=72 and V/K=1.6, respectively). Dehydrogenation of 3MI appears to be the primary bioactivation pathway for these two enzymes, but it is not known if 3MI is simply a good substrate for dehydrogenation or if certain cytochrome P450s responsible for 3MI bioactivation have unique active sites that only catalyze the dehydrogenation of the molecule. Therefore, product formation and kinetics by the CYP2F1 and CYP2F3 enzymes were compared to other cytochrome P450 enzymes. The enzymes tested were CYP1A1, CYP1A2, CYP1B1 and CYP2E1. The CYP1A1 and CYP1A2 enzymes produced three 3MI metabolites: the dehydrogenation product, 3-methyleneindolenine (V/K=0.7 and V/K=24, respectively), the hydroxylation product, indole-3-carbinol (V/K=49 and V/K=111, respectively) and the epoxidation product, 3-methyloxindole (V/K=5 and V/K=91, respectively). For both enzymes, the maximum rate of 3-methyloxindole production was 3-4 fold higher than the dehydrogenation product. CYP1B1 produced indole-3-carbinol (V/K = 171) and 3-methyloxindole (V/K=5). CYP2E1 only produced 3-methyloxindole (V/K=100). The ability of the various CYP1 family enzymes to catalyze the formation of different 3MI metabolites, along with the specific oxygenation by CYP2E1, illustrate that dehydrogenation is not solely directed by the substrate, but that the members of the CYP2F family may possess unique active sites that control the dehydrogenation pathway. (Supported by USHPS grant number HL13645.)

490 THE EFFECT OF DEXAMETHASONE ON THE HALF-LIFE OF CYP2E1 MRNA IN PRIMARY CULTURED RAT HEPATOCYTES.

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Although the half-life of the CYP2E1 protein in the absence and presence of substrates and inducers has been examined in several systems, little is known regarding CYP2E1 mRNA turnover. The synthetic glucocorticoid, dexamethasone (Dex), is known to elevate the mRNA and protein levels of several P450s in cultured hepatocytes, including CYP2E1 mRNA. Since the mechanism of this elevation of CYP2E1 mRNA is unknown, the effect of Dex on CYP2E1 mRNA stability in primary cultured rat hepatocytes was examined. Hepatocytes were maintained for 48 h on Vitrogen-coated 60 mm dishes in fortified Chee's media supplemented with 0.1 nM insulin and 10^{-7} M Dex, with media changes every 24 h. Two hours prior to actinomycin D addition, the dishes were separated into two treatment groups: control (no Dex) and 10^{-7} M Dex. Media for each group was supplemented with 5 μg/ml of actinomycin D, and samples harvested immediately and at 2.0, 4.0, 6.0, and 8.0 h post-treatment for mRNA isolation and Northern blot analysis. CYP2E1 band densities were determined and the best fit line was obtained using linear regression analysis, which was then used to calculate CYP2E1 mRNA half-life values. The results reveal that the half-life of CYP2E1 mRNA isolated from Dex-treated cells was 11.0 h, whereas CYP2E1 mRNA isolated from control cells exhibited a half-life of 5.7 h. Thus a 2-fold difference exists in CYP2E1 mRNA half-lives between Dex-treated and untreated hepatocytes. This is consistent with CYP2E1 mRNA gel shift assays in which cytosol from Dex-treated hepatocytes yielded greater band intensities relative to untreated cells. This supports the hypothesis that the Dex-mediated increase in CYP2E1 mRNA is likely the result of enhanced mRNA stability associated with proteins bound to CYP2E1 mRNA. (Supported by grants ES 03656 and EHS Center Grant ES 06639.)

491 SPECIES, STRAIN AND TISSUE SPECIFIC CYP1A1 INDUCTION IN MICE EXPOSED TO TOBACCO SMOKE.

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Induction of cytochrome P450 1A1 (CYP1A1) conventionally has been measured by the ethoxyresorufin-O-deethylase (EROD) assay. In order to assess differences among species and strains in CYP1A1 induction in various tissues, we exposed mice to tobacco smoke which contains several known inducers of CYP1A1. We also compared CYP1A1 activity as measured by the microsomal EROD assay with CYP1A1 activity measured by reverse transcriptase polymerase chain reaction (RT-PCR). We exposed two strains of laboratory mice (Mus musculus) AJ and C57; and a mouse species native to North America, (Peromyscus leucopus), to tobacco smoke in an enclosed chamber for six hours. Mice were sacrificed either immediately post-exposure or 24 hours post-exposure. CYP1A1 induction was measured in microsomal preparations of liver, lungs and kidneys via EROD assay, and in liver, lung and peripheral blood lymphocytes via RT-PCR. There was no significant difference in liver CYP1A1 activity among species/strains at either six or 24 hour sampling times. Peromyscus, but neither of the Mus strains, showed induction of CYP1A1 in the kidney at both 6- and 24-hour sampling times. Lung CYP1A1 activity was induced in both Mus strains and in Peromyscus.

492 MODULATION OF RAT PULMONARY CYTOCHROME P4501A1 (CYP1A1) EXPRESSION BY HYPEROXIA.

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We reported recently that exposure of rats to hyperoxia for 48 h leads to induction of hepatic CYP1A1/1A2 enzyme activities, followed by decline at 60 h. In this study we tested the hypotheses that: (i) Pulmonary CYP1A1 expression is modulated by hyperoxia, similar to the changes in hepatic CYP1A parameters; and (ii) hyperoxia-induced alterations in pulmonary CYP1A1 reflect cell-specific modulation of CYP1A1 expression, in relation to lung injury. Adult male Sprague-Dawley rats were maintained in room air (RA) or exposed to hyperoxia (> than 95% O2) for 24, 48, or 60 h, and lung microsomal ethoxyresofurin O-deethylase (EROD) (CYP1A1) activities, CYP1A1 apoprotein contents, and CYP1A1 mRNA levels were determined. Pulmonary EROD activities were enhanced 6.3-fold after 48 h of hyperoxia over RA controls, and this was accompanied by induction of CYP1A1 apoprotein. CYP1A1 enzyme activity and apoprotein content declined by 60 h. Hyperoxia exposure for 24 h showed a 58% increase in CYP1A1 mRNA over RA controls. CYP1A1 mRNA was no longer detectable after hyperoxia for 48 or 60 h. These results suggest that pre-translational mechanisms contributed to the modulation of CYP1A1 by hyperoxia. Lungs of hyperoxic animals showed an increase in CYP1A1 immunostaining in the bronchiolar epithelial cells, type II pneumocytes, and endothelial cells after 24-48 h. There was microscopic evidence of lung inflammation between 48 and 60 h. In conclusion, the early induction of CYP1A1 in the lungs in association with minimal lung injury in hyperoxia, followed by the terminal decline in CYP1A1 expression, which was temporally accompanied with severe lung inflammation, suggests that regulation of expression of pulmonary CYP1A1 by hyperoxia has mechanistic relationship(s) with hyperoxic lung injury. (Supported in part by NIH grant ES09132 and by ALA of Texas.)

493 SPECIFIC CYTOCHROME P450 INDUCTION IN FEMALE B6C3F1 MICE COMPARED TO FISCHER 344 RATS FOLLOWING PRETREATMENT OF 4-VINYLCYCLOHEXENE OR ITS TOXIC EPOXIDE METABOLITES.

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4-Vinylcyclohexene (VCH) is an intermediate in the production of rubber and styrene. Following repeated exposure, VCH is ovotoxic in B6C3F1 mice but not in F-344 rats. A crucial factor for the species differences in toxicity is the greater rate of formation of the toxic metabolites [1,2-vinylcyclohexene monoepoxide (1,2-VCHE), 7,8-vinylcyclohexene monoepoxide (7,8-VCHE), and ultimately, vinylcyclohexene diepoxide (VCD)], from VCH in mice as compared to rats. Hepatic microsomes isolated from mice or rats pretreated with VCH (7.5mmol/kg for 10d.) caused greater induction of bioactivation of VCH to epoxides in the mouse (3.5-fold in 1,2-VCHE formation, 2-fold in 7,8-VCHE formation, and detectable VCD formation) as compared to the rat (2.1-fold in 1,2-VCHE formation, 1.3-fold in 7,8-VCHE formation, and no detectable VCD formation). This parallels an induction of total cytochrome P450 levels in the mouse and not the rat. CYP2A and CYP2B protein immunoblots revealed induced expression of these isoforms following VCH pretreatment in mouse but not rat hepatic microsomes. Neither species pretreated with VCH demonstrated an induction of CYP2E1. To determine whether VCH and/or its epoxide metabolites were causing the inductions in CYP2A and CYP2B, mice and rats were dosed with either 7.5mmol/kg VCH, 1.4mmol/kg 1,2-VCHE, or 0.4mmol/kg VCD for 10 days (equitoxic doses of epoxides were used based on previous studies in the mouse). Microsomes from VCH-pretreated mice had elevated levels of CYP2A (2-fold) and CYP2B (3-fold), while those from 1,2-VCHE- and VCD-pretreated mice had only elevated levels of CYP2B (2.2-fold and 1.5-fold, respectively). CYP2E1 levels were not induced following pretreatment with VCH or the epoxide metabolites. Neither CYP2A, CYP2B, nor CYP2E1 levels were induced in rat microsomes following any of the pretreatments. In conclusion, at equitoxic doses in the mouse, the epoxide metabolites did not induce hepatic CYP levels to the same extent as VCH in the mouse, while neither VCH nor its epoxide metabolites induced any hepatic CYP levels in the rat. (This research was supported in part by NIEHS Training Grant (ES07091), Center Grant (ES06694) and Chemical Manufactures' Association.)

494 N-ALKYLPROTPORPHYRIN IX FORMATION IN RAT HEPATIC MICROSOMES AFTER INTERACTION WITH PORPHYRINOGENIC XENOBIOTICS.

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Several porphyrinogenic xenobiotics disrupt heme synthesis by eliciting mechanism-based inactivation of specific isozymes of cytochrome P450 (CYP). During inactivation, the CYP heme moiety is converted to an N-alkyprotoporphyrin IX (N-alkylPP), which is a potent inhibitor of the terminal enzyme in heme synthesis, ferrochelatase. We have already determined the major rat hepatic CYP isozyme sources of N-alkylPP after the administration of porphyrinogenic compounds *in vivo*. Previously we have determined the human CYP isozymes which undergo mechanism-based inactivation *in vitro* after interaction with several porphyrinogenic compounds. We did not, however, determine which of these isozymes were responsible for N-alkylPP formation. Thus, our objectives for the present study were to determine whether N-alkylPPs could be isolated from rat liver microsomes following interaction *in vitro* with porphyrinogenic xenobiotics, and if so, whether they are produced in amounts sufficient for studies utilizing microsomes containing human single cDNA-expressed CYP isozymes. The *in vitro* formation of N-alkylPP was observed following incubation of 3-[(arylthio)ethyl]sydnone (TTMS), allylisopropylacetamide (AIA), and 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethylpyridine (4-ethylDDC), but not 1-aminobenzotriazole (ABT), with rat hepatic microsomes. However the overall N-alkylPP yield per nmol CYP *in vitro* was much lower than previously observed *in vivo*. The N-alkylPP yield per nmol CYP was highest for TTMS, which elicited the formation of 0.043 nmol N-vinylPP/nmol CYP, and it was concluded that the use of microsomes containing single cDNA-expressed human CYP isozymes would be feasible for further study with TTMS, but not for AIA or 4-ethylDDC.

495 ALTERATION OF PARATHION AND CHLORPYRIFOS DESULFURATION BY METHOXYCHLOR *IN VITRO*.

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Methoxychlor (MXC), an organochlorine (OC) insecticide, was used to investigate the *in vitro* interaction of an OC insecticide with the desulfuration activity needed to activate the organophosphorus (OP) insecticides [chlorpyrifos (CPS) and parathion (PS)]. Adult rats were administered β-naphthoflavone (BNF) or phenobarbital (PB) to induce hepatic cytochrome P450s responsible for the activation of OP insecticides. Oil and saline (vehicle) controls and naive controls were also included. Microsomes were prepared from liver of male and female rats. Desulfuration activity was the same among control groups. Male rats treated with PB had 3.8- and 18-fold higher desulfuration activity of PS and CPS than the controls and when treated with BNF had 1.6- and 2.8-fold higher desulfuration activity of PS and CPS than controls. Female rats treated with PB had 3.6- and 13-fold higher desulfuration activity of PS and CPS than the controls and when treated with BNF had 1.2-and 2-fold higher desulfuration activity of PS and CPS than controls. MXC was added at an equimolar concentration concurrently with PS or CPS to initiate

desulfuration. In males, desulfuration of PS was decreased by 53%, 29%, and 39% for BNF, PB, and controls, respectively, and desulfuration of CPS was decreased by 74%, 26%, and 70% for BNF, PB, and controls, respectively. In females, desulfuration of PS was decreased by 50%, 29%, and 32% for BNF, PB, and controls, respectively, and desulfuration of CPS was decreased by 50%, 57%, and 51% for BNF, PB, and controls, respectively. MXC affected desulfuration in all groups and had the greatest effect on the desulfuration of CPS in BNF-treated and control males, and the least effect on PB-treated groups of both sexes.

496 EXPRESSION OF CYP2E1 IN ISOLATED OVARIAN FOLLICLES OBTAINED FROM B6C3F1 MICE.

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4-Vinylcyclohexene (VCH), an industrial chemical, causes ovarian toxicity in mice. Previous studies have shown that VCH is bioactivated to its ovotoxic metabolite, vinylcyclohexene diepoxide (VCD), by the cytochrome P450 (CYP450) enzyme system. In mice, VCD selectively destroys the smallest ovarian follicles (preantral; 25-100 mm). It is speculated that VCH is bioactivated in the liver, however, it is not known if the ovary also plays a role in the metabolism of VCH. The purposes of this study were to investigate a) whether the mouse ovary expresses mRNA encoding cyp2e1, b) the distribution of its expression in ovarian follicles, and c) the effect of VCD dosing on this expression. Female B6C3F1 mice (n=10) were dosed daily (15 d) with VCD (0.57 mmol/kg; ip) or control (sesame oil; ip), or 10 d with the cyp2e1 inducer, acetone (1% in drinking water; po). Following treatment, ovaries and livers were removed and ovarian follicles were separated into three distinct populations (fraction 1, small preantral, 25-100 mm; fraction 2, large preantral, 100-250 mm; and antral, >250 mm). Total RNA was isolated from these tissues. Expression of mRNA encoding cyp2e1 was visualized by reverse-transcriptase polymerase chain reaction (RT-PCR). Amplification products were detected in fraction 1, fraction 2, and liver, but not antral follicles, from control and acetone-treated mice. In VCD-treated mice, cyp2e1was detected in liver, but not in fraction 1 or fraction 2 ovarian follicles. These results demonstrate that expression of cyp2e1 in the mouse ovary is compartmentalized and expressed in preantral (25-250 mm) rather than antral (>250 mm) follicles. Furthermore, repeated dosing with VCD, known to be ovotoxic in those small follicles, may reduce ovarian expression of cyp2e1. This suggests that the ovotoxic metabolite, VCD, may affect expression of cyp450's in the ovary. (ADCRC 9809, ESO8979, ESO9246, NIEHS Center Grant 06694.)

497 AH RECEPTOR AND nf-κb INTERACTION: SUPPRESSION OF CYTOCHROME P4501A1 THROUGH ACTIVATION OF nf-κb.

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Inflammatory cytokines, such as TNF-α, IL-1β and bacterial endotoxin suppress the induction of cytochromes P450 1A1/1A2. However, the underlying mechanism(s) is not well understood. In an earlier study, we demonstrated a physical interaction and functional mutual modulation between the AhR and NF-κB signaling pathways. In the present studies, we showed that activation of NF-κB down-regulates the expression of cytochrome P450 1A1, thereby providing an underlying mechanism for the long-standing observations that inflammatory cytokines cause suppression of cytochromes P450 1A1/1A2. Specifically, NF-κB inducers, such as TNF-α and bacterial endotoxin, suppress *cyp1a1* gene expression in Hepa 1c1c7 cells, as determined by Western blot analysis, as well as transient transfection assays with a reporter gene whose expression is under the control of *cyp1a1* promoter. In transient transfection assays, we demonstrated that TNF-α-imposed repression of *cyp1a1* promoter activity is reversed by the NF-κB super repressor ΔIκBα, which is resistant to the TNF-induced degradation, thereby causing constitutive inhibition of NF-κB activation. These results suggest that the suppression of *cyp1a1* expression by cytokine TNF-α is mediated, at least in part, through the activation of NF-κB. Expression of RelA subunit of NF-κB through a tetracycline-regulated promoter also markedly suppresses the *cyp1a1* promoter activity in Hela cells; further strengthening the notion that NF-κB activation suppresses *cyp1a1* expression. (Supported in part by NIEHS Center Grant #ES05022.)

498 IMMUNOCHEMICAL CHARACTERIZATION OF RAT PREGNANE X RECEPTOR H.

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An orphan nuclear receptor, termed the pregnane X receptor (PXR), has recently been cloned from several species. Transient cotransfection experiments demonstrate that the PXR responds to structurally dissimilar compounds and activates a reporter construct containing a *cis*-element from the gene encoding cytochrome P4503A (CYP3A). Northern blotting analyses show that several prototypical CYP inducers markedly increase the accumulation of rat PXR mRNA. This study was designed to immunochemically characterize the rat PXR. A peptide derived from this receptor was synthesized and conjugated with the keyhole limpet hemocyanin. An antibody was raised against the conjugated peptide and subjected to affinity chromatography. This antibody detected a protein only in the PXR-transfected COS-7 cells but not in the control cells. This immunoreactive protein had a molecular weight of 51-kDa, similar to that calculated from the cDNA. Consistent with the tissue distribution of PXR mRNA, this 51-kDa protein was abundant in liver, intestine, and to a lesser extent, in kidney and lung. The level of this protein were markedly increased in rats treated with isoniazid, clofibrate and perfluorodecanoic acid in both hepatic and extrahepatic tissues. The antibody also detected a testicular protein which had a higher electrophoretic mobility than the 51-kDa protein. The testicular protein was abundantly expressed and little change was observed in xenobiotic-treated rats. Compounds that increase PXR expression (inducers) and compounds that activate the PXR (ligands) likely have synergistic effects on CYP3A induction, providing a novel molecular explanation for drug-drug interactions. (Supported by a grant from the Rhode Island Foundation.)

499 THE NORDIC EXPERT GROUP FOR CRITERIA DOCUMENTATION OF HEALTH RISKS FROM CHEMICALS.

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The main task of the Nordic expert group for criteria documentation of health risks from chemicals (NEG) is to produce criteria documents. These are used by the regulatory authorities in the Nordic countries as the scientific basis for setting occupational exposure limits (OELs) at the national level. Some of the NEG documents are also used internationally, e.g. by the European Union. NEG consists of scientific experts from the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). The project is financed by the Nordic Council of Ministers, an intergovernmental collaborative body for the five countries. The aim of the document is to establish dose-response /dose-effect relationships, to identify a NO(A)EL or LO(A)EL, and to define a critical effect based on the scientific literature. The draft document is reviewed and finally accepted by the NEG. The documents are written in English, and are printed and published by the National Institute for Working Life in Sweden in the scientific serial Arbete och Hälsa (http://www.niwl.se/ah/default_en.htm). In the near future, electronic short versions of the documents will be published on the web. During the past years NEG also had a bilateral cooperation with US NIOSH and the Dutch expert committee for occupational exposure standards (DECOS). The project started in 1978. Since then, approximately 130 criteria documents have been published. Recent documents include antimony, dichlorobenzenes and refractory ceramic fibers. The nordic collaboration in NEG is beneficial as it leads to a concordant view in risk assessment, a shared burden in producing documents and decreases duplication of work.

500 WORST-CASE BENZENE EXPOSURE SCENARIO FROM DIESEL LOCOMOTIVE EXHAUST IN A ROUNDHOUSE.

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An exposure assessment of benzene was used to estimate the carcinogenic hazard posed by a possible worst-case exposure scenario to diesel locomotive exhaust in a roundhouse. To understand the upper bound risk to benzene from diesel exhaust, a locomotive was allowed to run for four 30-minute intervals during an 8-hour workshift in a roundhouse to simulate conditions that are beyond worst-case. Full-shift and one-hour airborne concentrations of benzene were measured in the breathing zone between and during the emission episodes on two consecutive days. Carbon monoxide, elemental carbon (surrogate for diesel exhaust), and nitrogen dioxide/nitric oxide area airborne measurements were used to characterize these conditions. Carbon monoxide was measured continuously; elemental carbon was sampled with full-shift

area samples; and nitrogen dioxide/nitric oxide was sampled using full-shift and 15-minute (nitrogen dioxide only) area samples. The benzene concentrations were compared to the current occupational exposure limits and to benzene concentrations associated with cigarette smoking and filling vehicle gas tanks at service stations. The one-hour airborne benzene concentrations range from 1-15ppb with 65% of the measurements below the limit of detection (2-4ppb). Results indicate that the 8-hour time-weighted average for benzene in the roundhouse is approximately 100 fold less than the current threshold limit value (TLV) of 0.5ppm. Under this worst-case exposure scenario, benzene concentrations from diesel exhaust are significantly less than those reported in cigarette smoke and those associated with filling vehicle gas tanks. These data are consistent with other studies, which have indicated that benzene concentrations due to diesel emissions in a relatively confined environment are low.

501 ESTIMATION OF LUNG CANCER RISK IN POPULATION LIVING IN THE VICINITY OF ALUMINIUM SMELTERS IN QUEBEC.

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Aluminium smelters using the older Horizontal Stud Söderberg process have long been recognised as significant contributors to PAH emissions. The lung is one of the target organs after PAH inhalation. Therefore, we focused our attention on estimation of lung cancer risk in population living in the vicinity of aluminium smelters in Quebec. For our calculations we used individual PAH concentrations measured at 11 different locations in Quebec between 1989 and 1994 . Two approaches in the risk assessment of PAH mixture were used. In the first approach, benzo[a]pyrene (B[a]P) toxic equivalent concentrations were used as estimates for exposure to the mixture of PAH. The relative potency factors proposed by Malcolm and Dobson (Department of the Environment. Her Majesty's Inspectorate of Pollution, London, 1994) were used. The lifetime unit risk values for continuous inhalation exposure to BaP obtained from the animal data sets ranging between 0.37 x 10-6 and 4.8 x 10-6 per ng/m3 of BaP were used. The calculated upper bound lung cancer risk values ranged between 1.2 x 10-7 and 4.7 x 10-5 . An alternative approach assumes that the potency of the PAH fraction of any environmental mixture is proportional to the BaP content of the mixture. Public lifetime lung cancer unit risk values of 0.3 - 9.5 x 10-5 per ng/m3 BaP , recalculated by Gibbs (Ann.Occup.Hyg. 41, Suppl. 1, 49-53, 1997) were used. The estimated excess probabilities of lung cancer ranged between 2 x 10-7 and 3.3 x 10-4. It should be noted that both estimates are based upon numerous assumptions such that the confidence in calculated values is evaluated as low-to-medium. (This study was supported by ALCAN ALUMINIUM Ltd., Canada.)

502 HEALTH RISK ASSESSMENT FOR A FORMER ARMY AMMUNITION PLANT.

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As part of a remedial investigation and feasibility study at an army facility which produced and stored trinitrotoluene during World War II, a comprehensive human health risk assessment was conducted to evaluate the cancer risk and health hazard for potentially exposed on-site workers, an off-site residential population and recreational users. Due to the large site area and different operational activities at the facility, six remedial sites and the entire site as a whole were evaluated. Risk analyses indicated that five of the six sites posed unacceptable risks and hazards to on-site workers. Nitroaromatic explosives, PCBs and metals contributed most to the risk and health hazards. However, the potential exposure pathway that presented the largest calculated risk and hazard for the on-site assessment was dietary consumption of fish caught in on-site ponds, a non-occupational, recreational exposure scenario. Upon further investigation of the aquatic monitoring program, it was concluded that this former army ammunition facility was not the single contributor to the PCBs in the fish caught from the ponds. The cleanup goals are being developed for the site.

503 RELATION BETWEEN WORKING CONDITIONS AND EXPOSURE TO DIOXINS IN A MUNICIPAL WASTE INCINERATOR.

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The purpose of this paper is to report on the exposure assessment of MWI workers to dioxins. Subjects were 92 workers and were grouped to four categories. I; workers who did not work inside the facility. II; workers who had worked at the periphery of the facility and handled the non-flammable residues. III; workers who had worked inside the facility. IV; workers who had engaged in maintenance of the furnace, the electrical dust collector, and the smoke cleaning process. Total level of dioxins in I-TEQ (T-TEQ) of groups I, II, III, and IV were 34.2, 66.8, 93.3, and 323.3 pg I-TEQ/g blood-fat, respectively. The dioxin level of group IV was significantly higher than those of the rest of three groups and had a significant correlation between T-TEQ and working duration. Congener profiles of groups I, II, III, and IV expressed by PCDF/PCDD ratio were 0.3, 0.3, 0.7, and 1.6, respectively. From the high T-TEQ level and the positive correlation between T-TEQ and working duration, workers of group III and IV must have been exposed to dioxin emitted from the incinerator. PCDF rich congener profiles from excess exposure support this inference. Exposure to co-planer PCBs was also examined.

504 RISK ANALYSIS OF CANDLE EMISSIONS.

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Previous studies that reported significant public health risks from candle emissions have relied upon unsupported toxicity assumptions about candle soot and have based lifetime risk estimates on emissions from individual candles. Candle soot was assumed to exhibit the same cancer potency as diesel particulate matter (DPM). Carcinogenic PAHs, notable constituents of DPM, have not been detected, or are present only at low levels, in candle soot. Therefore, the assumption of equal potency is not warranted. The purpose of this study was to use generally accepted risk assessment methodology to evaluate the potential health risks associated with candle emissions. Probabilistic risk estimates were calculated using the fitted distribution of reported emissions as the source term and distributions from EPA's Exposure Factors Handbook for other exposure factors. A Monte Carlo analysis using Latin hyper cube sampling was performed. The 50th to 95th percentile risk estimates for benzene emissions ranged from about $2x10^{-8}$ to $1x10^{-6}$. Point estimates calculated using the 95% UCL emission rate, rather than individual candle emissions, resulted in risk estimates for benzene emissions ranging from $2x10^{-7}$ to $3x10^{-6}$. The potential risks estimated in this study are generally considered acceptable by USEPA, and are below the threshold (10-5) for California Proposition 65 listing and labeling. (Partially supported by NIOSH Grant T42/CCT412874.)

505 THE SCIENTIFIC BASIS FOR SETTING OCCUPATIONAL EXPOSURE LIMITS IN SWEDEN - THE SWEDISH CRITERIA GROUP.

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The Swedish Criteria Group (SCG) is an expert committee at the Swedish National Institute for Working Life (NIWL) and consists of about 15 scientists from inside as well as outside the institute. They represent different areas in toxicology and occupational medicine. Observers from the trade unions and the Swedish National Board of Occupational Safety and Health (NBOSH) also participate in the group. The main task of the group is to gather and evaluate data, which are then used as a scientific basis for NBOSH in their setting of administrative occupational exposure limits. An appointed scientist writes a draft document that summarises toxicological and medical data. Only material published (preferably peer reviewed) in the scientific literature is accepted. After review in the Criteria Group the drafts are published as a consensus report from the group. The task of the Criteria Group is not to propose a numerical occupational exposure limit value but to present dose-effect and

dose-response relationships, define the critical effect for occupational exposure, and identify a NOAEL or LOAEL. The consensus reports are published yearly, both in English and in Swedish, by the NIWL in the scientific serial Arbete och Hälsa (http://www.niwl.se/ah/default_en.htm). Since the start of the Criteria Group in 1978, about 250 consensus reports and 39 comprehensive criteria documents have been published. Several of the criteria documents have been published in collaboration with the Dutch Committee on Occupational Standards (DECOS) or the US National Institute for Occupational Safety and Health (NIOSH). The Criteria Group also has a close collaboration with the Nordic Expert Group.

506 HUMAN HEALTH RISK ASSESSMENT OF TOLUENE DIAMINE ISOMERS FROM POLYURETHANE FOAM USED IN AN IMPLANTABLE MEDICAL DEVICE.

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The long term implantation of a new polyurethane foam-containing medical device could potentially result in exposure of recipients to low levels of the biodegradation products, 2,4- and 2,6-toluenediamine (TDA) over time. In animal studies, 2,4-TDA has been reported to be carcinogenic in rodents, and thus, a quantitative risk assessment of possible exposure to TDA from this novel medical device was undertaken.Based upon a considerable amount of published data related to the degradation of polyurethane-coated breast implants and the *in vivo* disposition of 2,4-TDA, the lifetime average daily exposure to 2,4-TDA from the polyurethane foam used in the current device was estimated to be 7.3x10-7 mg/kg body weight/day. A cancer potency estimate of 0.21 (mg/kg body weight/day)-1 for 2,4-TDA was derived based on a National Cancer Institute carcinogenicity study in rodents, in which it was concluded that 2,4-TDA, but not 2,6-TDA, was carcinogenic in rats and female mice. The resulting upper limit of risk was calculated to be 1.5x10-7, representing a theoretical lifetime risk (over 35 years) of 1.5 in 10 million for developing cancer from exposure to 2,4-TDA released from the polyurethane foam. Based upon this assessment, and the application of a number of conservative assumptions, it is concluded that the theoretical risk of developing cancer from exposure to 2,4-TDA, potentially released from polyurethane foam used in the medical device, is negligible. Furthermore, in humans industrially exposed to 2,4-TDA, there has been no evidence of an increased incidence of cancer.

507 ASSESSMENT OF RISK FROM DIOXIN IN TAMPONS.

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We have examined claims originating on the Internet about potential harm from dioxin in tampons that have aroused concern in Congress. We have examined the potential for the processes used in the manufacture of tampons to generate dioxins and the available published analyses of dioxins in tampons. We have developed a likely exposure scenario based on typical useage patterns, and determined likely exposure levels. We compared these values to average daily doses and body burdens present in the US population. Tampons are made by mechanical means from rayon or cotton fibers. Cotton is bleached with hydrogen peroxide (or less commonly, with peracetic acid) and dioxins are not formed by this chlorine free process. Rayon is made from cellulose derived primarily from wood pulp which must be bleached to remove lignin. Chlorine gas bleaching generates a small amount of chlorinated dioxins; elemental chlorine free (ECF) bleaching can theoretically generate a very small amount of dioxin, but this amount is below the detection limit of current assay methods; totally chlorine free (TCF) bleaching generates no dioxins. Currently, ECF or TCF is used for all rayon in tampons sold in the US. Actual analyses in the literature report no TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin, the most toxic of the dioxin isomers) in tampons and very little TEQ (toxic equivalents, used to account for the relative toxicity of a mixture of dioxin isomers). Tampons are used ca. 4 days a month, and may be changed as often as every two hours. We calculate an average of 288 per year for 35 years or a lifetime usage of 10,080 tampons. Although both the theoretical assessment and actual measurements indicate that no TCDD is present in ECF or TCF bleached tampons, we assume as a worst case a dioxin (TEQ) content 2 times the maximum from actual measurements. We calculate that if dioxin were present at this amount, tampons would contribute, at a maximum, 3 thousandths of the average body burden and daily dose of dioxin found in the general US female population.

508 RISK ASSESSMENT-BASED APPROACH FOR THE BIOLOGICAL EVALUATION OF MEDICAL DEVICE MATERIALS.

M. E. Stratmeyer and R. P. Brown. *CDRH/FDA, Rockville, MD.* Sponsor: P. L. Goering.

Systemic toxicity tests of medical device materials are usually conducted using an extract obtained from the material. The use of relatively dilute extracts limits the ability of these tests to detect all but the most overt signs of toxicity. Further, these tests are often conducted with little or no knowledge of the chemical composition of the extract obtained from the device. As a result, it is not possible to anticipate the toxic effects that may be produced following exposure to the medical device material. To address these needs, a risk assessment-based approach has been developed to assess the potential for a medical device material to produce adverse systemic effects. The hallmarks of this approach are: 1) chemical characterization of the device, 2) estimation of the dose of each chemical constituent received by a patient, 3) derivation of a Tolerable Intake (TI) value for each constituent, and 4) comparison of the dose of each compound received by a patient to it's TI. While this proposed approach has not yet been formally accepted in CDRH as an alternative to systemic toxicity testing, it shows promise as a means to provide relevant data for regulatory decision making and should reduce the number of animals required for premarket testing. Further, this proposed approach is consistent with a number of regulatory initiatives currently underway in the FDA, including the use of knowledge bases and consensus standards for the biological evaluation of medical devices.

509 ESTIMATION OF MARGINS OF EXPOSURE: A PRELIMINARY RISK ASSESSMENT FOR OCTAMETHYLCYCLOTETRASILOXANE (D4) BASED ON REPRODUCTIVE TOXICITY STUDIES IN SPRAGUE DAWLEY RATS.

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The results for several endpoints evaluated in a reproductive toxicity study in which female Sprague-Dawley rats were exposed to D4 by whole body inhalation were selected for dose-response modeling using the Benchmark Dose (BMD) approach. Margins of Exposure (MOE), which are the ratio of the lowest lower bound on the BMD (BMDL) to the estimated intakes of D4, were calculated. Intake estimates were based on the exposure assessment conducted by Everest Consulting Assocates and differed only in the use of a more recently derived human dermal absorption value. These receptors included: 1) workers in the D4 manufacture, formulation of D4-containing products, or in the use of these products in professional settings; 2) consumers who use these personal care products; 3) consumers who may be exposed to silicone antifoams used in food processing; and 4) the general public living in the vicinity of a plant that produces or processes these materials. All MOEs calculated for the selected receptors were greater than 100 and with few exceptions were greater than 1000. When the impact of scientifically based assumptions regarding dermal absorption and route equivalence was considered, all MOEs for consumer use products were greater than 1000 and for many products, greater than 10,000. MOEs of even greater magnitude are expected when the species extrapolation uses the delivered dose at the target tissue rather than estimates of intake. MOEs may be further increased when the species- and strain-specific modes of action can be considered. (This work was supported in part by the Silicones Environmental Health and Safety Council.)

510 USE OF PBPK/PD MODELS AND FOLIAR TRANSFER COEFFICIENTS IN ASSESSING REENTRY INTO PESTICIDE TREATED CITRUS AND TURF.

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Physiologically-based pharmacokinetic and pharmacodynamic (PB-PK/PD) models describing the fate of dermally applied 14C-ring labeled ethyl parathion and isofenphos and inhibition of B-esterases (acetylcholin-, butyrylcholin- and carboxyl-) by oxons in the rat were used in conjunction with foliar transfer coefficients to assess reentry levels/intervals into treated citrus (parathion) and turf (isofenphos) to harvest fruit or for recreational use, respectively. Michaelis-Menten kinetics were used to describe the desulfuration of parathion and isofenphos to their oxons and hydrolysis of parent chem-

icals and oxons. Bimolecular rate constants, ki (pM-1 hr-1) were used to describe inhibition. Rates for enzyme synthesis, reactivation and aging were considered. A foliar transfer coefficient (slope of the regression line relating exposure in µg/h and foliar dislodgeable residue in µg/cm2, two-sided residue) of 10000 cm2/h was used in both models. According to the PBPK/PD models, 3% of the parathion and isofenphos foliar residues transferred to human skin were dermally absorbed over an 8 h work day in citrus or 2 h on turf. Exposure to foliar residues of 0.09 µg/cm2 (two-sided leaf residue) of parathion on citrus and 0.6 µg/cm2 (two-sided leaf residue) of isofenphos on turf resulted in no red blood cell cholinesterase inhibition. On the basis of ChE NOELs of 0.05 mg/kg/day for parathion and isofenphos in chronic studies, margin of exposures (MOEs) of 16 and 10, respectively, were calculated.

511 A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR METHYL *TERT*-BUTYL ETHER IN HUMANS.

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The gasoline oxygenate methyl *tert*-butyl ether (MTBE) is the most frequently used additive to reduce carbon monoxide in automobile emissions. To evaluate recent concern that MTBE poses a health hazard to humans, pharmacokinetic information is needed. The objective of this study was to verify the ability of a physiologically based pharmacokinetic (PBPK) model to predict MTBE blood levels in humans following inhalation exposure. The model structure for MTBE was flow-limited and had 6 essential compartments: lung, liver, rapidly perfused tissues, slowly perfused tissues, fat, and kidney. In this model, metabolism occurred in the liver and followed Michaelis-Menten kinetics. The PBPK model used ordinary differential equations to explain the change in the amount of MTBE over time. The physiological and anatomical parameters were obtained from the literature. MTBE-specific parameter values such as partition coefficients for kidney and fat were experimentally measured in human tissue. The human MTBE blood:air partition coefficient was obtained from the literature. Metabolic rate constants were measured *in vitro* in human liver microsomes from 10 subjects and extrapolated to *in vivo* whole-body metabolism based on microsomal protein content, body weight, and liver weight as a percentage of body weight. This PBPK model was analyzed using MATLAB® and Simulink® software packages. Model simulations were compared with data on blood concentrations of MTBE taken from 6 individuals after a 4-hour inhalation exposure to 4 or 40 ppm MTBE. The PBPK model accurately predicted MTBE pharmacokinetics at the high MTBE exposure levels for all time points. However, the model underpredicted early time points while adequately predicting later time points at the low MTBE exposure level. The ability of this model to predict blood levels of MTBE given an external MTBE exposure concentration is important in determining dose estimates for risk assessment.

512 THE KINETIC-DYNAMIC RELATIONSHIP OF THE EFFECT OF REPEATED GLYCYRRHIZIC ACID EXPOSURE ON THE URINARY CORTISOL-CORTISONE RATIO IN HUMANS.

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Glycyrrhizic acid (GD) is widely applied as a sweetener in food products and chewing tobacco and is also used clinically for treatment of chronic hepatitis. In some highly exposed subgroups of the population, side effects like hypertension and electrolyte disturbances have been reported. These clinical effects originate in the inhibition of 11-beta-hydroxysteroid dehydrogenase (HSD) by the main metabolite of GD, glycyrrhetic acid (GA). This renal enzyme converts the mineralcorticoid cortisol into the inactive compound cortisone. Long term elevation of renal cortisol induces symptoms of an apparent mineralocorticoid excess. For the health risk assessment of GD exposure, the toxicokinetic–dynamic relationship of GD was studied. An assay was developed for quantification of *in vivo* HSD inhibition by determining the ratio between cumulative urinary cortisol-cortisone levels in 24 hour urine samples. In a pilot study, in which two healthy volunteers consumed an aqueous suspension of 130 mg GA once daily for three days, a dose dependent increase of this ratio was found. The ratio returned to its basal level within 3 days after the GA exposure was stopped. The determined onset of the effect of GA on the urinary cortisol-cortisone ratio is consistent with the results of other clinical studies on this effect. Using both datasets, a relationship was found between the area under the GA plasma concentration-time curve, as forecasted by a previously reported human GA PBPK model, and the absolute change in the urinary cortisol-cortisone ratio. The PBPK/PD model shows that especially in people having a prolonged gastrointestinal residence time, the urinary cortisol-cortisone ratio will rise rapidly after daily liquorice consumption, indicating that this subgroup has a higher potential of developing liquorice induced hypertension.

513 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODEL AND HUMAN HEALTH RISK ASSESSMENT FOR COUMARIN.

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Coumarin, a fragrance ingredient, is a mouse lung and rat liver toxicant. Long-term administration of coumarin has been associated with an increased incidence of lung tumors in B6C3F1 mice in chronic oral gavage studies conducted by the NTP and liver tumors were observed in male Sprague-Dawley rats chronically fed coumarin in the diet. Species differences in the cytochrome P450-mediated metabolism of coumarin are profound, and are integrally linked to the toxicity of this chemical. In humans, coumarin is metabolized primarily to the non-toxic 7-hydroxycoumarin metabolite. In contrast, coumarin 3,4-epoxidation predominates in rodents. To better understand the relationship between coumarin bioactivation, toxicity, and carcinogenicity, a physiologically-based pharmacokinetic model for coumarin has been developed. Simulation of the pharmacokinetics of coumarin associated with administered doses from the animal toxicity and carcinogenicity data was conducted in order to derive dose metrics to identify the most likely metabolite associated with the observations in animals. This model was then extended in order to simulate dose metrics in the human following likely exposure situations. Mode of action of the carcinogenicity of coumarin was also determined, based on integration of the available qualitative and quantitative kinetic and toxicity information. The dose metrics were then used, in conjunction with the most appropriate dose-response model based on the proposed mode of action for coumarin, to conduct a human health risk assessment for coumarin.

514 INVESTIGATION OF THE IMPACT OF BENCHMARK AND PBPK MODELING ON THE DERIVATION OF MRLS.

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This effort investigated the potential impact of physiologically based pharmacokinetic (PBPK) modeling and benchmark dose (BMD) modeling on the derivation of minimal risk levels (MRLs) for 10 chemicals: perchloroethylene, trichloroethylene, vinyl chloride, benzene, 1,1,1-trichloroethane, carbon tetrachloride, toluene, acetone, chromium, and lead. PBPK modeling was also used for route-to-route extrapolations in order to estimate MRLs for which no data appropriate for MRL derivation are currently available, or to compare the MRLs predicted across routes using the PBPK model versus current MRLs. The impact of drinking water patterns was also investigated, by incorporating a human drinking water profile into the PBPK models. There was no clear trend in terms of the direction of the impact of using these techniques; that is, both higher and lower MRLs were obtained. Twelve MRLs were estimated using route-to-route extrapolation for which no MRL currently existed. These included MRLs for perchloroethylene, trichloroethylene, vinyl chloride, benzene, cadmium, 1,1,1-trichloroethane, and toluene. The results of the comparison of predicted MRLs across routes demonstrated that for some chemicals, a more sensitive endpoint for one route of exposure could be used for the estimation of an MRL in place of a less sensitive endpoint by the other route. The incorporation of the drinking water profile had a significant impact on dose metrics that were representative of a peak target tissue concentration, but had relatively little impact on dose metrics that were representative of cumulative exposure. Because of the uncertainty in actual drinking water patterns in humans, deriving MRLs from dose metrics based on such patterns is not recommended at this time.

515 ASIAN & PACIFIC ISLAND SEAFOOD CONSUMPTION STUDY IN KING COUNTY, WASHINGTON.

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Described are Asian and Pacific Islander (API) seafood consumption rates, species, and seafood parts commonly consumed and cooking methods. Phase I focused on identifying target ethnic groups and developing an appropriate questionnaire (see Asian and Pacific Islander Seafood Consumption Study, EPA 910/R-96-007, August 1996). Phase II, detailed in this report, was the characterization of seafood consumption patterns of ten API ethnic groups (Cambodian, Chinese, Filipino, Hmong, Japanese, Korean, Laotian, Mien, Samoan, and Vietnamese) within King County, Washington. Participants were first or second generation members, ≥ 18 years of age and were seafood consumers. A survey questionnaire was translated into the respondents' native languages. Surveys were administered by trained bilingual API community members. Phase III was the development of culturally appropriate health messages regarding seafood consumption risks and its dissemination to the API community. The majority of the 202 respondents (89%) were first generation (i.e., born outside the United States). There were slightly more women (53%) than men (47%), and 35% lived under the 1997 Federal Poverty Line. The average overall consumption rate for all seafood combined was 1.891 grams/per kilogram body weight/day (g/kg/day), with a median consumption rate of 1.439 g/kg/day. The predominant seafood consumed was shellfish (46% of all seafood). Seafood consumption based on gender, age, income, and "fishermen" status did not differ significantly.

516 ESTIMATES OF HUMAN EXPOSURE TO PCBS AND ASSOCIATED HEALTH RISKS FROM DIETARY SEAFOOD EXPOSURE.

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PCBs have been detected in all species of fish monitored in the past ten years by the Puget Sound Ambient Monitoring Program. Recent surveys of Native Americans and Asian and Pacific Islanders (API) in the Puget Sound area have demonstrated seafood consumption rates far greater than the 6.5g/day adopted for use in the Clean Water Act. Median consumption rates are 53 to 66g/day for men and 25 to 34 g/day for women in the Tulalip and Squaxin Tribes and 89 g/person/day for API. There is concern about possible health risks from PCB exposure through seafood for these groups, but estimation of exposure is difficult due to inadequacies of environmental and consumption data. Our current study assesses confidence in available seafood PCB data for each species consumed as a percent of total seafood consumption and as a percent of estimated total seafood PCB exposure. A prioritization scheme based on species specificity, data quality, and location compatibility was developed to rate confidence in seafood PCB data. For API, 45.9% of total seafood consumed is shellfish; the geographic source of only 11% of this is known. 72-80% of anadromous fish, which dominate Tribal seafood diets, is harvested in Puget Sound. To explore the value of specific contaminant and consumption data for human health risk assessments, risk assessments based consumption of Puget Sound crab for the API and salmon for the Tribes were performed. These included consideration of cooking and preparation and use of Monte Carlo techniques. Results suggest that while PCB exposure from most seafood consumed by these groups cannot be well characterized, risk estimates based on consumption of species for which comprehensive environmental data exist still contain considerable variability and uncertainty.

517 ART MATERIALS RISK ASSESSMENT FOR DYED PAPER PRODUCTS.

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A chronic human health risk assessment was conducted on six types of dyed paper products in order to comply with the requirements of the Labelling of Hazardous Art Materials Act (LHAMA; Public Law 100-695). LHAMA requires that certain types of art materials be reviewed to determine the potential for causing a chronic hazard, and that appropriate warning labels be put on those art materials found to pose a chronic hazard. The procedure for evaluating art materials is described in the American Society for Testing and Materials (ASTM D-4236). The first step involved collecting information on the composition of the paper products. Ingredients included pulp and various additives, such as corn starch, titanium dioxide, calcined clay, filter clay, aluminum sulfate, aluminum silicate, biocides, defoamers, dye fixatives, colored pigments and dyes. The suppliers of the additives provided Materials Safety Data Sheets (MSDSs) that listed individual ingredients and provided toxicity information. A list of the potentially most toxic compounds was developed and evaluated in a risk assessment. Two types of exposures were considered in the risk assessment – children using the paper for drawing in classrooms, and adult office workers using the paper for printing and copying. It was assumed that children and adults could be exposed to the paper products through incidental ingestion, dermal contact and inhalation of volatilized chemicals. Using these exposure assumptions and chemical-specific toxicity values, screening concentrations that represented safe levels of exposure were calculated for each of the chemicals. These screening concentrations were then compared to measured or predicted concentrations of these chemicals in the dyed paper products. The predicted concentrations were upper limits on the amount of chemical that could be present in the final paper product. The comparison showed that all of the measured and predicted levels were lower than the screening concentrations. These results showed that the paper products contained no materials in sufficient quantitities to cause health problems, and that no warning labels were required.

518 DEMOGRAPHICS OF CHRONIC DISEASES IN THE UNITED STATES.

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Under the Safe Drinking Water Act amendments of 1996, the US EPA is directed to give increased attention to sensitive human populations. Individuals suffering from chronic diseases are, in some instances, more sensitive to the toxic effects of specific environmental contaminants. Accordingly, the US EPA Office of Water collected background demographic information on the prevalence of 12 chronic conditions in the US population. The National Health Interview Survey, National Health and Nutrition Examination Survey and Centers for Disease Control were the primary sources of this information. The data were compiled by age, gender, race, ethnicity, and geographic area. As expected, the prevalence of chronic diseases differed for children (<11 years) and adults (>20 years). Immunological hypersensitivity, including asthma and allergic disorders, were the leading chronic condition in both children and adults. Cardiovascular disease ranked third for both adults and children whereas hypertension ranked second for adults and fifth for children. Anemia ranked second among children but much lower in adults. The collected data provide useful background information in the risk assessment for any contaminant with an adverse impact on one or more of the populations characterized. (The opinions expressed in this abstract are those of the authors and not necessarily those of US EPA.)

519 ARE CONCENTRATIONS OF ARSENIC IN FILL SOILS AT MARE ISLAND ELEVATED?

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Arsenic occurs naturally in soils and has industrial, agricultural, forensic, and medicinal uses. However, arsenic poses a human health risk at low concentrations in soils (0.38 ppm). An initial assessment of metals in soils at Mare Island Naval Shipyard (Vallejo, California) indicated that ambient concentrations of arsenic were 36 ppm in artificial fill and 16 ppm in materials native to the original island. Regulatory agencies were concerned that this disparity was due to Navy operations at Mare Island. To determine the sources of elevated arsenic we 1) re-evaluated the initial arsenic datasets for Mare Island, 2) reviewed the ambient arsenic literature for the Bay Area, and 3) conducted statistical comparison tests. Most of the Mare Island fill is composed of dredged estuarine deposits, so this study compared the fill and the native estuarine material. The initial datasets were expanded and compared statistically using the Wilcoxon Rank Sum (WRS) test. The study concluded that ambient arsenic concentrations in California consistently exceed the EPA's preliminary remediation goal by at least an order of magnitude and high ambient arsenic concentrations characterize much of the natural environment. The WRS test showed that fill and native arsenic concentrations at Mare Island are statistically indistinguishable. Average arsenic concentrations for fill and native data sets are 14.51 and 14.53 ppm, respectively; Z statistic equals 1.81, and double-sided p-value is 0.07. The elevated arsenic on Mare Island is likely a product of natural runoff from arsenic-bearing minerals exposed at the source

region of the Sacramento-San Joaquin and Napa River drainages. The data we present indicate a common origin for the arsenic present in soils at Mare Island and do not suggest that an environmental release due to Navy operations has occurred.

520 DETERMINATION OF ACUTE REFERENCE EXPOSURE LEVELS FOR AIRBORNE TOXICANTS.

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We have developed acute inhalation reference exposure levels (RELs) applicable to the general public routinely exposed to hazardous substances released in the environment. The acute REL is an exposure that is not likely to cause adverse effects in a human population, including sensitive subgroups, exposed to that concentration on an intermittent basis. Acute RELs were developed for 51 compounds or classes of compounds, predominantly for one-hour exposures. The adverse effects were categorized as either mild (e.g., eye and respiratory irritation, mild cardiovascular or CNS effects, and immune responses) or severe (e.g., reproductive and developmental effects, hematological effects, and serious CNS effects). 34 of the RELs were based on human data. All acute exposure levels are designed to protect the most sensitive individuals in the population by identifying NOAELs and by inclusion of uncertainty factors (UFs). UFs ranged from 1 to 1000, although most of the UFs were 10 or less. Where the benchmark dose approach was applied, a log dose vs. probit response model identified the 95% LCL concentration expected to produce a 5% increase in toxic response, which was defined as a NOAEL. When the duration of experimental exposure (t) differed from the desired exposure duration, the concentration (C) was adjusted using $C^n \times t = K$, where K is a constant and $1 \leq n \leq 4.6$.

521 A PROPOSAL FOR INCORPORATING ORAL BIOAVAILABILITY IN HUMAN HEALTH RISK ASSESSMENTS.

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Bioavailability is that fraction of the external dose of a substance that is available to contribute to the physiological or toxicological response of the substance. Oral bioavailability, a complex issue that impacts diverse scientific disciplines, can be conceptualized as three processes: (1) bioaccessibility (the fraction of the dose that is available for absorption), (2) absorption, and (3) distribution/metabolism. Once the substance reaches the target organ, toxicodynamic processes dominate. For each process, knowledge gained from experiments or literature searches can be used to modify the dose or exposure estimate (bioaccessibility) and/or the interspecies uncertainty factor (absorption, distribution/metabolism, toxicodynamics) normally used in non-cancer risk assessments. The accepted default value of the interspecies uncertainty factor is 10. Based on the literature examining bioavailability and uncertainty factors, we suggest that the interspecies uncertainty factor comprises three components: absorption (1.0 - 2.24), distribution/metabolism (1.0 - 2.24), and species sensitivity (1.0 - 2.0). The product of the maxima of these components is 10. When good-quality data exist for any component, they should be used in the risk assessment, and the numerical value of the corresponding uncertainty factor component can be reduced. Incorporating bioavailability data will likely have a small impact on the numerical outcome of risk assessments, but the confidence in the output will be strengthened because scientifically defensible values rather than default assumptions were used. (This presentation is an extension of a U.S. EPA, Office of Water project funded through the ILSI Risk Science Institute. These views do not reflect the policy or opinions of the USEPA or the Risk Science Institute.)

522 CHEMICAL POLLUTANTS PRESENT IN YOUNG BODY, RISK FOR CANCER DISEASE.

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The authors present the research results obtained in 1998-1999 period of some chemical pollutants with risk for cancer disease [nitrates/nitrites, pesticide residues, metals] in human body, in relation with these chemical pollutants from foods [vegetables, meat, fish, milk, bread] and in total diets from Iassy, Bacau and Botosani districts. The investigation were carried on urine samples from 120 children (2-16 years old) and 120 young (17-20 years old) which living in Iassy, Bacau and Botosani districts. The pesticide residues in urine and foods were analyzed by gas-chromatographic method, nitrates/nitrites were analyzed by colorimetric method and metals by atomic absorbtion spectrophotometric method. In all analyzed samples these chemical pollutants were found. The mean levels of organochlorine pesticide residues in urine varied between 0.57μg/l (3-5 years old) and 0.92 μg/l (6-10 years old). The mean levels of nitrite in urine varied between 0.72 mg/l (3-5 years old) and 1.2 mg/l (11-16 years old). The mean levels of zinc in urine varied between 0.7 μg/l (11-16 years old) and 1.51 μg/l (6-10 years old). The mean levels of copper in urine varied between 0.08 μg/l (3-5 years old) and 0.34 μg/l (11-16 years old). We haven't found correlation between the levels of these chemical pollutants present in urine with the levels of foods used in these districts. Determination of these pollutants in young body are important in clinical routine analysis and environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health and epidemiology.

523 CHARACTERIZATION OF FURAN-MEDIATED APOPTOTIC AND NECROTIC CELL DEATH IN ISOLATED RAT HEPATOCYTES.

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Furan is a nonmutagenic, hepatotoxic compound that induces hepatocellular carcinomas in rats and mice through mechanisms involving cell death and proliferation. Previous studies in our laboratory have shown that exposure of isolated F-344 rat hepatocytes to furan produced concentration- and time-dependent increases in endonuclease-mediated DNA double-strand breaks (dsbs) prior to cell death. To characterize the role of apoptosis in cell death, F-344 rat hepatocytes were treated with 100 μM furan or 50 μg/ml cycloheximide for 4 hr in suspension and analyzed for evidence of apoptotic and necrotic cell death using flow cytometry (Annexin V binding/propidium iodide staining) and with morphological techniques (Hoechst staining). While cycloheximide produced a 3-fold increase in apoptosis over controls (6 ± 3% vs. 18 ± 2%) at the four hr time period, 100 μM furan did not cause a significant increase in apoptosis or necrosis, despite causing a significant increase in DNA dsbs. After 24 hr culture of furan exposed cells, predominantly necrotic cell death with some apoptosis was observed with 100 μM furan, while 10 μM furan did not produce these effects. These data are consistent with *in vivo* studies in mice demonstrating that necrosis is the predominant mode of cell death induced by furan. The dose-dependence of these effects in isolated hepatocytes is consistent with prior data showing DNA dsbs induced by low concentrations of furan (10 μM) were repaired after 24 hr in culture. Erroneous repair of DNA dsbs may be involved in the induction of mutations leading to furan-induced liver cancer.

524 PROTECTIVE EFFECTS OF D-TAGATOSE AND D-FRUCTOSE AGAINST CYCLOSPORINE A-INDUCED APOPTOSIS IN RAT HEPATOCYTES.

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Lipid peroxidation, a general cytotoxicity mechanism of many xenobiotics is dependent on the presence of catalytic amounts of iron ions. Since cyclosporine A (CsA) induces an oxidative stress in rat hepatocytes, it was the purpose of the present study to investigate the role of iron-mediated lipid peroxidation in the cell damaging process by means of potential cytoprotective ketohexose- and desferrioxamine-type iron chelators. Rat hepatocytes were cultured for 4 and 20 hours with 0, 10, 25 and 50 μM CsA in the absence or presence of 4 mM D-tagatose, D-fructose, D-glucose or 5 mM desferrioxamine. Whereas the ketohexoses D-tagatose and D-fructose significantly attenuated CsA-induced LDH release, as well as inhibited caspase-3 activation and chromatin condensation and fragmentation, the aldohexose D-glucose did not have any inhibitory effect. The iron chelator desferrioxamine did not protect against CsA-induced LDH release and apoptosis. These data indicate that iron catalyzed lipid peroxidation is not directly involved in the cyclosporine cytotoxicity and apoptosis, and that the cytoprotective and anti-apoptotic effect of the ketohexoses are probably mediated by inhibition of caspase-3.

525 ENHANCED PANLOBULAR HEPATOTOXICITY TO ACETAMINOPHEN IN BCL-2 OVEREXPRESSING TRANSGENIC MICE.

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The mitochondria play an important role in apoptosis and drug-induced cell death. Previously, we have shown that acetaminophen (APAP)-mediated hepatotoxicity and cell death is associated with translocation to mitochondria of the BCL-2 family member, BAX. Mitochondrial translocation of BAX is consistent with its proapoptotic role initiating collapse of the mitochondrial inner membrane potential. Based on the antiapoptotic characteristics of the intracellular regulator protein BCL-2, we hypothesized that BCL-2 overexpression in transgenic mice would be hepatoprotective. Mice with a human BCL-2 transgene (-/+) and wild type (WT: -/-) were dosed with 500 mg/kg (ip) APAP or N-acetyl-m-aminophenol (AMAP). Early pathological evidence for APAP-induced zone 3 necrosis was seen at 6 hours in BCL-2 (-/+) mice while AMAP-treated BCL-2 (-/+) mice showed only microvesicular steatosis without progression to extensive necrosis. By 24 hours, APAP-treated BLC-2 (-/+) mice demonstrated severe panlobular necrosis with hemorrhagic infiltration. In contrast, WT mice dosed with APAP showed characteristic centrilobular necrosis. AMAP-treated BCL-2 (-/+) mice demonstrated only scattered necro-inflammatory foci. The evaluation of plasma transaminase activities was also consistent with the enhanced hepatotoxicity of APAP in BCL-2 (-/+) animals. To further evaluate the role of mitochondria in APAP-mediated hepatotoxicity, Complex III activity was assessed. In BCL-2 (-/+) mice only APAP led to decreased Complex III activity which correlated strongly with plasma enzyme activities. The specificity of the correlation between Complex III and hepatic damage is being examined. In conclusion, BCL-2 overexpression unexpectedly enhances APAP-associated hepatotoxicity in mice but has no effect on the toxicity of AMAP. (Supported by NIH grants #GM25418 to SDN, #GM51916 to SAB, NIEHS Center Grant P30-ES07033, and #CA74131 to NF.) The views expressed in this article are those of the author(s) and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the US Government.

526 THE ROLE OF APOPTOSIS IN PHENOBARBITAL MODULATION OF COCAINE-INDUCED LIVER DAMAGE.

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Liver damage, following cocaine administration, has been generally attributed to necrosis. The purpose of this study was to evaluate a possible apoptotic component in cocaine hepatotoxicity. Male ICR mice were either pretreated with phenobarbital (80 mg/kg, i.p.) for three days and subsequently administered cocaine (60 mg/kg, i.p.), or treated with cocaine (60 mg/kg, i.p.) alone. Blood and liver were harvested at 0.5, 1, 2, 4, 6, 12, 24, 48, 72 hours and 7 days following cocaine treatment. Serum alanine aminotransferase activity was used to verify hepatotoxicity and liver tissue from treated and control mice was analyzed to determine amount and area of apoptosis. The histopathological evaluation of cocaine-induced damage revealed patterns of necrosis similar to those reported in previous studies. However, using the TUNEL method, apoptotic cells were observed in the same hepatic compartments in which necrosis was identified. Thus, in mice treated with cocaine alone apoptosis was observed in midzonal and centrilobular regions, while in mice treated with cocaine following phenobarbital pretreatment, apoptosis was observed exclusively in midzonal and periportal areas. Treatment with phenobarbital protected the centrilobular region from both apoptosis and necrosis. Also, histopathological evaluation suggested that cocaine altered the normal trafficking pattern of hepatocytes. Thus, hyperplastic hepatocytes, originating in midzonal regions, appeared to migrate to adjacent regions where significant cell loss had occurred. Together these results indicate that apoptosis contributes significantly to cocaine-induced liver damage, that hepatocyte traffic may play an important role in restoring liver function, and that phenobarbital treatment may alter the type, the intensity and the course of cocaine-induced hepatic injury. (Supported in part by NIOSH Grant 412874-03.)

527 EFFECTS OF GALACTOSAMINE AND TNF-α ON CASPASE-MEDIATED APOPTOSIS IN ISOLATED RAT HEPATOCYTES.

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Release of tumor necrosis factor (TNFα) alpha following lipopolysaccharide treatment has been shown to induce apoptosis in galactosamine (GalN)- sensitized mice leading to hepatic failure. GalN's ability to inhibit mRNA and protein synthesis sensitizes hepatocytes to the apoptotic process by depleting uridine. The objective of this study was to evaluate the role of caspases 3 and 8 in cell killing by GalN and TNFα alone and in combination. Primary isolated F344 rat hepatocytes were pretreated with varying amounts of GalN (0.5 - 7.5 mM) for 30 minutes followed by 40 ng/mL TNFα treatment. TNFα and/or GalN treatment was given for 4, 24 or 48 hours. Endpoints evaluated were caspases 3 and 8 activity along with LDH leakage. Rat hepatocytes sensitized with GalN displayed increased caspase 3 activity 24 hours after treatment with 40 ng/mL TNFα. TNFα alone had no effect on caspase 3 activity. N-acetyl cysteine (NAC) treatment enhanced the activity of caspase 3 in GalN sensitized hepatocytes following 24 hour 40 ng/mL TNFα treatment. Caspase 8 activity was unaffected by GalN and TNFα treatment. Caspase 3 activity was elevated following 48 hours of treatment with GalN alone. This study suggests that GalN sensitized TNFα treated hepatocytes undergo apoptosis through a caspase 3 mediated pathway that does not appear to involve caspase 8 activation. Treatment with NAC potentiated the caspase 3 activity. These data are in contrast to effects seen in monocytic cell lines where NAC offers protection against apoptosis via its anti-oxidant properties. In addition, previous studies with actinomycin-sensitized hepatocytes have described an apoptotic pathway that includes caspase 8 activation.

528 EFFECT OF P53 STATUS ON 7H-DIBENZO[C,G]CARBAZOLE-INDUCED APOPTOSIS AND CLONOGENIC SURVIVAL.

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7H-Dibenzo[c,g]carbazole (DBC) is a potent hepatotoxin and liver carcinogen in rodents following various routes of exposure. While p53 mutations have been observed in DBC-induced murine liver tumors, the biological consequences of these mutations are unknown. The aim of this study was to determine the effects of p53 status on DBC-induced apoptosis and cell survival, *in vitro*. Hepatoma cells harboring point mutations in the p53 gene (HLF, Mahlavu) were markedly resistant to DBC-induced apoptosis relative to wild-type cells (HepG2) measured by DNA laddering, DNA content and TUNEL analyses. In contrast, colorectal carcinoma cells (RKO) overexpressing the HPV16 E6 protein underwent p53-independent apoptosis following exposure to DBC. In the HLF, Mahlavu and RKO-E6 cells, significant increases in clonogenic survival were measured following exposure to DBC. Additional factors were examined that could contribute to the marked differences observed in the hepatoma cell lines. There were no differences in 14C-DBC uptake or ROS generation by DBC in any of the cell lines. However, high concentrations of DBC (80 microM) were found to maximally deplete intracellular ATP levels within 6 hours in HepG2 cells (<50% of controls) en route to membrane leakage and necrosis. HPLC analysis of 14C-DBC-exposed cultures revealed the greatest quantity of DBC species detected in HepG2 cells suggesting that differential metabolism may be a contributing factor. While these results suggest that the role of p53 in DBC-induced apoptosis is cell type specific, the clonogenic survival following DBC exposure is dependent upon p53 status.

529 SUPPRESSION BY ZINC OF DOXORUBICIN-INDUCED APOPTOSIS: INHIBITION OF CASPASE-3 ACTIVATION.

J. C. Lambert, G. W. Wang and Y. J. Kang. *University of Louisville, Louisville, KY.*

The prevention of apoptosis by zinc has been observed in several cultured cell systems. The anti-apoptotic action has generally been attributed to its effects on late events of caspase-3-mediated apoptotic pathway, including inhibition of caspase-3 action and endonuclease activities. In this study we investigated the effect of zinc on activation of caspase-3 by doxorubicin, an effective anti-cancer agent, in HeLa cells. Cultured cells were treated with ZnC_{l2} prior to exposure to one μM doxorubicin for 12 hrs. DOX caused massive apoptosis and zinc significantly inhibited the apoptotic effect as determined by TUNEL assay and Annexin V-FITC staining. Examination by a laser confocal microscope using an antibody against the active form of caspase-3 revealed that zinc dramatically diminished DOX-activated caspase-3 activities. The effect of zinc on the processing of pro-caspase-3 by DOX was then examined by

Western blot. This process was also significantly inhibited by zinc. The results obtained from this study thus demonstrates that zinc specifically inhibits caspase-3 activation, suggesting its anti-apoptotic effect is mediated by multiple actions on the mitochondrion-controled apoptotic pathway. (Supported in part by NIH grants CA68125 and HL59225.)

530 DOXORUBICIN-INDUCED HEPATOTOXICITY MAY INVOLVE APOPTOTIC CELL DEATH BY MODULATING EXPRESSION OF BCL-XL AND P53.

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The cytotoxic effect of Doxorubicin (DOX) on malignant cells and its toxic effects on various target organs such as liver, heart, and kidney are well known. This study examined the potential of DOX to induce or enhance apoptotic cell death, and to influence the expression of apoptosis-regulatory genes such as bcl-XL and p53 in the liver. Male ICR mice, fed ad libitum, were treated ip with single doses of DOX (20, 40, 60, 80 and 120 mg/kg) and sacrificed 36 hours later. Blood was collected for analysis of serum chemistry, and liver samples for histopathology and Western blot analysis. DOX produced liver injury (>ALT levels) and DNA fragmentation in a dose-dependent manner. Higher doses of DOX (80 and 120 mg/kg) were lethal to mice. Lower doses (0, 20, 40, 60 mg/kg) inhibited bcl-XL expression in a dose-dependent manner (nearly blocking it at 40 and 60 mg/kg), and increased apoptotic death, whereas the same doses of DOX enhanced p53 gene expression. Although p53 is known to induce either cell cycle arrest or apoptosis, these findings suggest that p53 facilitates apoptotic death. (Supported by Div. of Pharmacol. & Toxicol., AMS Coll. of Pharm. & Hlth. Scs.)

531 OXIDATIVE STRESS IN KERATINOCYTES: ROLE IN APOPTOTIC SIGNALING.

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We studied the mechanism(s) through which oxidative stress participates in apoptotic signaling in keratinocytes. We exposed normal human keratinocytes (NHEKs) to cumene hydroperoxide (Cu-OOH, 50-200μM) for 1 h and found significant and dose-dependent depletion of antioxidant reserves, oxidation of GSH and protein sulfhydryls. We metabolically labeled membrane phospholipids in NHEKs with oxidation-sensitive cis-parinaric acid (PnA) to detect selective oxidation of specific phospholipid classes. We found that incubation of NHEKs with Cu-OOH resulted in dose-dependent oxidation of PnA-phospholipids: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Most importantly, PS was significantly more sensitive to Cu-OOH oxidation than other phospholipids. This selective oxidation of PS did not occur in liposomes prepared from PnA-labeled keratinocytes phospholipids. Exposure to Cu-OOH induced externalization of PS in NHEKs, as evidenced by its chemical labeling with fluorescamine. These oxidative modifications of NHEK phospholipids were not accompanied by gross changes of the phospholipid composition as evidenced by our HPTLC determinations. Together with other assays of apoptosis (caspase-3 activation and DNA laddering), our results suggest that Cu-OOH-induced selective PS oxidation may represent a signaling pathway linked to PS externalization in keratinocytes undergoing apoptosis.

532 ENHANCED GLUTATHIONE BIOSYNTHESIS RETARDS APOPTOSIS IN SPITE OF CASPASE-3 ACTIVATION IN HEPA-1 CELLS OVEREXPRESSING GLUTAMATE-CYSTEINE LIGASE.

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Glutathione is a major antioxidant that is responsible for maintaining thiol redox potential. Glutamate-Cysteine Ligase (GLCL) is the rate limiting enzyme in glutathione biosynthesis. Increased glutathione has been implicated in the resistance of certain cell types to apoptotic stimuli. We have stably transfected mouse liver Hepa-1 cells (CR17) which overexpress both the catalytic (GLCLc) and regulatory (GLCLr) subunits of GLCL, to test the hypothesis that increased glutathione synthesis is important in preventing apoptosis. Previously we have shown that CR17 cells when treated with TNF plus actinomycin D conserve their glutathione levels, NADPH levels and mitochondrial membrane potential, whereas plasmid vector alone transfectants (Hepa-V cells) did not. Caspase-3 is activated in both Hepa-V and CR17 cells with TNF+ActD treatment at 12 hrs. Western blot analysis shows substantial cleavage of GLCLc in Hepa-V cells, whereas only minimal cleavage of GLCLc was seen in CR17 cells at 22 hr. The increased glutathione biosynthesis capacity of CR17 cells not only allows them to resist TNF+ActD induced glutathione depletion, but may also inhibit apoptotic events secondary to caspase-3 activation. (This work was supported by NIH Grants ES04696, ES07033 and ES07032.)

533 COMPARATIVE *IN VITRO* STUDIES OF CADMIUM AND ARSENIC-INDUCED APOPTOSIS IN RENAL TUBULE EPITHELIAL CELLS.

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Cadmium and arsenic are nephrotoxic elements which frequently occur together in Superfund sites. The mechanism(s) by which these elements produce cell death are not completely understood. The present studies were undertaken to examine differences in the inter-species induction of apoptosis in renal tubule epithelial cells from rats, hamsters and humans following *in vitro* exposures to Cd^{2+} or As^{3+} over concentration ranges between 10-7M to 10-4M. The TUNEL assay was utilized to examine the induction of apoptosis in relation to cell injury as monitored by changes in ALAMAR Blue fluorescence. Results of these studies indicated that both elements were capable of producing apoptosis in cells from all 3 species but that the cellular mechanisms appear different for Cd^{2+} and As^{3+} since there were marked morphological response differences for each element. Arsenic (As^{3+}) exposure also produced necrosis in some cells so that both necrosis and apoptosis mechanisms were operating. The results of the ALAMAR Blue assay showed a dose-related decreases in cell metabolism following both cadmium and arsenic exposure. Overall the results of these studies indicate that renal tubule epithelial cells from all 3 species are susceptible in a similar manner to apoptosis induced by Cd^{2+} and As^{3+} exposure. It is hypothesized that the As^{3+} is operating via mitochondrial apoptotic inducing factors while the mechanism for cadmium is presently unclear. (Supported by USEPA STAR Grant ER 827161-01-0.)

534 ACROLEIN ENHANCES MECHLORETHAMINE-INDUCED APOPTOSIS.

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Acrolein, a highly electrophilic α,β-unsaturated aldehyde is, along with phosphoramide mustard, a metabolic product of cyclophosphamide (CP). Although acrolein is generally considered to be a toxic metabolite, at low concentrations it may enhance the activity of CP by sensitizing cells to apoptosis. To test this theory, B lymphocytic FL5.12 cells were incubated for 0.5 h with 2.5 μM acrolein followed by 150 or 250 nM mechlorethamine. Acrolein depleted GSH by ~80% at 0.5 h. Apoptosis was morphologically distinguished from necrosis by the acridine orange/ethidium bromide assay. At 24 h post-treatment, acrolein alone caused 15% apoptosis and 6% necrosis, whereas mechlorethamine alone caused 23 and 33% apoptosis and 5 and 2% necrosis at 150 and 250 nM, respectively. When acrolein and 150 nM mechlorethamine were combined there was 44% apoptosis and 4% necrosis. Acrolein with 250 nM mechlorethamine caused 60% apoptosis and 5% necrosis. Enhanced apoptosis was more evident using an ELISA to assess nucleosomes in the cytoplasm. To test the possibility this sensitization effect was working through GSH depletion, FL5.12 cells were treated with 1 mM diethyl maleate (DEM) (lowering GSH by ~70%). Surprisingly, DEM alone caused a significant amount of necrosis, making it impossible to assess the effect of co-treatment with mechlorethamine. These data demonstrate that acrolein enhances apoptosis induced by subsequent treatment with an alklyating agent. At comparable levels of GSH depletion, the effects of acrolein and DEM differ suggesting mechanistic differences. The ability of acrolein to inhibit NF-κB activation at 4 h may be a factor in the observed apoptosis. (Supported by HL48035, ES09791 and ES07784.)

535 DIFFERENTIAL VINCRISTINE-INDUCED ENGAGEMENT OF APOPTOSIS IN DRUG RESISTANT AND SENSITIVE LYMPHOMA CELL LINES.

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Resistance of cancer cells to chemotherapeutic drug treatments can involve several different mechanisms. Although most drug treatments induce apoptosis, it is less clear in what cases differential triggering of this process plays a prominent role in drug resistance. We used a panel of drug resistant and sensitive cell lines to assess the role of apoptosis induction in cellular responses to the lymphoma drug and spindle poison vincristine (VCR). Avian and human lymphoma cell lines were exposed to graded concentrations of VCR and studied for the extent of mitotic inhibition and apoptosis at 6, 12, 24, and 48 hours using a combination DNA and viability fluorescence assay. Drug resistant and sensitive lymphoma cell lines were shown to be arrested in mitosis to a similar extent, about 50% blocked cells at 6 hours. VCR induced apoptosis quickly and extensively in the avian DT40 B lymphoma cells, 80% apoptosis at 12 hours, but only modestly (less than 10%) in the CU159 T lymphoma cells. CU159 cells persisted in a mitotically blocked state without entering an apoptotic cascade. The Burkitt's lymphoma cell lines also showed differential sensitivity to VCR-induced apoptosis. The ST486 cell line underwent apoptosis readily whereas the CA46 cell line survived in a mitotically blocked condition without engaging in apoptosis. The EW36 cell line showed intermediate sensitivity to VCR-induced apoptosis. Caspase-3 activation was found to be coincident with the engagement of apoptosis observed morphologically. These results suggest a prominent role for differential triggering of apoptosis, associated with caspase-3 activation, underlying drug resistance. In lymphoma cells, regulation of differential apoptosis induction by VCR appears to be upstream of caspase-3 activation.

536 CELLULAR GLUTATHIONE STATUS MODULATES PCB-INDUCED STRESS RESPONSE AND APOPTOSIS IN PORCINE ENDOTHELIAL CELLS.

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Exposure to environmental contaminants, such as polychlorinated biphenyls (PCBs), may severely compromise normal function of vascular endothelial cells (EC). We have previously shown that PCB 77 (3,3'4,4'-tetrachlorobiphenyl), an arylhydrocarbon receptor (AhR) agonist, can induce oxidative stress in cultured EC. We now show that PCB 77 can activate EC and induce a cellular stress response which is reflected by the activation of c-Jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK). Our data suggest that this PCB 77-mediated stress response can be modulated by the intracellular glutathione (GSH) content, which was initially decreased in cells exposed to PCB 77. EC pretreated with buthionine-sulphoximine (BSO), an inhibitor of GSH synthesis, delayed the late recovery of PCB-induced JNK/SAPK activity. In contrast, media supplementation with the glutathione precursor N-acetyl-cysteine (NAC) was sufficient to inhibit PCB 77-induced JNK/SAPK. Although PCB 77 alone did not induce apoptosis, combined treatment with PCB 77 and BSO induced the activation of caspase 3, annexin V and DNA fragmentation. The caspase-3-specific inhibitor DEVD-CHO protected cells against PCB 77/BSO mediated-apoptosis and inhibited the caspase activity without affecting JNK/SAPK activation or cellular glutathione levels. These results suggest that AhR ligands, such as PCB 77 cause vascular EC dysfunction by modulating intracellular GSH, which subsequently leads to activation of stress-specific kinases. Furthermore, inhibition of glutathione synthesis by BSO or possibly other prooxidants can further potentiate the PCB 77-induced stress response, and ultimately lead to apoptotic cell death. (Supported in part by grants from NIEHS/EPA, and the KY Agr. Exp. Station.)

537 3-METHYLINDOLE (3MI) CAUSES BOTH APOPTOSIS AND NECROSIS IN CULTURED HUMAN LUNG CELLS.

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The immortalized lung cell line (BEAS-2B) is susceptible to cytotoxic effects of the pneumotoxicant 3MI. Bioactivation of 3MI by cytochrome P450 enzymes is required to produce a reactive metabolite that is responsible for the formation of protein and DNA adducts. Earlier studies by our laboratory have shown that 3MI can cause cytotoxicity based on the release of lactic dehydrogenase into the media during incubations of human and rabbit lung cells with this toxicant. Evidence of apoptosis as well as necrosis has been demonstrated by fluorescence microscopy using acridine orange and ethidium bromide. An increase in apoptotic nuclei has been observed within 18 hr for BEAS-2B cells incubated with 3MI. In addition, an increase in DNA damage is observed within 24 hr by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling. Although most cells appear to undergo necrosis, there is a significant degree of cells induced to undergo apoptosis in the presence of 3MI. These studies indicate that some cellular injury induced by 3MI promotes both apoptosis and primary necrosis in cultured human lung cells incubated with this toxicant. (Supported by USPHS grant HL13645 from NHLBI.)

538 THE JNK AND P38MAPK ARE NOT ACTIVATED IN BREAST CANCER CELLS MCF-7 BY GENISTEIN.

S. Nomoto and F. Kayama. *Jichi Medical School, Kawachi, Japan.* Sponsor: T. Yoshida.

Breast cancer is the most common cancer among American women, whereas Asian women, particularly Chinese and Japanese, have a relatively low incidence. It is believed that genistein, a prominent isoflavonoid contained in soy products, may be responsible for reducing the rate of breast cancer in Asian women, due to high rate of soy products in Asian diet. Genistein was shown to arrest cell cycle at the G2-M phase and induce apoptosis in breast cancer cell lines. However the signal transduction pathway(s) activated by the treatment of genistein has not been clearly identified. We investigated the effects of genistein on the MAP kinase cascades, the JNK and p38MAPK pathways. When we treat the cells with 30μM of genistein, the rate of cell death reached 65% after 24 h and 81% after 48 h, indicating that genistein treatment at this concentration causes apoptosis. Under the same condition, we performed Western blotting analysis using phospho-specific antibodies against JNK and p38MAPK that recognize the kinases phosphorylated as the result of activation of upstream kinases. The phosphorylated forms of JNK and p38MAPK were not identified after genistein treatment, suggesting that the JNK and p38MAPK pathways were not activated by genistein in MCF-7 cells. The involvement of other possible signaling pathways is currently under investigation.

539 EFFECT OF METALLOTHIONEIN ON DOXORUBICIN-INDUCED APOPTOSIS IN CARDIOMYOCYTES: ROLE OF CYTOCHROME C-ACTIVATED CASPASE-3.

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In previous studies, we have demonstrated that metallothionein (MT), a potent antioxidant, functions in cardiac protection against doxorubicin (DOX)-induced injury. The present study was undertaken to investigate possible mechanisms by which MT functions in this cardioprotection. Because apoptosis is a constant feature of myocardial damage following oxidative stress and reactive oxygen radicals generated from DOX are highly responsible for the drug's cardiotoxicity, this study focused on effects of MT on DOX-induced myocardial apoptosis using primary neonatal cardiomyocyte cultures. Cardiomyocytes isolated from MT-overexpressing transgenic neonatal mice and non-transgenic controls were treated with DOX at different concentrations for varying periods of time. Cardiomyocyte apoptosis was detected by a TUNEL assay and an Annexin V-fluorescein isothiocyanate binding. To investigate cellular and molecular mechanisms leading to DOX-induced apoptosis, the activation of mitochondrion-controlled apoptotic pathway was examined, including Western blot analysis of mitochondrial release of cytochrome c and caspase-3 activation, the latter was further confirmed by laser confocal microscope using an antibody against the active form of caspase-3. DOX markedly induced apoptosis in non-transgenic cardiomyocyte cultures as a function of time in a dose-dependent fashion. This apoptotic effect was significantly suppressed in the MT-overexpressing transgenic cardiomyocyte cultures. Corresponding to the apoptotic effect of DOX and the inhibitory effect of MT, increased mitochondrial release of cytochrome c and activation of caspase-3 were observed in the non-transgenic cardiomyocytes following DOX treatment. The activation of this mitochondrion-controlled apoptotic pathway was almost completely blocked in MT-overexpressing transgenic cardiomyocytes. These results demonstrate that MT suppresses DOX-induced apoptosis in cardiomyocytes through at least in part inhibition of mitochondrion-controlled apoptotic pathway. (Supported in part by NIH grants CA68125 and HL59225, American Heart Association EI award (9640091N) and Jewish Hospital Foundation, Louisville, Kentucky.)

540 CHARACTERIZATION OF THE ARSENITE DOSE RESPONSE CURVE ON PROMYELOCYTIC LEUKEMIA CELLS (U-937).

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Arsenic is a known environmental toxicant and carcinogen. Chronic arsenicism leads to several cancers, yet arsenic trioxide treatment has been found recently to induce clinical remission in patients with acute promyelocytic leukemia (APL). While some data suggest that the potential therapeutic value of arsenicals may lie in triggering apoptosis, it has not been established that cytotoxicity is the sole mechanism of action. We have used a promyelocytic leukemia cell line (U-937) to characterize the dose dependent effects of arsenite on cell growth, viability, apoptosis and differentiation. Arsenite has diverse effects on U937 cells that are dependent on concentration. Low concentrations of arsenite (i.e., 1μM) potentiate the vitamin-D3-induced differentiation of U937 cells towards monocytic maturation. Both Mac-1 expression and NBT reduction, markers of monocyte differentiation, are increased in arsenite-exposed, D3-co-stimulated cells. Intermediate concentrations of arsenite (i.e., 5 to 10 μM) are cytostatic but not cytotoxic. Cell cycle analysis using elutriated, synchronous cell populations revealed that arsenite delays cell cycle transit by affecting both the G1 and G2phases of the cell cycle. Higher concentrations (i.e., >10 μM) or prolonged (i.e., 24 hr) incubation with intermediate concentrations of arsenite induced apoptotic cell death based on both morphological and biochemical criteria. Arsenite-induced apoptosis was caspase-3 dependent, as caspase-3 was activated in a time- and concentration-dependent fashion. Moreover, arsenite induced cell death was prevented in the presence of the broad caspase inhibitor, z-vad-fmk. The caspase inhibitor did not reverse arsenite-induced cytostasis, suggesting that the cell cycle and apoptotic effects of arsenite are separable. Our studies show that arsenite has a differential dose effect on promyelocytic leukemia cells, and that cell cycle control and differentiation are more sensitive parameters than is cell death. (Supported by P30-ES06639.)

541 ROLE OF BCL-2 AND BCL-X_L IN MITOCHONDRIAL PERMEABILITY TRANSITION INDUCED BY THE RADIOSENSITIZER CI-1010.

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The mitochondrial permeability transition (MPT) is a high conductance transmembrane pore that opens prior to apoptosis *in vitro*. Expression of antiapoptotic proteins such as Bcl-2 and Bcl- X_L stabilize the pore in the closed conformation, conferring protection against neurotoxicants. CI-1010 ((R)-α [(2-bromoethyl)-amino]methyl]-2-nitro-1H-imidazole-1-ethanol monohydrobromide), is a nitroimidazole that produces progressive retinal neuronal degeneration with sparing of adjacent glial cells. Differential cellular susceptibility to CI-1010 is mediated by opening of the MPT and correlates with differential expression of Bcl-2 and Bcl-X_L in astrocytes and neurons. The present study uses human SY5Y neuroblastoma and rat C6 glioma cells as a model of retinal astrocytes and neurons: CI-1010 treated SY5Y cells undergo apoptosis in culture. Following exposure to 750 μM CI-1010 at 1, 2, 4, and 24 hours, expression of Bcl-2 and Bcl-X_L was monitored using western blot analysis and opening of the MPT imaged by confocal microscopy (TMRM, calcein AM, propidium iodide). Loss of mitochondrial reducing potential was determined by the MTT cytotoxicity assay. C6 glioma cells express high constitutive levels of Bcl-2 and Bcl-X_L, however, increased expression of both proteins was detected in both cell types 24h after exposure to CI-1010. CI-1010 also induced rapid mitochondrial depolarization in SY5Y neuroblastoma cells and C6 glioma cells within 10 min. Viability assays revealed a lower LC_{50} for SY5Y approximately half that of C6 treated with CI-1010. The precise role of Bcl-2 and Bcl-X_L in the regulation of MPT-induced cell death remains to be determined. However, these results suggest that constitutive expression of Bcl proteins may confer increased protection against nitrocompound-induced neurotoxicity.

542 DYSREGULATION OF CD95-MEDIATED APOPTOSIS BY MERCURY.

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Exposure to inorganic mercury (Hg^{2+}) has been associated with autoimmune disease; however, the underlying mechanisms are poorly understood. Since dysregulation of CD95/Fas-mediated apoptosis has been implicated in autoimmune disorders, we have hypothesized that Hg^{2+} could contribute to autoimmunity by dysregulating apoptosis in autoreactive lymphocytes. Using Jurkat T cells we have established that 5-10 μM $HgCl_2$ attenuates CD95-mediated apoptosis by targeting a molecular component upstream of caspase3 or caspase3, itself. Hg^{2+} does not appear to block the activation of caspase9 or caspase8, suggesting that mercury does not dysregulate the CD95 pathway by direct interaction with caspases. Furthermore, using a caspase3 enzyme activity assay we have ruled-out the possibility that caspase3 is directly targeted by mercury. Hg^{2+} loses its ability to block caspase3 activity when added after anti-CD95 in a time dependent fashion. Moreover, Hg^{2+} does not block TNF-mediated activation of caspase3 indicating that the molecular component responsible for the inhibition of caspase3 by Hg^{2+} in the CD95 pathway is not present in the TNF pathway. Experiments using human peripheral blood lymphoblasts are being conducted to corroborate the above findings. This study is the first to correlate Hg^{2+}exposure with dysregulation of the CD95 death pathway. It represents a model to test the hypothesis that modulation of the CD95 pathway by Hg^{2+} contributes to a breakdown in peripheral tolerance leading to autoimmunity. (Supported by P30-ES06639 and R21-ES10351.)

543 METHYLMERCURY AND MERCURIC CHLORIDE AFFECT NEURONAL APOPTOSIS IN PC12 CELLS IN A DOSE- AND NGF-DEPENDENT FASHION.

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Methylmercury (CH3Hg), an ubiquitous environmental toxicant, has a profound effect on the developing central nervous system. Gestational exposure to CH3Hg has led to neurological abnormalities resulting from death of neurons and altered cytoarchitecture in the developing CNS. We examined the effects CH3HgCl and HgCl2 on cell death in rat pheochromocytoma (PC12) cells. PC12 cells were exposed to 0, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mM of either CH3Hg or HgCl2 in the presence or absence of 50 ng/ml NGF for 24 hours. Following exposure, fragmented DNA, an endpoint of apoptosis, was quantified by ELISA. Determinations of alterations in cell size and morphology were also made. In the presence of NGF, CH3Hg caused increased levels of fragmented DNA at concentrations of 1, 3 and 10 mM, while HgCl2 had no significant effect on fragmented DNA at any of the concentrations tested. In the absence of NGF, control levels of fragmented DNA were approximately 8-12 times higher than in the presence of NGF and both mercury compounds increased fragmented DNA at the higher concentrations (3 and 10 mM). Effects on cell size were also observed following exposure to CH3Hg. In the presence of NGF, a significant decrease in cell size was observed at 0.3-10 mM of CH3Hg, while no significant change in cell body size was seen following exposure to HgCl2. These data suggest that, in the presence of NGF, CH3Hg but not HgCl2, induces apoptotic cell death. (This abstract does not necessarily reflect EPA policy.)

544 PARP EXPRESSION AND CLEAVAGE PRECEDE NEURONAL DEATH FOLLOWING OXIDATIVE INJURY IN NEONATAL RATS.

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Background: Nitric oxide (NO) and peroxynitrite have been implicated as mediators of neuronal death following focal brain ischemia. Studies in adult human and rat brain have implicated DNA damage and the activation of poly (ADP-ribose) polymerase (PARP) in this pathway to neuronal death. Neuronal vulnerability to insult peaks at post-natal day 7 in the rat, a period associated with rapid brain growth and the formation of synaptic connections. Death of neurons during the comparable period in humans is known to have long-term consequences, for example Cerebral Palsy. PARP expression in neonatal rats following oxidative injury has not been previously examined and, if present, may be a target for therapeutic intervention. **Objective:** We tested the hypothesis that PARP expression is rapidly increased in response to a hypoxic/ischemic NO-mediated insult, and that the cleavage of PARP would precede the onset of morphological changes consistent with cell death. **Experimental Design:** Hypoxic-ischemic injury was induced by left common carotid artery ligation of 7 day old rats and was followed by a 90 or 120 minute exposure to 7.6% oxygen. Extent of injury and expression of PARP were examined 1, 2, 6 and 12 hrs post-hypoxia. Hematoxylin and eosin stained coronal brain sections were examined for morphological changes and TUNEL staining was employed to identify neuronal death. PARP cleavage was detected with anti-PARP antibodies by Western blot. **Results:** Significant morphological changes such as vacuolization of the neuropil, cell shrinkage,

pyknotic nuclei and increased cytoplasmic eosinophilia were observed primarily in the ipsilateral neocortex in rats with a 12-hr survival period but not in brains of rats who survived <12 hrs. PARP cleavage was detectable in rats surviving 6 hrs post-hypoxia. **Conclusions:** Our observations of PARP expression prior to the appearance of morphological changes suggest a window in which PARP inhibitors could be administered to block subsequent neuronal death and minimize brain damage. (Supported by NIEHS T32 ES07254.)

545 INVOLVEMENT OF NMDA-RESPONSIVE NEURONS IN STAUROSPORINE AND OXYGEN/GLUCOSE DEPRIVATION-INDUCED CELL DEATH.

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Cell death in primary rat cortical neuronal cultures was investigated using the excitotoxin N-methyl-D-aspartate (NMDA) or the pro-apoptotic agent staurosporine (STS). The mode of cell death was compared/contrasted to an *in vitro* model of ischemia which utilized 5 hr of oxygen and glucose deprivation (OGD) followed by re-oxygenation. Cultures exposed to NMDA undergo necrotic cell death typified by lactate dehydrogenase (LDH) leakage with little caspase-3 activation or oligonucleosome formation. Cells exposed to STS exhibit rapid, extensive activation of caspase-3 with coincident LDH leakage and generation of oligonucleosomes. Both LDH leakage and oligonucleosome content were significantly greater at 48 hr than at 20 hr with STS treatment. Pretreatment of the cultures with NMDA for 4 days prior to STS effectively killed NMDA-responsive neurons, prevented caspase-3 activation and diminished LDH leakage and oligonucleosome formation. Following OGD with re-oxygenation, extensive oligonucleosome formation and LDH leakage is observed in the absence of caspase-3 activation. Depletion of NMDA-responsive neurons prevented OGD-induced LDH leakage and oligonucleosome formation. Partial protection was provided by the NMDA channel blocker MK-801 which also restored sensitivity to STS-induced caspase-3 activation. The data with OGD are consistent with 1) a non-apoptotic cell death in NMDA-responsive neurons which resembles excitotoxicity and 2) a caspase-3 insensitive apoptosis in remaining neurons. Thus, OGD induces both necrotic and apoptotic cell injury and the modality of cell death in NMDA-sensitive neurons is dependent upon the initiating event and the time over which the phenomenon is studied.

546 EXPRESSION OF THE MITOCHONDRIAL PROTEIN SM-20 PROMOTES DEATH IN NERVE GROWTH FACTOR-DEPENDENT SYMPATHETIC NEURONS.

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The withdrawal of nerve growth factor (NGF) from sympathetic neurons results in cell death. Since the death of these neurons is blocked by inhibitors of RNA and protein synthesis, we hypothesize that genes specifically expressed following NGF withdrawal are likely to be important in the apoptotic pathway. Using the technique of differential display, we identified a gene called SM-20 that is preferentially expressed in NGF-deprived (dying) versus NGF-maintained (healthy) sympathetic neurons. To begin characterization of the SM-20 protein, we performed subcellular localization studies. In immunofluorescent experiments, SM-20 colocalized with Mitotracker Green FM, a mitochondria-selective dye, and cytochrome oxidase I, a mitochondrial protein. We found that the first 25 amino acids of SM-20 are sufficient to target dihydrofolate reductase (DHFR), a cytosolic protein, to the mitochondria. To explore the significance of SM-20 in the apoptotic pathway, we overexpressed SM-20 in sympathetic neurons maintained in the presence of NGF. Microinjection of SM-20 expression vectors resulted in decreased cell survival as compared with b-galactosidase injected controls. These results suggest that increased SM-20 protein may contribute to neuronal death by affecting mitochondrial function. Recent microinjection studies have demonstrated that SM-20(51-355), a truncated form of SM-20 lacking the first 50 amino acids, can elicit cell death comparable to full-length SM-20 when overexpressed in sympathetic neurons. Thus, it is conceivable that localization of SM-20 to the mitochondria is not necessary for its death-promoting activity. Currently, we are investigating the effects of tetracycline-inducible expression of SM-20 on the survival of rat PC12 cells. (Supported in part by NIH Grant NS34400 and NIH Toxicology Training Grant ES07026.)

547 ROLE OF CYTOCHROME C IN CYANIDE-INDUCED APOPTOTIC CELL DEATH.

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Previous studies show that cyanide (KCN) can induce neuronal apoptotic cell death, which is mediated by oxidative stress following NMDA receptor activation and involves the caspase-3-like proteinase. Release of the apoptotic factor cytochrome C from mitochondria into the cytosol is an early signaling factor for a variety of apoptotic stimuli. In the present study, cerebellar granule cells were used to investigate the early signaling events responsible for the induction of apoptosis in cyanide-induced cell death. In this cell model, exposure to cyanide at different concentrations (100-500 μM) for 24 hrs induced maximum release of cytochrome C into the cytosolic fraction as assessed by western blot. This was associated with enhanced caspase 3-like proteinase expression, followed by apoptosis as determined by western blot and TUNEL staining, respectively. It was found that induction of cytochrome C release into the cytosol occurred as early as 3 hrs of cyanide exposure, which proceeded the initiation of caspase activation. Elevation cytosolic cytochrome C was blocked by pretreatment with uric acid, a scavenger of peroxinitrite, indicating that oxidative stress plays a role in the release of cytochrome C from the mitochondria. This process was initiated by NMDA receptor activation since MK-801 blocked the elevation of cytosolic cytochrome C. However, the caspase-3 inhibitor, Ac-DEVD, did not prevent cytochrome C release, indicating the induction of cytochrome C release is upstream to caspase activation in cyanide- induced cell death. (Supported by NIH grant ESO4140.)

548 DIETHYLDITHIOCARBAMATE INDUCES APOPTOSIS IN RAT HIPPOCAMPAL ASTROCYTES.

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Diethyldithiocarbamate (DDC), a potent metal chelator and pro-oxidant has been shown to be neurotoxic. This may be due to its chelating ability resulting in an increase of intracellular metal concentration or by forming disulfide linkages with essential proteins. The pathogenesis of thiocarbamate toxicity is not clearly understood. Mammalian cells have evolved numerous responses to toxicity. Apoptosis, a form of cell death, is an important regulatory elimination pathway in the development of many animal cell types. The purpose of this experiment was to determine if DDC caused apoptosis in rat hippocampal astrocytes. Astrocytes grown in Dulbecco's modified Eagle's essential media were treated with 35 and 350 μg/ml DDC at subconfluency for 1 hr, washed and refed with normal media. Cells were collected at 24, 48 and 72 hr following DDC post-treatment. Apoptosis was detected using an *in situ* staining method for DNA strand breaks detected by TUNEL and visualized by light microscopy. Apoptosis was observed at 48 and 72 hr post-treatment at the 35 μg/ml DDC dose and observations were dramatically increased at the 350 μg/ml dose. No apoptotic cells were seen at the 24 hr post-treatment time period regardless of dose. This investigation suggests that cell death resulting from DDC toxicity may be due to an apoptotic event and not solely due to necrosis.

549 DOPAMINE AND CYANIDE-INDUCED NEURONAL APOPTOSIS IN RAT MESENCEPHALON CULTURES.

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Dopamine (DA) neurotoxicity has been implicated in the degeneration of dopaminergic neurons. The goal of the present study was to examine DA toxicity in rat mesencephalon cells. Since neurons may be more susceptible to injury under energy impaired conditions, cyanide (KCN) was use to inhibit mitochondrial respiration. Cells were incubated with DA (300 mM) and/or KCN (100 mM) for 24 hrs. TUNEL staining revealed that DA-induces apoptosis and KCN increased this effect, suggesting that impaired energy metabolism potentiates DA toxicity. Intracellular generation of reactive oxygen species (ROS) increased significantly in DA-treated cells and was potentiated by KCN. This effect was attenuated with glutathione and uric acid, indicating involvement of ROS and NO in DA toxicity. Furthermore, DA and DA + KCN produced an immediate increase in Ca++. The increase in intracellular ROS and Ca++ preceded depolarization of the mitochondrial membrane. Cells exposed to DA and/or KCN for 1, 2, and 4 hrs exhibited mitochondrial depolarization. Western blot analysis demonstrated that cells incubated with DA had increased levels of cytosolic cytochrome c. Cytochrome c release was potentiated by KCN and blocked by pre-treatment with glutathione and uric

acid. Exposure of cells to DA, and/or KCN resulted in caspase 3 activation, which was maximal at 12-18 hrs. It was concluded that mesencephalon cells incubated with DA undergo apoptosis, which is potentiated by compromised neuronal energetics. DA-induced apoptosis is mediated by ROS and involves the initiation of an intracellular cascade characterized by; a rise in intracellular Ca++, depolarization of the mitochondrial membrane, the release of cytochrome c and activation of caspase 3. (Supported by NIH grant ESO4140.)

550 PYRIDOSTIGMINE-INDUCED CEREBELLA GRANULE CELL DNA FRAGMENTATION VIA LOSS OF MITOCHONDRIAL MEMBRANE POTENTIAL AND ACTIVATION OF CASPASE-LIKE PROTEASES.

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Pyridostigmine bromide (PB) is a reversible cholinesterase inhibitor that has been used for treatment of myasthenia gravis and for prophylactic protection against organophosphate nerve agent. We have previously shown that PB can induce apoptotic cell death in the rat brain. In this study we investigated the role of loss of mitochondrial membrane potential and caspase-like protease activity in PB-induced DNA fragmentation in rat cerebellar granule cell. DNA fragmentation was determined by gel electrophoresis of extracted DNA and TUNEL staining cerebella granule cells. Exposure of cerebellar granule cells to PB(250 μM) for 24 hr caused apoptotic cell death. Pretreatment of the cells with the caspase inhibitor Z-Val-Ala-DL-Asp-Fluoromethyl keton (zVAD-flk 100 μM) and the muscarinic receptor antagonist atropine (10 μM) blocked cellular chromatin condensation and the DNA fragmentation induced by PB. To confirm whether caspase-like proteases were involved in the apoptotic events, we treated granule cells with varying concentrations of PB (50-500 μM) for 24 hr. PB caused a dose-dependent increase in the activity of caspase-like proteases. Time studies showed that caspase-like protease activity was first elevated at 4 hr and was markedly increased at 16-24 hr. Both zVAD-flk and atropine blocked caspase-like protease activity induced by PB exposure. Using RH123 as a probe to determine the changes of mitochondrial membrane potential, PB induced a dose-dependent loss of mitochondrial membrane potential in the cells, which was blocked by atropine. These results show that PB-induced cerebellar granule cell DNA framentation involves loss of mitochondrial membrane potential and activation of caspase-like proteases. (Supported by U.S. Army grant DAM 17-97-71354.)

551 METABOLITES OF CHLORPYRIFOS INDUCE APOPTOSIS IN PC12 CELLS.

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Previously we reported that neurite outgrowth was inhibited by the cholinesterase-inhibiting pesticide chlorpyrifos (CPF) and its metabolites, CPF-oxon and trichlorpyridinol (TCP) in differentiated PC12 cells. These effects of CPF and TCP were observed in the absence of cholinesterase inhibition. In the present study, we evaluated the effects of CPF, CPF-oxon and TCP on apoptosis in differentiated and undifferentiated PC12 cells. PC12 cells were differentiated for one week with 50 ng/ml of hrNGF followed by replating cells at 1x105 cells in 24 well plates. Following NGF withdrawal, 5 ng/ml of hrNGF prevented 50% of the cell death in differentiated cells, and 0.1 ng/ml was equally effective in undifferentiated cells. Apoptosis was examined in the presence or absence of different doses (0.1, 1, 10, 100 μM or nM) of CPF, TCP, or CPF-oxon with or without hrNGF at 37 C for 24 h. Cells were lysed and fragmented DNA was assessed using a cell death ELISA. Our results show that, i) TCP (1 μM) or CPF-oxon (1 nM) alone induced apoptotic death, ii) in the absence of NGF, CPF-oxon or TCP showed a dose-dependent induction of apoptotic death, and iii) exposure to NGF (10 ng/ml) protected against cell death at all doses of TCP and CPF-oxon used except at 100 μM and 100 nM, respectively. These results suggest that differentiated PC12 cells are more vulnerable to apoptotic cell death following NGF withdrawal than undifferentiated cells and that NGF under certain conditions can protect against this pesticide-induced apoptosis. (This abstract does not necessarily reflect EPA policy.)

552 INDUCTION OF MUTATION AND APOPTOSIS IN MOUSE PRIMORDIAL GERM CELLS BY ENU.

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Primordial germ cells (PGC) are the ancestor cells of both male and female gamete. If a mutation is induced in a PGC, all germ cells derived from it possess the same mutation (cluster mutations). We have found that N-ethyl-N-nitrosourea (ENU) induces recessive mutations at a relatively high rate in male mice primordial germ cells (PGC) at 8.5, 10.5 and 13.5 days of development (G8.5, G10.5 and G13.5) using a specific locus test (Shibuya et al., 1993 and 1996). An apparent stage-specificity have been observed on the induction of mutations, i.e., G10.5 is the highest, followed by G8.5 and then G13.5. The cluster mutations have been easily obtained in G8.5 and G10.5 PGC. The high mutation rate in G8.5 and G10.5 are resulted from incidences of cluster mutations. Furthermore, a dose-dependent induction of mutations was also observed in both G8.5 and G10.5 PGC. It is known that the numbers of PGC at these developmental stages are very small and PGC proliferate rapidly with a doubling time of about 16 hr at these developmental stages. Therefore, the induction of cluster mutation by ENU might result from complex phenomena. Furthermore, the cell killing effect may modify the induced mutation rate. To elucidate the cell kinetics on mutation induction in PGC by ENU, we studied on apoptosis of PGC G13.5 in genital ridge of male treated with ENU at G10.5. ENU induced apoptosis dose-dependently in PGC. Therefore, apoptosis induced by ENU is one of an important factor to induce cluster mutations.

553 PARTICIPATION OF THE P53 PROTEIN ON THE MEMBRANE EXPRESSION OF FAS IN THE GC-2SPD(TS) GERM CELL LINE.

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The tumor suppressore p53 is a transcription factor that is involved in the regulation of cell growth and apoptosis. Recent observations suggest that the p53 protein can influence the re-distribution of the Fas receptor (Fas) from a cytosolic location to the cell membrane by a transcription-independent mechanism. The Fas-system has been previously shown participate in the regulation of testicular germ cell apoptosis. In this study, we evaluate the influence of p53 activation on the membrane expression of Fas in a p53-termperature-sensitive germ cell line (GC-2spd). In these cells, p53 is activated and found exclusively in the nucleus at the permissive temperature of 32°C, Both western blot analysis and immunocytochemistry were used to examine the membrane expression of Fas in these cells. In cells maintained at 32°C, significant increase in the membrane expression of Fas was observed compared to those maintained at 37°C. The sensitivity of GC-2spd cells to Fas-mediated apoptosis was assessed by the addition of a Fas-activating antibody (5ug/ml, Jo-2, PharMigen) to the culture media. Apoptosis was assessed by erminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL). In GC-2spd cells maintained at 32°C, a large increase in TUNEL-positive cells was observed after 24 h and few viable cells remained attached to the culture matrix after 48 h. Taken together, these data indicate the involvement of p53 in the localization of Fas to the germ cell membrane. (Supported by NIEHS grants ES09145 & ES07784.)

554 DIFFERENTIAL SENSITIVITY OF YOUNG AND ADULT FASml MUTANT *GLD* MIDE TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP)-INDUCED TESTICULAR GERM CELL APOPTOSIS.

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Apoptosis of testicular germ cells is essential for functinonal spermatogenesis. The Fas-signaling system has been proposed as a mechanism by which Fas ligand (FasL)-expressing Sertoli cells initiate apoptosis of Fas receptor (Fas)-positive germ cells. Exposure of C57/b16 mice to the Sertoli cell toxicant MEHP (1 g/kg, p.o.) induces a rapid increase in the incidence of germ cell apoptosis. Previous work of our group revealed the insensitivity of prepubertal gld mice, which express a non-functional form of FasL, to MEHP-induced germ cell apoptosis for up to 24 h after exposure. Regardless of this insensitivity, the gld mice exhibit no significant testicular-related abnormalities (e.g.,m massive hyperplasia, infertility, etc) compared to the parent strain. Therefore, we tested whether the decreased sensitivity of germ cells to MEHP-induced apoptosis extends beyone 24 h and if it is dependent upon the

age of the gld mice. Young 28-day-old gld and wild-type mice wer given an acute oral dose of MEHP (1 g/kg) and testes collected aftger 48, 72, 96 and 120 h. Adult mice were given a single dose of MEHP (2 g/kg) and testes collected after 6, 12, 24 and 48 h. Characteristic apoptotic DNA collected after 6, 12, 24, and 48 h (slightly elevated compared to 24 h) whereas a 3-fold increase was seen in wild-type mice (similar to 24 h level). In adult gld and wild-type mice an identical 3-fold increase in TUNEL-positive cells was observed 24 h aftger MEHP exposure. These data suggest that germ cell was observed 24 h after MEHP exposure. These data suggest that germ cell apoptosis occurs in adult, and to a lesser degree in young, gld mice via a Fas-independent signaling mechanism. This mouse model may be useful in providing new insights into additional signaling pathways regulating testicular germ cell apoptosis. (Supported by NIEHS grants ES089145 and ES07784.)

555 SALICYLIC ACID AND CHLOROFORM-INDUCED NEPHROTOXICITIES MAY INVOLVE GENOMIC DNA FRAGMENTATION AND CELL DEATH BY APOPTOSIS.

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Chloroform (Chfm) and salicylic Acid (SA), two well known nephrotoxins, show apoptogenicity towards the liver. The objectives of the present investigation were to determine whether Chfm and SA have the potential to induce genomic DNA fragmentation in kidneys, and if so, whether they turn on apoptotic cell death in addition to necrosis *in vivo*. In order to assess these events, male ICR mice (25-45g) were treated with nephrotoxic doses of Chfm (200-550 mg/Kg, i.p.) and SA (200-500 mg/Kg i.p.) for 24 hours. Blood was collected for the determination of plasma BUN (nephrotoxicity) and malondialdehyde levels (MDA: malondialdehyde, a marker of lipid peroxidation), and superoxide dismutase activity (SOD: a marker of oxidative damage). Kidney tissues were histopathologically evaluated for apoptotic/necrotic deaths and analyzed for DNA fragmentation. Results show that both Chfm and SA induce significant DNA fragmentation and cause cell death by apoptosis. Although, Chfm and SA both were equally effective in inducing nephrotoxicity, SA was slightly more potent in causing DNA damage. In addition, both these agents showed increased levels of MDA and SOD activity indicating possible involvement of oxidative stress. Collectively, these studies establish links between oxidative-stress-mediated nephrotoxicity, DNA fragmentation, and apoptotic cell death in kidneys.

556 DICLOFENAC-INDUCED NEPHROTOXICITY MAY INVOLVE OXIDATIVE STRESS AND MASSIVE GENOMIC DNA FRAGMENTATION *IN VIVO*.

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Diclofenac (DCLF) is a nonsteroidal anti-inflammatory drug, that is widely used for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute muscle pain conditions. The goals of this investigation were to determine: (i) potential of DCLF to induce nephrotoxicity, (ii) its power to induce oxidative stress and genomic DNA damage, and (iii) its ability to induce apoptotic cell death in addition to necrosis. Male ICR mice (25-45 g),*fed adlibitum*, were administered nephrotoxic doses of DCLF (100 & 300 mg/Kg, orally) and sacrificed 24 h later. Blood was collected to evaluate renal injury (BUN), lipid peroxidation (MDA: malondialdehyde levels), and superoxide dismutase (SOD) activity (a marker of oxidative stress). Kidney tissues were examined histopathologically, and analyzed for DNA damage. Results show that diclofenac is a potent nephrotoxin (at 100 and 300 mg/kg: 4 and 5 fold increase in BUN respectively) and a powerful inducer of oxidative stress (significant increase in MDA levels) leading to massive DNA fragmentation (100 and 300 mg/kg: 3 and 10 fold increases compared to control) coupled with apoptotic and necrotic cell deaths. A dose-dependent increase in MDA level and SOD activity were also observed. Collectively, these data suggest that, in addition to its nephrotoxic potential, DCLF at toxic doses, may induce genomic instability *in vivo*.

557 HPLC ANALYSIS OF CYTOCHROME C USING 393NM DETECTION.

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The release of cytochrome c from the mitochondria can induce apoptotic cell death. Previous methods to detect cytochrome c release from mitochondria have relied upon immunoblotting, a procedure that can be limited by non-linearity of signal, epitope masking, and impracticality for large numbers of samples. In order to circumvent these limitations, we have developed a reverse-phase HPLC method for cytochrome c detection and quantitation by taking advantage of a novel acid-induced absorbance maximum at 393 nanometers for cytochrome c in buffer containing 0.1% trifluoroacetic acid. With a C4 reverse-phase analytical column, this assay had a limit of quantitation of 10 ng (0.8 pmoles) of cytochrome c. HPLC detection of cytochrome c was then compared against immunoblot detection of cytochrome c. Both methods demonstrated a good linearity between signal response and cytochrome c loaded (10 ng to 100 ng). However, in contrast to the HPLC method, the immunoblotting signal plateaued at 150 ng of cytochrome c. We demonstrated the detection and quantitation of cytochrome c from isolated mitochondria. HPLC quantitation of cytochrome c is rapid and can be performed on the raw sample. This method of cytochrome c analysis may be useful for the study of agents that cause mitochondrial dysfunction and apoptotic cell death.

558 DIFFERENTIAL INHIBITION OF RIBOSOMAL RNA SYNTHESIS BY TOPOISOMERASE INHIBITORS.

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The genes encoding ribosomal RNA (rRNA) are highly sensitive to the inhibition of transcription by topoisomerase inhibitors compared to genes encoding mRNA transcripts. Because transcription of rRNA accounts for a significant proportion (~50%) of total RNA synthesis in growing cells, such preferential drug interactions may be a potentially important determinant of drug-induced cell death. We examined rRNA transcriptional inhibition by several topoisomerase inhibitors, including camptothecin and actinomycin D, in B- and T-lymphoma cells that are highly sensitive and resistant to the induction of apoptosis by these agents, respectively. We found that the mode of rRNA synthesis inhibition varied among the particular agents used and, in some cases, was concentration-dependent. At relatively low concentrations of actinomycin D, and at all concentrations of camptothecin tested, premature termination of rRNA transcription was detected, resulting in the accumulation of small, truncated transcripts. The sites of termination of transcription were localized within the 5'external transcribed region of the pre-rRNA and corresponded to a region in the gene that containes several topoisomerase I sites. In contrast, exposure of cells to higher concentrations of actinomycin D, which were required to inhibit mRNA synthesis, resulted in the inhibition of initiation of rRNA transcription. We also found that the mode of inhibition of rRNA transcription correlated with different forms of cell death. For example, concentrations of actinomycin D that lead to premature termination of transcription resulted in the induction of apoptosis whereas cells exposed to higher concentrations of this drug, in which transcriptional initiation was blocked, were unable to execute an apoptotic pathway but instead underwent delayed cell death by necrosis. Thus, topoisomerase inhibitors show interactions with rDNA that may influence the mode and extent of cell death.

559 EVALUATION OF THIOBARBITURIC ACID REACTIVE SPECIES INDUCTION AND MEASURES OF APOPTOSIS FOLLOWING ACUTE AND REPEATED ORAL EXPOSURE TO METHYLMERCURY.

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The purpose of this research is to develop a battery of end points to be used in neurotoxicological hazard identification. Putative mechanisms of methylmercury-induced neurotoxicity include the induction of reactive oxygen species and apoptosis. In this study, an attempt was made to determine if oral administration of methylmercury induced such responses. Male Long-Evans rats were administered dosages of methylmercury known to induce neuropathology. Areas of the central nervous system associated with methylmercury-induced dysfunction, spinal cord, cerebellum, and neocortex, were assayed for lipid peroxidation at selected time points after acute oral

administration (0, 0.12, 0.37, 1.11, 3.33, and 10 mg/kg) or after a seven day repeated oral regimen. Lipid peroxidation was determined measuring thiobarbituric reactive species (TBARS). To characterize the generation of TBARS and assay sensitivity, lipid peroxidation was induced, in a dose-dependent manner, via ex vivo addition of ferrous ammonium sulfate. In addition, tissues from these regions were assayed for DNA fragmentation with an enzyme linked immuno-sorbant assay (ELISA). Acute and repeated exposure regimens resulted in some lethality and decreased body weight gain. In contrast to previous studies involving subcutaneous and intraperitoneal administration of methylmercury, significant increases in TBARS were not observed. DNA fragmentation following acute administration of methylmercury chloride resulted in a suggested increase in apoptosis. These results may indicate that oral administration of methylmercury chloride does not induce lipid peroxidation. However, given the trends observed with DNA fragmentation, further studies concerning the time course and potential for methylmercury-induced apoptosis might be justified. (This abstract does not necessarily reflect EPA policy.)

560 DELAYED-TYPE HYPERSENSITIVITY TO SULFAMETHOXAZOLE IN MICE: ROLE OF METABOLITES.

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Little is known of the mechanisms involved in delayed-type hypersensitivity (DTH) to drugs. The skin adverse effects of sulfamethoxazole (SMX) are probably immune mediated and caused by metabolites. A murine model combining *in vivo* and *in vitro* endpoints was used in an attempt to predict DTH reactions to SMX. Mice injected intra-dermally with SMX for 3 days were challenged at day 7 with SMX alone or incubated with a microsomal enzymic activation system (S9mix). No *in vivo* positive DTH responses were obtained using several mouse strains, varying SMX and S9mix dose levels, challenge sites, or phenobarbitone pretreatment. In contrast, the secondary proliferation of spleen cells *in vitro* from SMX- or SMX/S9mix-treated mice evidenced SMX-specific lymphocytes, which could be stimulated only after incubation of SMX with S9mix. As no proliferation was obtained after incubation with S9mix alone, a mitogenic effect or sensitization to microsomal proteins can be excluded. In addition, *in vitro* cell proliferation only occurred following prior metabolite production, whereas sensitization of mice was achieved after treatment with either SMX alone or SMX/S9mix. These results confirm the crucial role of metabolites in SMX hypersensitivity in mice as already shown in human patients. It remains to test whether similar ex vivo findings can be obtained in mice to predict DTH reactions to other medicinal products.

561 REACTIVITY OF T CELLS TO DRUGS AND THEIR METABOLITES.

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Drugs represent a group of low molecular weight non-peptide antigens which can be recognized by alpha beta TCR-positive T cells. Over the past years, specific T cell clones have been generated from patients allergic to the antibiotic sulfamethoxazole (SMX) suggesting an important role for T cells in the manifestation of drug hypersensitivity. Allergic reactions to SMX are usually thought to be a consequence of bioactivation to the hydroxylamine metabolite (SMX-NHOH) and further oxidation to the ultimative reactive metabolite, nitroso-sulfomethoxazole (SMX-NO). SMX-NO is then able to covalently modify self proteins which in turn might be recognized as neo-antigens by the immune system. Recent investigations, however, revealed additionally a MHC-restricted but processing- and metabolism-independent pathway of drug presentation. Antigen-presenting cells (APCs) preincubated with the drug over night and subsequently washed were not able to activate SMX-specific alpha beta TCR+ T cells. The drug could, however, be efficiently presented even by glutaraldehyde-fixed APC when it was continuously present in the culture. Moreover, T cell clones responded to non-covalently presented SMX by a rapid downregulation of their T cell receptors. These observations were best explained by a labile, low-affinity binding of SMX to MHC-peptide complexes on APCs and a processing-independent antigen presentation. In order to study the role of covalent versus non-covalent drug presentation in SMX-allergy, a) we analyzed whether T cells from allergic and non-allergic individuals react with SMX and the metabolites SMX-NO or SMX-NHOH; b) we generated T cell clones to SMX and the chemically reactive metabolites and investigated the crossreactivity in detail; and c) we assessed the kinetics of TCR downregulation in T cell clones crossreactive with SMX/SMX-NO. Our data indicate, that crossreactive T cells can be detected in and isolated from the peripheral T cell pool of drug-allergic individuals but that their appearance differ between individuals. The overall pattern of antigen-reactivity suggests that in SMX-hypersensitivity already the primary stimulation can be directed to the non-covalently bound SMX.

562 IMMUNOMODULATION BY MEDICINAL DRUGS ASSOCIATED WITH ANAPHYLAXIS IN HUMANS: SELECTIVE INDUCTION OF TH-2 RESPONSES DURING PRIMARY IMMUNE STIMULATION IN MICE.

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Hypersensitivity reactions involving the immune system may be the most frequent adverse effects associated with pharmaceutical agents. Anaphylaxis, immediate-type hypersensitivity, is a relatively rare adverse drug-reaction but is one of concern due to its potential severity. Current pre-clinical animal models have little value, if any, in predicting drug-induced anaphylaxis. Anaphylaxis is a type 2 IgE mediated reaction. In the mouse IgE production is regulated by the availability of IL-4, which is produced by Th-2 cells (T-helper cell type 2) and is antagonized by INFγ. This study was conducted in BALB/c mice and used the conjugate TNP-OVA (trinitrophenyl-ovalbumin) in the reporter antigen popliteal lymph node assay (RA-PLNA) to test the following hypothesis: pharmaceutical agents associated with anaphylaxis in humans produce a positive response in the RA-PLNA and selectively induce type 2 antibody (IgE and IgG_1) to TNP. The analgesic glafenine (GF), the NSAID zomepirac (ZO) (both withdrawn from the market due to anaphylaxis) and the NSAID diclofenac (DF), which is associated with anaphylaxis, were tested. Phenobarbital (PB) was used as a negative control, and streptozotocin, which selectively induces a type 1 (INFγ, IgG_{2a}) response in the TNP-OVA RA-PLNA, was used as an isotype control. GF, ZO, DF and STZ produced dose-dependent increases in the lymph node diameter and cellularity while PB failed to produce a response. The four immunostimulating compounds also significantly increased TNP-specific IgM production. However, GF, ZO and DF selectively induced IgE and IgG_1 TNP-antibody production while STZ selectively induced IgG_{2a}. Although it is unrealistic to suggest that one assay based on a limited number of test compounds can independently identify all drugs with the potential to induce anaphylaxis in humans, this study does suggest the mouse RA-PLNA should be further investigated.

562A PRIMARY AND SECONDARY IMMUNE RESPONSES TO THE NSAID DICLOFENAC IN BALB/C MICE: EVIDENCE FOR DRUG-SPECIFIC SENSITIZATION.

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The NSAID diclofenac (DF) is associated with idiosyncratic hepatotoxicity and several distinct hypersensitivity reactions. The mechanism(s) are unknown but evidence suggests both cell-mediated and antibody-mediated immune effector systems may be involved. This suggests DF can haptenize protein *in vivo* and can be recognized as an immunogen/antigen. This study tested the hypothesis that a single dose of DF, unconjugated and administered without adjuvant, can sensitize BALB/c mice. It used the reporter antigen popliteal lymph node assay (RA-PLNA) with the conjugate TNP-Ficoll (trinitrophenyl-Ficoll) to characterize a DF primary and secondary immune response. The specificity of the secondary immune response was determined by injecting the irrelevant hapten D-Penicillamine (D-PEN). Following a footpad injection, the primary RA-PLNA reaction using DF produced a dose- and time-dependent increase in cellularity and was dominated by TNP-specific IgM antibody production on days 4-8 with minimal IgG (IgG_1, IgG_3, IgG_{2a} and IgG_{2b}) produced on days 5-8. Next, mice were divided into 2 groups and gavaged (1x) with either 1.0 mg DF or vehicle (ddH_2O). Then 21 days later all animals were injected with DF - TNP-Ficoll. Compared with vehicle treated controls, animals gavaged with DF produced a secondary response that was significantly increased both in magnitude on day 4 (based on cellularity and TNP-specific IgM production) and kinetics on day 4 (based on IgG_1 production). Additionally, the secondary response was dominated by IgG_1 and IgG_3 isotype production, relative to IgM, on day 7. In separate groups of animals, the oral dosing with DF or vehicle was repeated. Then 21 days later all groups were injected with D-PEN. Based on cellularity, IgM and IgG antibody production the RA-PLNA response to D-Pen on days 4 and 7 did not differ between treatment and control groups. These results suggest that a single

oral dose of DF can sensitize BALB/c mice. Additionally, this data demonstrates the potential of the mouse RA-PLNA in detecting drug-specific immunomodulation without the need to generate drug-conjugates or identify relevant *in vivo* carrier proteins.

563 CYTOKINE LEVELS AND CELL POPULATION CHANGES AS PREDICTIVE MEASURES OF IMMUNOSTIMULATORY EFFECTS OF PHOSPHOROTHIOATE OLIGONUCLEOTIDES (PS ODN) IN MICE.

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Female B6C3F1 mice were used to develop more predictive measures for distinguishing between stimulatory and non-stimulatory PS ODNs. Previous estimates had been based on splenomegaly and *in vitro* B cell assays. In addition to spleen weight, serum cytokine levels, spleen cytokine mRNA levels, ODN concentrations in spleen, and splenocyte population identification were investigated. With a single iv dose of 20 mg/kg ISIS 12449 (high stimulator), spleen weights increased approximately 2-fold. Cytokines including MIP-1α, MIP-2, MCP-1, IL-10, IL-12, IFN-γ, IL-1β, IL-6, IL-1Ra, TNF-α/β, MIF, and RANTES were increased in serum or splenocyte mRNA. Relative numbers of macrophages and endothelial cells were increased; B and T cells were unaffected. In comparison, an equal dose of ISIS 12450 (low stimulator) had no effect on spleen weight, cytokines were unchanged for serum or mRNA, and spleen cell populations were similar to saline dosed mice. The concentration of ISIS 12449 in spleen, as determined by CGE analysis, was slightly greater at early time points (< 1h) but not at the later experimental time points (2 - 144 h). Thus, a stimulatory ODN induced a spectrum of cytokine and cell population changes consistent with non-specific systemic immune stimulation. These end points will provide a comparative baseline by which the immune stimulation of other ODNs can be assessed.

564 ANALYSIS OF IMMUNE-RELATED ADVERSE EVENTS ASSOCIATED WITH THERAPEUTIC USE OF DRUGS.

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Immune-related adverse responses to therapeutic drugs make up approximately 10% of all adverse events reported to the FDA. Existing nonclinical assays appear to be poorly predictive of drug-induced immune-related systemic hypersensitivity. We extracted immune-related adverse event data from the FDA database and obtained usage data for up to the first five years of actual sales for ~380 drugs approved for marketing between 1978 and 1997. The selected drugs included only small, organic, single active ingredient formulations. Proteins, salts, metals, combination products and polymers were excluded. We calculated incidence of reported adverse events per million shipping units of drug. The data were then ranked by individual incidences and groups of related incidences. The incidence values ranged from over 1000 to 0 events per million units. Over 75% of reported adverse events were in two categories: rash and urticaria. The remaining events were mostly anaphylaxis, angioedema, Stevens-Johnson syndrome, photosensitivity, vasculitis, and eosinophilia. A plot of the ranked incidence values demonstrated a multi-exponential shape with <5% of the drugs being "highly positive". The data could be used to select drugs for evaluation of assays proposed to predict systemic drug allergy.

565 INVESTIGATION OF IMMUNOSTIMULATORY POTENTIAL WITH A MODIFIED MURINE POPLITEAL LYMPH NODE ASSAY.

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The suitability of a modified murine popliteal lymph node assay (PLNA) to investigate the immunostimulatory potential of test compounds was investigated. Groups of six female BALB/c mice received a s.c. injection of either Oxazolone (0.25%), D-Penicillamine (0.2, 2 and 20%), Carbamazepine (0.2, 2 and 10%), Procainamide (0.2, 2 and 20%), Procainamide (20%) + S-9 mix, or Diphenylhydantoin (3%), into the left foot pad. The popliteal lymph nodes (LN) draining the site of application were excised 5 days later (peak of response). The evaluation consisted of assessing LN weights and cell counts, LN macrophage activity and cell surface marker expression (CD4 T cells, CD8 T cells, B cells, antigen presenting cells and CD4 T cells with an enhanced expression of IL-2 receptor). The immunostimulatory substances induced LN hyperplasia (increased LN weights and cell counts), as well as an augmented macrophage activity (oxidative burst) upon PMA-restimulation *in vitro* in LN cell suspensions, reflecting the onset of an inflammatory process. The specificity of the immune responses was confirmed through the characterization of the immune cell activation pattern: an increased CD4 blastocytosis, as well as an upregulation of IL-2R expression on CD4 T cells could be observed. The monitorization of multiple endpoints in the present system represents an advantage with respect to the standard PLNA, which determines only LN cellularity: the specificity and the potential to identify weak immunostimulatory responses are enhanced. In conclusion, these results support the suitability of the modified PLNA to assess the immunostimulatory potential of test substances.

566 ALLERGENICITY OF HAZELNUT PROTEINS IN THE BROWN NORWAY RAT MODEL OF FOOD ALLERGY.

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Food induced pollen-associated oral allergy syndrome (OAS) has become a well established clinical entity. At present there is limited information on the nature of food proteins responsible. We have employed the Brown Norway (BN) rat model of food allergy to study the potential of selected food allergen extracts to induce sensitization with the production of specific IgE. We have measured the potential of the same food extracts to induce mediator release in a mast cell surrogate (the rat basophil cell line RBL-2H3) when passively sensitised with specific rat IgE. RBL-2H3 cells were sensitised using an optimal dilution of sera (1:50) containing IgE raised in BN rats against hazelnut proteins. We found that sensitized cells respond to challenge with native hazelnut protein in a dose-dependent manner, releasing up to 75% of total β-hexosaminidase. Challenge with hazelnut protein heated for 40 min at 140°C also elicited a comparable response, suggesting that certain allergenic epitopes within hazelnut proteins can survive high temperature. Challenge with both birch pollen extract and purified Bet v1 also elicited a significant response, signifying cross reactivity between the hazelnut and birch pollen allergens, observations that were confirmed by inhibition immunoblotting studies. These results suggest that the BN rat model would be a suitable surrogate for man in the investigation of the sensitisation phase of OAS and that the RBL-2H3 cell line is suitable for studying cross reactivity of food protein allergens. (Supported by MAFF, UK and EU FAIR contract CT97-3224.)

567 EVALUATION OF HUMAN CONTACT ALLERGENS IN THE MURINE LOCAL LYMPH NODE ASSAY.

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ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) has recently endorsed the murine local lymph node assay (LLNA) as a stand-alone method for skin sensitization hazard identification. The investigations described here were designed to explore further the ability of the LLNA to identify accurately those chemicals that cause allergic contact dermatitis in humans. To that end we have measured, in three laboratories, LLNA responses induced by 18 test chemicals, 11 of which are known to cause skin sensitization and 7 of which are not associated to a significant extent with allergic contact dermatitis in humans. Three concentrations of each material were tested and were chosen to provide the highest possible concentration based on either solubility in the selected vehicle or lack of systemic toxicity. The LLNA correctly classified 16 of the 18 materials. All of the 11 chemicals tested which are associated with allergic contact dermatitis in humans were found to be positive in the LLNA. Of the seven materials believed to be non-sensitizers, five were negative in the LLNA and two elicited weakly positive results at relatively high test concentrations (50 and 100%). In a comparison of the LLNA and human classification with this data set, the LLNA was found to have an accuracy of prediction of 89%. This is consistent with the 72% accuracy derived by ICCVAM in their independent analysis of the performance of the LLNA using a data set of 74 chemicals. Collectively these data provide additional evidence that the LLNA is able to discriminate skin sensitizers from those chemicals which do not possess a significant skin sensitization potential and thus provides a stand-alone method for hazard identification that offers important animal welfare benefits.

568 PREDICTING THE SKIN SENSITIZING POTENTIAL OF PHARMACEUTICALS AND PHARMACEUTICAL INTERMEDIATES USING THE LOCAL LYMPH NODE ASSAY.

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The murine Local Lymph Node Assay (LLNA) was recently recommended by a US interagency peer review panel for regulatory acceptance as a stand-alone alternative to guinea pig skin sensitization tests. The purpose of this collaborative study was to evaluate more extensively the performance of the LLNA, as a predictor of the skin sensitization potential of pharmaceuticals and pharmaceutical intermediates. Over 65 compounds belonging to highly diverse chemical classes were tested using both the LLNA and a guinea pig test method, and the results were compared for each compound. LLNA performance was also compared with compound molecular weight (MW), as well as with the choice of vehicle used in the assay. For most compounds, the presence or absence of positive structural alerts for skin sensitization, as identified by a commercially-available SAR modeling program, was investigated. Overall, the LLNA did not demonstrate strong agreement with either guinea pig test, due to a high incidence of false negative results. No overt relationships were observed between LLNA performance and either MW or vehicle, and no key structural alerts were identified. The results suggest that the LLNA might not be effective as a stand-alone alternative to guinea pig test methods, particularly when used to predict the skin sensitizing potential of pharmaceuticals and pharmaceutical intermediates. Further investigation is recommended in order to evaluate this assay for use in the pharmaceutical industry.

569 UTILITY OF THE LLNA FOR THE INVESTIGATION OF THE IMPACT OF VEHICLE MATRIX ON SKIN SENSITIZATION POTENCY.

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The identification of chemicals with contact allergenic activity is often followed by estimation of the potency of that hazard, so that a risk assessment for a defined use situation can be made. However, methods for quantitative assessment of skin sensitizing potency are not widely available. Recently, it has been proposed that the murine local lymph node assay (LLNA) may provide such information via interpolation of dose response information to give an estimate of the quantity of a chemical necessary to generate a threshold positive response (the EC3 value). It is recognized that the perceived potency of a chemical sensitizer may be influenced to a considerable degree by the vehicle matrix/formulation in which skin exposure occurs. Using the LLNA, EC3 values can be used to assess the impact of vehicle upon sensitizing potency. Dihydroquinone (HQ), ethyleneglycol dimethacrylate (EGDMA), dibromodicyanobutane, cinnamic aldehyde, isoeugenol, dimethylaminopropylamine and chloromethylisothiazolinone (CMI) were tested in the standard range of vehicles proposed for the LLNA (acetone/olive oil [4/1 v/v], dimethyl formamide, methylethylketone, propylene glycol and dimethylsulfoxide). The results revealed substantial vehicle related variations in potency; eg EC3 values for HQ ranged from 0.08%->1.0%; for EGDMA the range was 15%-36%; for CMI the range was 0.0049%-0.048%. Chemical dependent effects of vehicle on potency were observed. In summary, these results demonstrate that the matrix in which a chemical contacts skin can have a substantial impact on its sensitization potential. This effect can be quantitated successfully by estimation of the EC3 value in the LLNA.

570 QUANTITATIVE ESTIMATION OF SKIN SENSITIZING POTENCY USING THE LOCAL LYMPH NODE ASSAY.

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Risk assessment in skin sensitization demands that information on the relative potency of the chemical under evaluation is available. Until recently, this has been assessed only in a qualitative or semi-quantitative manner using guinea pig data and/or limited information from predictive human testing. The development of the murine local lymph node assay (LLNA) has afforded an opportunity to derive a quantitative estimate of sensitizing potency via a mathematical interpolation of dose response data. In this way, the concentration of a test chemical necessary to stimulate a 3-fold increase in activity in lymph nodes draining the site of chemical application is obtained; the EC3 value. This parameter has been calculated for a range of substances and compared with what is understood of the relative sensitizing activity of these chemicals in humans. Twenty chemicals were placed into 5 categories on the basis of their human sensitizing potency. LLNA dose response data for these chemicals (in a standard vehicle) were used to derive EC3 values. The strongest 4 allergens (Class 1) had EC3 values in the region of 0.001%-0.01%, while for the weakest 8 allergens, EC3 values were typically >25%. The LLNA provided a rank order comparable to that in humans. All the strongest sensitizers and non-sensitizers were identified correctly. Of the remaining 8 chemicals with significant sensitizing activity, the LLNA assessment of relative potency was of the correct order for 7 when an EC3 value of 10% was used to distinguish between Classes 2 and 3. As expected, the LLNA was not able to assign a potency estimate for the weakest sensitizers (Class 4). In conclusion, derivation of an EC3 value for a skin sensitizer may provide an objective and quantitative estimate of its relative potency.

571 PRIMARY EAR IRRITATION AND LYMPH NODE HYPERPLASIA INDUCED BY CONTACT ALLERGENS AND IRRITANTS IN THE MURINE LOCAL LYMPH NODE ASSAY.

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Sponsor: A. Wolf.

To improve the predictive differentiation of contact allergy (CA) and photoallergy (CPA) from irritancy (IR) and photoirritancy (PI), induced by low molecular weight compounds, we established a murine local lymph node assay (LLNA) with two endpoints in different tissues: ear-draining lymph node (LN) hyperplasia and primary ear irritation. Groups of female BALB/c or NMRI mice received compound solution or vehicle (40% Dimethylacetamid, 30% Acetone, 30% Ethanol) alone on the dorsum of both ears on three consecutive days. Immediately after compound application indicated groups of mice were exposed to a UVA light-dose of 10J/cm². Ear swelling was determined before treatment and immediately before necropsy. Weights of the apical area (0.5 cm²) of the right ear as well weights and cell counts of the auricular lymph nodes draining the ear tissue were determined at necropsy 24 hr following the last exposure. Tested chemicals were oxazolone, dinitrochlorobenzene, dinitrofluorobenzene, eugenol, isoeugenol, mercaptobenzothiazol as CA, tetrachlorosalicylanilide, chlorpromazine as CPA, croton oil, sodium dodecyl sulfate as IR and 8-methoxypsoralen as well as anthracene as PI. The principle findings of this study were: (I) both CA and CPA as well as IR and PI induce hyperplasia of draining LN, (II) irritants predominantly induce skin irritation, which in turn increases draining LN hyperplasia, (III) some CA and CPA induce only marginal skin irritation, but a vigorous LN hyperplasia. The ratio of relative increases in ear thickness/weight and LN hyperplasia were used to differentiate between (photo)allergic and (photo)irritant reactions (IMDS: integrated model for the differentiation of chemical-induced allergic and irritant skin reactions). A ratio fare above 1, obtained with a certain concentration of the test chemical, indicate a (photo)allergic reaction, whereas a ratio below 1 points to (photo)irritation. Values around 1 indicate that the test chemical is likely to possess both considerable irritative and allergenic potential and, thus has to be evaluated in further testing. Our results show that this model contributes to the differentiation of CP/CPA from IR/PI by investigating simple endpoints in a short study conduct.

572 ROLE OF GLUTATHIONE IN CONTACT SENSITIZATION: APPLICATION OF A FLOW CYTOMETRY-BASED LOCAL LYMPH NODE ASSAY.

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The Local Lymph Node Assay (LLNA) is an animal-based test employed throughout the cosmetic and pharmaceutical industries to screen and characterize the immunotoxic properties of chemicals. Although the LLNA is effective at detecting chemical irritants and sensitizers, it has limited discriminating power to differentiate distinct subclasses of sensitizing agents (i.e., irritants vs. contact allergens). To improve the specificity and sensitivity of this important screening test, we have applied flow cytometry to the standard assay. Using this modified LLNA, we examined the role of glutathione levels in the induction of contact sensitization. In these studies, dinitrochloroben-

zene (DNCB, a known sensitizer), Vitamin E or vehicle were applied to the dorsum of both ears of mice for three consecutive days. Subsets of each treatment group were co-treated without or with the glutathione synthesis inhibitor, buthionine sulfoximine (BSO), dosed in the drinking water. Prior to sacrifice the mice were injected with bromodeoxyuridine (BrdU, i.p.) and the number of proliferating (BrdU-positive) cells of the auricular lymph nodes was determined using flow cytometry. Both DNCB and Vitamin E were found to induce lymphocyte proliferation (> 3 fold over vehicle control) and thus were positive as sensitizers in this modified LLNA. These agents were also found to increase cellular levels of reduced glutathione in the epidermal keratinocytes of mouse skin. BSO was found to dramatically inhibit agent-induced increases in cellular glutathione as determined using flow cytometry and the (fluorescent) glutathione-specific dye, monochlorobimane. Interestingly, the ability of these sensitizers to induce proliferation of lymphocytes was dramatically inhibited by treatment with BSO, and both DNCB and Vitamin E failed to function as sensitizers in BSO-treated mice. Results of these studies indicate that cellular levels of reduced glutathione may play a critical role during the induction phase of contact sensitization.

573 UTILIZATION OF SAR AND THE MURINE LOCAL LYMPH NODE ASSAY (LLNA) TO ASSESS THE SENSITIZATION POTENTIAL OF HAIR COLORANTS.

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Both oxidative (permanent) and direct (semi-permanent) dyes have an acknowledged potential to cause contact sensitization, however, this is regarded as a manageable health issue for the general population. The use of computational toxicology and the local lymph node assay (LLNA) allows us to evaluate the sensitization potential of new dye candidates "upstream" of the more traditional guinea pig assays (i.e., Buehler or GPMT methods). A number of aromatic/nitro-aromatic amines used commercially as oxidative or direct hair dyes were evaluated for allergic contact sensitization potential using the LLNA. Test materials included 10 direct and 2 oxidative dyes. The application vehicle for all materials was dimethyl sulphoxide (DMSO). Relative potency was compared to para-phenylenediamine, a more relevant although less potent allergen as compared to other OECD recommended positive controls, such as 2,4-dinitrochlorobenzene (DNCB), oxazolone, or hexyl-cinnamic aldehyde (HCA). Using a Computer Automated Structure Evaluation (CASE/MultiCASE) customized database constructed of aromatic and nitro-aromatic amines, SAR predictions and LLNA data were compared with historical data obtained with traditional guinea pig methods. In addition, the data generated from the LLNA was also used to derive a quantitative SAR model for predicting sensitization. When comparing actual LLNA and guinea pig data, an overall concordance of 77% (10/13) was achieved. Sensitivity and specificity were 92% (12/13) and 85% (11/13) respectively. These results indicate the LLNA may be successfully used to evaluate the contact sensitization potential of new and existing hair colorants.

574 A TWO-TIERED MURINE LOCAL LYMPH NODE ASSAY TO IDENTIFY CONTACT (PHOTO)ALLERGENIC POTENTIAL.

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Sponsor: A. Wolf.

To improve the predictive differentiation of contact allergy (CA) and photoallergy (CPA) from irritancy (IR) and photoirritancy (PI), induced by low molecular weight compounds, we developed a non-radioactive, two-tiered protocol of the murine local lymph node assay (LLNA). Tier I testing covering a broad concentration range of the test chemical consists of two endpoints: primary ear skin irritation and lymph node (LN) hyperplasia. The ratio of the relative changes in the ear-draining LN and in the ear skin provides information about the preferential mode of action the tested chemical. A ratio fare above 1, obtained with a certain concentration of the test chemical, indicate a (photo)allergic reaction, whereas a ratio below 1 points to (photo)irritation. Values around 1 indicate that the test chemical is likely to possess both considerable irritative and allergenic potential and, thus has to be evaluated in a tier II test schedule. For tier II testing mice were sensitised with selected tier I concentrations of chemicals or treated with vehicle (40% Dimethylacetamid, 30% Acetone, 30% Ethanol) on the shaved back on 3 consecutive days with subsequent UVA exposure in case of CPA and PI studies. Challenge was performed 12 days later on the ears on 3 consecutive days in order to reveal a possible allergy-related increase in ear swelling and ear weight as well as to investigate the expression of relevant cytokines at two stages of the evolution of an allergic response, the induction and the challenge phase. Tested chemicals were oxazolone, dinitrochlorobenzene, dinitrofluorobenzene, eugenol, isoeugenol as CA, tetrachlorosalicylanilide, chlorpromazine as CPA, croton oil as IR and 8-methoxypsoralen as well as anthracene as PI. Exclusively in case of CA and CPA, allergy-related ear swelling and ear weight increase as well as a marked increase in IL-4 and IL-10 expression by ex vivo restimulated LN CD4 T cells was observed in the challenge groups. These results were in line with the finding of high ratios between LN hyperplasia and primary skin irritation. Ratios around 1, which do not precisely indicate the mode of action of a chemical - CA/CPA or IR/PI, can occurr at high concentrations of chemicals with CA/CPA potential, but also with compounds like croton oil consisting of different ingredients. One of these ingredients of croton oil are phorbol esters affecting lymphocytes in a non-specific manner, and thus, causing LN hyperplasia. Especially in the latter case tier II testing provided the necessary information to predict or deny a CA or CPA potential.

575 ASSESSMENT OF THE SUBCUTANEOUS ROUTE OF TEST ARTICLE ADMINISTRATION USING THE MOUSE LOCAL LYMPH NODE ASSAY.

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The mouse local lymph node assay (LLNA) measures lymphocyte proliferation in draining lymph nodes of mice after topical application of a test material to the intact, non-occluded skin of both ears. There is a need to develop methods to evaluate the sensitization potential of chemicals and/or materials that are implanted or injected into the body (e.g. drugs, implanted medical devices, biologics), for which topical application in not a relevant route. In addition, it is important to distinguish between IgE mediated and cell mediated sensitizers. To evaluate the sensitization potential of implanted/injected materials, the LLNA was conducted using the subcutaneous route rather than the topical route. Two irritants (sodium lauryl sulfate (SLS), nonanoic acid), a T-cell mediated sensitizer (oxazolone), and a protein that is foreign to the mouse (human albumin) were evaluated using the subcutaneous route of administration. An IgE-mediated sensitizer (toluene diisocyanate; TDI) and a T-cell mediated sensitizer (DNFB) were used as positive controls. The irritant nonanoic acid did not induce lymphocyte proliferation compared to the vehicle control group. However, as seen with topical administration, SLS induced low level, significant lymph node cell proliferation at the two highest concentrations. Oxazolone and human albumin caused a significant concentration-dependent increase in lymphocyte proliferation versus the vehicle control. Both DNFB and TDI were associated with significant lymphocyte proliferation compared to the vehicle control. Phenotypic analysis conducted for groups of mice treated subcutaneously with vehicle (polyvinyl pyrrolidone; PVP), nonanoic acid, oxazolone, DNFB and TDI revealed a significant increases in the percent B220+ lymphocytes for the oxazolone, DNFB and TDI groups, whereas the nonanoic acid group was similar to the control. The percent IgE+B220+ lymphocytes increased from least to greatest in the oxazolone, DNFB and TDI groups, respectively whereas the nonanoic acid group was similar to the vehicle control group. TDI was associated with a 2-8 fold increase in the percent IgE+B220+ lymphocytes as compared to oxazolone or DNFB.

576 ASSESSMENT OF A MODIFIED LOCAL LYMPH NODE ASSAY TO EVALUATE THE IRRITANCY/SENSITIZATION POTENTIAL OF CHEMICALS EXPOSED TO BREACHED SKIN.

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There is a need to develop methods to evaluate the sensitization potential of chemicals and/or materials that contact breached skin (e.g. polymeric tissue adhesives, wound dressings, occupational exposure to chemicals through wounded or irritated skin). For this purpose a modified local lymph node assay (LLNA) was conducted using standardized methods for breaching the skin at the dose application site. Female BALB/C mice were exposed for three consecutive days on the ear to vehicle (.9% sterile saline) or a foreign protein (25% human albumin) via the topical route, breached skin route utilizing either a Simplate (Organon Teknika, Corp., Durham, NC) or a Multi-Test (Lincoln Diagnostics, Inc., Decatur, IL) device, or injected in the subcutaneous tissue between the ears. In order to investigate the potential for enhanced sensitivity of the assay by occlusion, for each test condition

(excluding the subcutaneous group) the exposure site was occluded using Blenderm (3M Corp., St. Paul, MN) tape in one group of animals and left unoccluded in a second group. Two days following the final exposure animals were injected intravenously with 3H thymidine, sacrificed five hours later and the draining lymph nodes excised and processed as for a standard LLNA. When unoccluded, no difference in proliferation occurred following topical application to intact skin. However, an increased proliferative response (>5 fold) was seen following both methods of breached skin with no significant differences between methods. With occlusion, exposure by all routes induced significant proliferation, however breaching with the Multi-Test device induced significantly higher (>3 fold over topical and >2 fold over Simplate) proliferation than by the other routes and induced proliferation similar to that observed following subcutaneous administration. Results indicate that the Multi-Test may provide a useful method for breaching the skin in testing compounds where exposure is anticipated to occur to compromised skin.

577 DERMAL SENSITIZATION EVALUATION OF MALEIC ANHYDRIDE AND SUBSEQUENT REACTION MATERIALS USED IN THE DEVELOPMENT OF A NOVEL SYNTHETIC FIBER.

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Maleic anhydride is a highly reactive chemical intermediate that has widespread use in the manufacture of polyester resins in the textile industry. The irritation properties of this material are well known, however the dermal sensitization potential has not been well documented. As part of a worker safety monitoring program, the dermal sensitization potential of maleic anhydride and that of several downstream synthesis materials used in the manufacture of a novel high nitrile content fiber were evaluated in a series of guinea pig dermal sensitization studies utilizing a standard closed-patch test method. Rechallenge techniques were used to selectively determine cross-reactivity between materials. The starting reaction material, maleic anhydride, was shown to be a dermal sensitizer. Reaction products of maleic anhydride and alcohols used as a stabilizer in the polymer formulation were also determined to be dermal sensitizers. The latex precursor of the novel high nitrile content polymer (before final stripping and addition of the maleate stabilizing agent) was also determined to be a weak dermal sensitizer. The polymer products (raw polymer with stabilizer prior to pellet formation, in pellet form, and in spun fiber form) were all shown to be non-sensitizers. Rechallenge applications determined that 1) animals previously sensitized to maleic anhydride were not cross-reactive to the maleate polymer stabilizer; 2) animals previously sensitized to the maleate polymer stabilizer were not cross-reactive to the final polymer; and 3) animals sensitized to the high nitrile polymer prior to addition of the maleate stabilizer were not cross-reactive to the raw polymer (with stabilizer prior to pelleting). Animals that had been previously sensitized to the stabilizer had equivocal responses when rechallenged with the raw polymer (with stabilizer prior to pelleting) indicating potential cross-reactivity. We conclude that maleic anhydride, the reaction product of maleic anhydride and alcohols and the raw nitrile polymer latex are dermal sensitizers, but no evidence of sensitization potential by the finished polymer was detected in these assays.

578 TOPICAL EXPOSURE OF MICE TO RESPIRATORY SENSITIZING ACID ANHYDRIDES STIMULATES THE EXPRESSION OF INTERLEUKIN 5.

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We have shown previously that prolonged topical exposure of BALB/c strain mice to chemical contact or respiratory allergens such as 2,4-dinitrochlorobenzene (DNCB) or trimellitic anhydride (TMA) elicits cytokine secretion patterns consistent with the selective activation of type 1 and type 2 T lymphocytes, respectively. Certain respiratory sensitizing acid anhydrides including phthalic anhydride (PA) and maleic anhydride (MA) stimulate comparable levels of the type 2 cytokine interleukin (IL)-10 to those provoked by TMA, but induce somewhat lower levels of the type 2 product IL-4. We have therefore examined the production by allergen-activated lymph node cells (LNC) of IL-5, a type 2 cytokine which plays an important role in the differentiation and recruitment of eosinophils. Exposure to TMA, PA or MA resulted in each case in the expression by LNC of relatively high levels of IL-5, with peak production ranging from 50 to 200pg/ml. In contrast, LNC derived from DNCB-treated animals failed to elaborate detectable amounts of this cytokine (<20pg/ml) under conditions where vigorous secretion of the type 1 cytokine interferon γ was observed. Furthermore, negative selection (by complement depletion) of $CD4^+$ or $CD8^+$ lymphocytes revealed that $CD4^+$ T helper (Th) 2 type cells were the exclusive source of IL-5, in addition to the other type 2 cytokines. These data demonstrate that exposure to respiratory sensitizing acid anhydrides activates predominantly Th2 type cells, resulting in the expression of IL-5, which presumably plays a central role in the lung eosinophilia which is a key feature of chemical respiratory allergy.

579 CYTOKINES FROM BRONCHOALVEOLAR LAVAGES AND THEIR *IN VITRO* PRODUCTIONS IN ASTHMATIC RATS INDUCED BY TOLUENE DIISOCYANATE.

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To investigate the status and the role of cytokines in occupational asthma induced by toluene diisocyanate (TDI), we carried out a quantitative determination of cytokine profiles from bronchalveolar lavages (BAL) and their *in vitro* productions, as well as from splenocytes and sera in an asthmatic murine model induced by TDI. Male rats were sensitized with two courses of intranasal application of 5% TDI in ethyl acetate for seven consecutive days each time separated by a one week rest. The control group of rats were similarly treated with vehicle. A week after second sensitization, both groups of rats were provoked by applying 2.5% of TDI in vehicle. BAL fluids and sera from all rats were collected, and BAL-adherent cells and splenocytes were cultured for determination the levels of IL-2, IL-4, IL-5 and IFN-r. After lavation, the lungs were intratracheally fixed and removed for histologic examination. Differential cell counts were performed on BAL fluids and peripheral bloods. The results showed that the TDI-sensitized group of rats exhibited the asthmatic symptoms. TDI exposure resulted in elevated IL-4, IL-6 in BAL fluids, IL-4, IL-6 and IFN-r in BAL-adherent cells and splenocytes, and IL-6 in sera. A marked infiltration of the peribronchial regions with eosinophils was observed in lung sections from TDI-exposed rats. The cell counts revealed that the increases of the numbers of neutrophils and eosinophils were found in BAL fluids and peripheral bloods from asthmatic rats. These findings indicate that IL-4, IL-6 and IFN-r may play an important role in the pathogenesis of TDI-induced occupational allergic asthma.

580 CYTOKINE REGULATION OF T CELL DIFFERENTIATION AND ANTIBODY ISOTYPE PRODUCTION IN A MOUSE MODEL OF OVALBUMIN SENSITIZATION.

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A mouse model featuring key components of human allergic asthma, including eosinophil recruitment into airwalls and Th2 pathway predominant antibody production has been validated. Intraperitoneal sensitization to ovalbumin followed by inhalation challenge resulted in eosinophil infiltration into peribronchial regions of the lung at 48h post-challenge. Levels of Th2 pathway antibodies (IgE, IgG1 and anti-ovalbumin antibodies AntiOVS IgE and AntiOVS IgG1) increased following sensitization, or sensitization and challenge, while Th1 pathway antibodies (IgG2a/b and anti-ovalbumin antibody AntiOVA IgG2a/b) were unchanged. This pattern of cellular and humoral change is characteristic of Th2 cell predominance leading to release of cytokines which are chemoattractant to eosinophils and stimulate B-cell switching to IgE and IgG1 isotype production.Preventive treatment of naïve mice with IFNy, Anti IL 4 (low and high doses), Anti IL 5 (low and high doses), or IL 12 (low and high doses) was performed both before the sensitization injections and before the ovalbumin aerosol challenge, to examine their preventive effect on Th2-cell pathway commitment caused by sensitization. Anti IL 5 significantly decreased peribronchial eosinophil infiltration at 12 and 48h post aerosol challenge, however it had no effect on altering antibody production even when given before sensitization. Both IFNy and Anti IL 4 pretreatment had little effect on preventing inflammatory eosinophil infiltration following aerosol challenge, but caused a dose-related switching of T-cell differentiation away from Th2 predominant B-cell AntiOVA IgE production to Th1 predominant B-cell AntiOVA IgG2a/b production. IL 12 treatment before sensitization caused both a consistent trend to decreased eosinophil infiltration and a dose-related B-cell isotype switching from AntiOVA IgE to AntiOVA IgG2a/b.In conclusion, it appears that IFNy, Anti IL 4 and IL 12, when given preventively before antigen exposure, can regulate the direction of T-cell differentiation and B-cell antibody isotype production from Th2 predominance to Th1 predominance. IL 12 appears to have additional benefit in

amelioration inflammatory cell infiltration when given preventively before sensitization. These cytokines or anti-cytokine antibodies therefore are potentially strong tools to modify cellular events and/or alter the direction of immune-mediated responses in allergic asthma.

581 THE ROLE OF TUMOR NECROSIS FACTOR (TNF) IN AIRWAY REACTIVITY TO TOLUENE DIISOCYANATE (TDI).

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Nearly 9 million workers are exposed to chemical agents associated with asthma; and, isocyanates represent the most common cause of occupationally-induced asthma. We hypothesize that inflammation is a pre-requisite of TDI-induced asthma and that the regulation of this inflammation by TNF is important in isocyanate-asthma development. To explore this hypothesis, TNF receptor knockout (TNFR) and anti-TNF (x-TNF) treated C57BL/6J mice were sensitized (s.c., 20µl, Day 1), boosted (s.c., 5µl, Days 4 and 11), and challenged (inhalation, 100ppb, Days 21, 23 and 25) with TDI (80:20, 2,4:2,6 TDI). Twenty-four hours following the last challenge, non-specific airway reactivity to methacholine challenge was reduced to baseline levels in x-TNF treated mice. Serum titers of TDI-specific IgG antibody were significantly reduced in TNFR and x-TNF mice compared to exposed wild-type mice and these antibodies were primarily of the IgG_1 isotype. Airway inflammation, goblet metaplasia and epithelial damage were reduced in the TNFR and x-TNF treated mice. In addition, x-TNF treatment modulated IL-4 and IFNγ message levels in airway tissues, while significantly decreasing TNF, IL-4 and IFNγ levels in splenic supernatants. Furthermore, TNF neutralization markedly reduced the number of FITC containing cells in local draining lymph nodes in an intratracheal instillation model. These results suggest that TNF plays a role in the airway inflammatory response to an irritant such as TDI and that one of the controlling steps may be modulation of the migration of antigen presenting cells to the local lymph node.

582 FURTHER EXPERIENCE WITH THE ASSESSMENT OF THE RESPIRATORY SENSITIZING POTENTIAL OF PROTEINS USING THE MOUSE INTRANASAL TEST.

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Certain proteins are capable of driving the immune system to form antigen specific immunoglobulin E (IgE) as part of a type-I hypersensitivity response. Thus if such proteins become airborne they can act as human respiratory allergens. Toxicologists have used a variety of protocols to model the relative ability of different proteins to induce respiratory sensitisation. The mouse intranasal test (MINT) has been proposed recently as an alternative to the hisorical guinea pig models, which although producing results predictive for man suffer from a number of limitations. The MINT has proven quite robust in interlaboratory evaluation of a positive control allergen (Alcalase) and a number of other proteins. However, it has been our experience that this method has produced estimates of relative sensitizing potency, compared to Alcalase, which in some instances have been substantially different to those assessed in the guinea pig. Proteins demonstrated to be approximately an order of magnitude more potent than Alcalase in guinea pig models have proven less allergenic than Alcalase in the MINT. Further MINT evaluation of a number of proteins in another laboratory has confirmed the differential between the guinea pig and mouse models for some proteins. The present studies have therefore provided additional data indicating that the MINT has limitations as a reliable predictive tool for the assessment of relative respiratory sensitizing potency and thus requires further evaluation. In particular, discrepancies in the potency assessments mean that occupational exposure guidelines established on the basis of MINT data may be flawed.

583 ALLERGIC INFLAMMATION AND ENZYME SPECIFIC IGG1, IGE ANTIBODY TO VARIOUS ENZYMES IN THE MOUSE INTRANASAL TEST (MINT).

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The MINT has been proposed for use for the assessment of immunogenic potential of enzymes. BDF1 mice receive an intranasal dose of enzyme protein on days 1, 3, 10 with serology on day 15. IgG1 antibody (AB) response to several enzymes has been well characterized in this model. IgG1 has been used as a surrogate marker for IgE antibody. The goal of the present work was to characterize allergic inflammation, IgG1 and IgE AB after exposure to various enzyme allergens following an extended dose schedule. Mice were exposed to Alcalase, Savinase, Termamyl and an endocellulase in detergent matrix on days 1, 3, 10, 17 and 24. Sera and nasal tissue were collected on day 29. Since exposure guidelines exist for Alcalase, this enzyme was used as the reference in all studies. From the IgG1 antibody response, endocellulase and Termamyl were estimated to be 3.8x to ~5x more potent than Alcalase. This is consistent with the potency estimates made from the regular dose regimen (Robinson, 1998 ToxSci, 43:39). Savinase and Alcalase were considered equipotent following both dose regimens. A dose-dependent increase in eosinophil infiltrate was noted in the nasal tissue of animals exposed to the enzymes. The infiltrates were noted in the front, mid and posterior sections of the nasal cavity. Mild eosinophilia was noted in the nasal cavity of control animals (detergent matrix). Enzyme specific IgE AB was detected by the rat passive cutaneous anaphylaxis test. IgE antibody to Termamyl was present at protein doses that did not induce IgG1 or IgE antibody to Alcalase or Savinase. The IgE antibody response to endocellulase was greater than the IgE antibody response to Alcalase. Based on the IgE antibody response, Termamyl and endocellulase were judged to be more potent than Alcalase. Therefore, the IgE and IgG1 responses were consistent in their ability to rank enzymes as more potent than Alcalase. These data support the use of IgG1 antibody as a surrogate marker for IgE when making immunogenic or allergenic assessments of enzymes in the MINT.

584 ASPIRATION (ASP) *VS.* INTRANASAL (IN) INSTILLATION LEAD TO COMPARABLE IMMUNE RESPONSES TO ALCALASE IN BDF1 MICE.

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The mouse intranasal test (MINT) has been proposed for use for the assessment of immunogenic potential of enzymes. IN instillation of protein led to little exposure to the lung (Robinson, etal, FAAT 1996, 34:15) yet led to a robust IgG1 and IgE antibody (AB) response to the enzyme allergen Alcalase. Early work by Kawabata, etal (1996, FAAT 29:238) showed that IgG1 and IgE AB could be induced in mice after 6 weeks of intratracheal exposure to Alcalase. The objective of this study was to compare the immune response to Alcalase following IN and direct lung exposure (ASP). Aspiration was used instead if intratracheal instillation due to the ease of the technique. BDF1 mice were exposed to Alcalase protein in saline or detergent (3mg/ml) on days 1, 3, 10 with serology on day 15. The IgG1 AB response to Alcalase was potentiated by the detergent 3.4x after ASP exposure. The same degree of potentiation (3.5x) was observed after IN exposure (Robinson, 1996). The response to Alcalase in detergent following ASP was similar to the response obtained after IN instillation (ASP ED50=0.18µg, IN ED50=0.22µg where ED50 is defined as the effective dose to generate ½ the maximum AB repsonse). Extension of the dose schedule to days 1, 3, 10, 17 and 24 with serology on day 29 led to higher IgG1 titers and AB responses to lower doses of Alcalase in both the IN and ASP exposed animals. The dose-IgG1 AB response was very similar between IN and ASP exposed animals. Alcalase specific IgE AB was detected by the rat passive cutaneous anaphylaxis test. Extension of ASP exposures to days 1, 3, 10, 17 and 24 induced IgE antibody to Alcalase. The extended dose schedule also allowed us to detect IgE antibody to Alcalase following IN instillation. These data show that exposure to enzyme allergen via the nasal mucosa or the lung will lead to similar IgG1 and IgE antibody responses in the BDF1 mouse.

585 STUDY ON THE ALLERGENIC POTENTIAL OF A FIRE-EXTINGUISHER POWDER AT TWO TIME POINTS IN BROWN NORWAY RATS.

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This study was performed to evaluate the respiratory allergenicity of a fire-extinguisher powder (FEP; siliconized powder on ammonium phosphate/ammonium sulfate basis). Male Brown Norway rats were given s.c. either FEP, ovalbumin (OA) plus alum, or vehicle on days 0, 2, and 7 to induce hypersensitivity. An antigen challenge was performed under anesthesia on day 14 or 21, respectively, during simultaneous recording of lung function. The FEP-treated animals were challenged with either 14 or 290 µg deposited FEP aerosol (low FEP, high FEP), or they were not challenged (neg-

ative control, NEG). The OA-sensitized animals were challenged with OA aerosol (40 μg; POS). The vehicle-treated group was exposed to the high FEP aerosol dose to assess non-specific reactivity (NFEP). A novel dose control system was used which processes data on respiratory minute volume and aerosol concentration. Airway hyperreactivity was assessed 22-24 h after OA challenge using acetylcholine provocations with evaluation of the inhalational dose causing 150 % increase in lung resistance. Bronchoalveolar lavage (BAL) was performed and blood withdrawn for later IgE analysis 44-48 h after challenge. At both time points (14/21 days), the early reaction after OA aerosol challenge was determined as a marked increase in airway resistance, and a marked hyperreactivity was found in the OA vs. NEG animals 24 h after challenge. In the FEP groups as well as in the NFEP group only a moderate increase in resistance and a slight to moderate hyperreactivity were detected. Significant eosinophilia was only detected in the POS group. The OA-effects were more pronounced after 21 than after 14 days. We conclude: the tested FEP did not induce respiratory allergenicity but moderate non-specific airway reactions in a rat model of allergic asthma. The OA-results indicate that allergenicity should be assessed after 21 days.

586 DEVELOPMENT AND VALIDATION OF AN IMMUNOENZYMOMETRIC ASSAY FOR TOTAL MURINE IGE: APPLICATION TO THE IDENTIFICATION OF RESPIRATORY HYPERSENSITIVITY POTENTIAL IN C57BL/6J AND BALB/C MICE.

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The aim of this study was to: (1) refine and validate assay methodology for total serum IgE in mice and (2) assess possible strain differences in detection of respiratory sensitisers using this assay. The immunoassay methodology has been redeveloped with substitution of immunoreagents, optimisation of assay conditions and selection of suitable standard matrix. The method has been validated to GLP standards with regard to precision, accuracy, matrix effects, specificity and analyte stability. The assay has a lower and upper limit of quantification of 9 ng/ml and 1000 ng/ml respectively (CV and relative error ≤ 20%). Untreated BALB/c mice showed a 7-fold higher baseline level of IgE than the C57BL/6J strain (266.5 compared with 36.2 ng/ml). Proportional, dose-dependent, statistically significant ($P<0.001$) increases in IgE occurred in both strains in response to the administration of moderate (TMA) and strong (HDI) respiratory sensitisers. Greater proportional increases, over the vehicle control group level, were observed with C57BL/6J mice in comparison with BALB/c (55-fold versus 8-fold, elicited by HDI at a high-dose). Although the variability in IgE levels within each dose group was similar for both strains, the use of C57BL/6J may allow greater potential for detection of weak or moderate respiratory sensitisers, in view of the low baseline control IgE values in this strain.

587 INFLUENCE OF TOPICAL EXPOSURE TO RESPIRATORY AND CONTACT ALLERGENS ON SERUM IGE LEVELS IN THE BROWN NORWAY RAT.

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Topical exposure to chemical respiratory allergens such as trimellitic anhydride (TMA) has been shown previously to induce increases in the total serum concentration of IgE in BALB/c strain mice. Contact allergens such as 2,4-dinitrochlorobenzene (DNCB), which apparently lack respiratory sensitizing potential, fail to provoke similar changes. We have now examined the influence of topical exposure to TMA and DNCB on serum IgE levels in the Brown Norway (BN) rat. Such animals can be bled serially and thus it is possible to perform longitudinal analyses of changes in serum IgE concentration. The kinetics of IgE responses can therefore be followed on an individual animal basis, allowing discrimination between transient and sustained increases in serum IgE concentration and in addition the exclusion of those animals with pre-existing high IgE levels. Rats (n=5) were exposed on shaved flanks to 50% TMA, to 1% DNCB or to vehicle (acetone: olive oil; 4:1) alone on day 0 and received a repeat dose at half the initial concentration on day 7 on the dorsum of each ear. Total IgE was measured by enzyme-linked immunosorbent assay (ELISA) in serum samples taken prior to, and from 14 to 28 days following, initial exposure. Pre-exposure IgE levels ranged from 0.18-0.7μg/ml. The levels of serum IgE in the majority of rats exposed to DNCB or vehicle alone remained relatively stable throughout the duration of all experiments conducted (0.18-0.87μg/ml), although some animals displayed transient increases in serum IgE. In each study exposure only to TMA resulted in a marked and persistent elevation in serum IgE with levels peaking between days 21 to 28 (1.11-2.69μg/ml). These data suggest that the measurement of induced changes in serum IgE concentration in the BN strain of rat is able to differentiate between different classes of chemical allergen and support the use of DNCB and TMA as controls against which to assess the activity of other test materials.

588 TRIMELLITIC ANHYDRIDE (TMA) INDUCED RESPIRATORY HYPERSENSITIVITY IN THE GUINEA PIG DOES NOT DIFFER IN SEXUALLY IMMATURE OR MATURE ANIMALS OF EITHER GENDER.

C. P. Larsen and J. F. Regal. *University of Minnesota, Duluth, Duluth, MN.*

The prevalence of asthma in humans is higher in children than in adults, and higher in males than in females until the age of puberty. We determined if TMA-induced cellular infiltration into the lung differed among four groups of Hartley guinea pigs (Charles River Lab.): sexually immature females (n=24; 274 ± 4 g at sensitization) and males (n=23; 300 ± 4 g), and sexually mature females (n=12; 435 ± 5 g) and males (n=12; 587 ± 5 g). Animals sensitized with 0.3% TMA were intratracheally challenged 21 d later with 4 mg of either TMA conjugated to guinea pig serum albumin (TMA-GPSA) or GPSA. Twenty-four hr after GPSA challenge, numbers of eosinophils in both the bronchoalveolar lavage fluid (BAL) and lung tissue were greater in mature females than in mature males, while numbers of neutrophils in lung tissue were greater in mature males. Twenty-four hr after TMA-GPSA challenge, numbers of eosinophils, neutrophils and mononuclear cells in the BAL, eosinophils and neutrophils in lung tissue, microvascular leakage of protein and lung hemorrhage increased significantly over GPSA challenged animals in all four groups. The magnitude of the TMA-GPSA induced response did not significantly differ due to sexual maturity or gender ($p<0.05$, three-way ANOVA). Thus, TMA-induced respiratory hypersensitivity is similar in guinea pigs of either gender regardless of sexual maturation. (Supported by NIH ES 07406.)

589 EVALUATION OF ANTIGENS IN THE ELISA INHIBITION ESSAY FOR NATURAL RUBBER LATEX PROTEINS.

V. J. Tomazic-Jezic and A. D. Lucas. *USFDA, CDRH/OST/DLS/HSB, Rockville, MD.* Sponsor: P. L. Goering.

In the process of development of a ELISA Inhibition assay for the quantitation of the natural rubber latex (NRL) proteins, we investigated properties of proteins from ammoniated (AL) and non-ammoniated (NAL) raw latex, as coating antigens, immunizing antigens and reference antigens. The anti-NRL sera were produced by immunizing rabbits with AL, NAL and a mix of both antigens. Determination of affinity of rabbit antisera for binding to these antigens indicated strong preference of both anti-AL and anti-NAL antibodies to bind the immunizing antigen, while anti-Mix antiserum bound similarly to both antigens. Evaluation of the three sera in the ELISA inhibition assay with AL and NAL proteins as coating and reference antigens, further confirmed this finding. Anti-AL serum demonstrated a strong preference in inhibiting and binding to AL antigen, as compared to either NAL antigen or mix. In the case where NAL was a coating antigen, standard curves for both inhibition antigens (AL and NAL) with all three sera were more linear and uniform. Next we investigated the capacity of different anti-sera to inhibit proteins in glove extracts, comparing two coating antigens, AL and NAL. We observed a significant difference in the appearance of the inhibition curves of glove extracts with these two coat antigens. When NAL was used as a coat antigen, the inhibition curves by all three sera were similar. The results indicate that the selection of an appropriate combination of anti-serum, inhibiting and coating antigen is critical for the accurate measurements of the NRL proteins in the finished products.

590 A STUDY OF LATEX ALLERGY USING THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY.

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Some researchers have suggested that, as a result of latex glove use, latex sensitization rates in health care workers (HCW) may be far higher than in the general population. However, measurements of latex sensitization rates in HCW have varied considerably and are difficult to interpret because of biases. These include methodological problems (biased and low recruitment rates), not controlling for important confounders (atopy), combining different

latex exposures (powdered and non-powdered), and failure to include a control group. The National Health and Nutrition Examination Survey (NHANES) is periodically conducted by the National Center for Health Statistics to provide estimates of the health and nutritional status of the US population. In this paper, we analyze data on latex allergy from NHANES III for the period of 1988-91. Demographic and socioeconomic factors along with complete medical and dietary history are available for the surveyed subjects. In addition, spirometry, fundoscopy, and laboratory data on blood and urine are also available. We analyzed the prevalence of elevated (at least 0.35 IU/ml) IgE levels specific for latex, as determined by the AlaSTAT assay results reported in NHANES III. We found that the prevalence of elevated anti-latex IgE among HCW and non-HCW was not statistically significantly different. Odds ratios computed for current HCW vs non-HCW, and historically longest job HCW vs. non-HCW were 1.24 and 0.98, respectively (p-values>.05). Adjustments for age and sex did not significantly affect the results. Based upon the results of allergy skin tests reported in NHANES III, HCW were more likely to show positive responses to common allergens. In summary, the NHANES data suggest that the prevalence of latex sensitization in HCW is no greater than in the general population.

591 RETINOID BINDING PROTEINS AND RETINOID TOXICITY.

E. Li. *University of Washington School of Medicine, St. Louis, MO.* Sponsor: D. Acosta.

Vitamin A is indispensable for growth, reproduction, differentiation and vision of vertebrates. Vitamin A deficiency is a major cause of childhood morbidity and mortality worldwide. On the other hand, the pharmacological use of retinoids can result in severe fetal malformations and in the adult most commonly causes hyperlipidemia. Two classes of non-steroid nuclear hormone receptors mediate retinoid signaling, the retinoic acid receptor and retinoid x receptor. In addition, the retinoid x receptors play a central role in regulating gene transcription through formation of homodimers and formation of heterodimers with other nuclear receptors other than the retinoic acid receptors, such as the peroxisome proliferator-activated receptors, vitamin D3 receptors, the thyroid hormone receptors. Intracellular trafficking of retinoids is mediated by specific cytoplasmic carrier proteins, which influence ligand availability for binding with the nuclear retinoids. Retinoid signaling involves a complex interplay between the nuclear receptors, cytoplasmic carrier proteins and sites of metabolic processing. Our laboratory has been interested in studying the structural basis of ligand protein interactions and in modeling intracellular trafficking of retinoids between various retinoid binding proteins. We have purified fully functional bacterially expressed nuclear retinoid receptors and cytoplasmic retinoid binding proteins and analyzed the interactions of these proteins with retinoids in solution using a number of biophysical techniques, most notably nuclear magnetic resonance. Using this approach we have modeled how the physical properties of these binding proteins affect vitamin A homeostasis and toxicity.

592 ASSESSING THE SAFETY OF GENE THERAPY.

J. E. Sanders[1] and M. E. I. Leibbrandt[2]. [1]*Rhône-Poulenc Rorer, Collegeville, PA and* [2]*Chiron Corp., Emeryville, CA.*

The ability to add a gene to a specific cell type is of great potential for the practice of medicine since it provides the opportunity to alter cellular function or structure. Doing so allows us to treat disease from the "inside out." Genes can be delivered by many routes of administration in viral or non-viral vectors; the individual characteristics of the vector transgene combination create unique challenges for the science of toxicology. For example, all viral vectors have the potential to become replication-competent and thus the molecular structure of the gene therapy should be chosen to minimize this risk. The potential for toxicity due to insertion into the host genome should be evaluated when integrating vectors are used (retroviral, adeno-associated viral) to confer long term expression of the transgene. Host immune responses associated with adenoviral vectors, on the other hand, create different challenges for toxicologists. The acute toxicity of the vector particle should be assessed in concert with the toxicity of the expressed transgene. Since there is currently limited ability to target gene therapies to individual cell types, the consequences of inappropriate transduction and expression should be evaluated with reference to biodistribution *in vivo*. The level and localization of such inadvertent transduction in tissues such as the testis and ovary must be closely examined in order to ensure negligible risk of germline transmission.

593 OVERVIEW OF GENE THERAPY.

P. Delaere. *Rhône-Poulenc, Vitry-Sur-Seine, France.* Sponsor: J. E. Sanders.

Gene Therapy is a great opportunity for the Pharmaceutical Industry since it is a new way to deliver new drugs which are produced locally and can act either locally or systemically. Viral and non viral vectors can transfer genes into various tissues (tumor cells, skeletal and cardiac muscles, vascular wall, brain tissue...). Currently different types of vectors are used in clinical trials: retrovirus, adenovirus, lentivirus, AAV, Poxvirus, naked DNA combined or not with lipofection or electroporation. The genes are transferred and expressed; they can evoke biologic responses that are relevant to human diseases. Adverse events have been rare and have been related mostly to the dose and the manner in which the vectors were administered. Improvements of vectors reside in the efficiency of gene delivery, the selective physical and/or genetic targeting, the decrease in the inflammation associated with some viral vectors, and the control and the stability of expression. A broad survey of gene therapy will be presented with the aim of answering the questions: What is gene therapy, how is it being used in clinical and in preclinical studies? What are some of the generic and specific safety, issues associated with gene therapy? Regarding the type of vectors, how do they achieve therapeutic effect? What are the vector specific advantages and issues. The potential side effects of specific delivery systems will also be discussed. Although different types of vectors will be presented with their advantages and limitations, a focus will be provided on adenovirus as viral vectors and on non viral approaches which exhibit complementary advantages. This presentation will also provide the audience with an appreciation of the technical challenges to measure and localize vector tissue distribution and gene expression using state-of-the-art assays.

594 PRE-CLINICAL EVALUATION OF THE HUMAN FACTOR VIII RETROVIRAL VECTOR.

M. E. I. Leibbrandt, H. Roehl, J. Greengard and M. Giedlin. *Chiron Corp., Emeryville, CA.*

Hemophilia A, characterized by absent or low levels of circulating functional factor VIII, occurs in 1 of every 10,000 live male births. Currently, this disease is treated by exogenous replacement of the deficient protein through administration of recombinant factor VIII or plasma-derived factor VIII. Successful gene therapy would confer long term, endogenous expression of factor VIII at levels sufficient to provide prophylaxis from spontaneous joint bleeds. A retroviral vector expressing human factor VIII (hFVIII(V)) has been developed for the treatment of patients with hemophilia A. Intravenous administration of hFVIII(V) to adult or juvenile rabbits at doses of $0.6–21 \times 10^8$ cfu-eq/kg was well-tolerated and led to the expression of therapeutic levels (=14 ng/ml) of factor VIII. Factor VIII levels varied widely between individual animals, possibly due to the presence of anti-human factor VIII antibodies. Expression followed a lag phase of 2-3 weeks and continued until animals were sacrificed between 3 months and 2 years post-treatment. There was a correlation between the dose of hFVIII(V) and circulating levels of FVIII. Acute toxicology studies in mice and rabbits showed no effects of hFVIII(V) treatment on hematology or clinical chemistry parameters, body weights, food consumption or macroscopic observations at necropsy at doses up to 96×10^8 cfu-eq/kg. Hemophilic dogs treated with hFVIII(V) showed shortened whole blood clotting time although response was highly variable between individual animals. Biodistribution of hFVIII(V) by PCR showed that vector- specific sequences localized primarily to the liver and spleen with occasional signal in other tissues including testis. A 16-week study is underway in rabbits to determine whether localization of hFVIII(V) to testis is associated with the presence of vector-specific sequences in semen.

595 PRE-CLINICAL STUDIES ON DNA PLASMID DISTRIBUTION AND INTEGRATION.

W. Nichols, B. Ledwith, S. Manam, P. J. Troilo, A. B. Barnum, C. J. Pauley, T. G. Griffiths II, L. B. Ludlow, H. B. Schock, H. Zhang, J. E. Faris, P. A. Way, C. M. Beare and W. J. Bagdon. *Merck Research Labs, West Point, PA.* Sponsor: J. E. Sanders.

The primary safety concerns for DNA plasmids being used for vaccines or gene therapy are integration into the genome of the recipient, the induction of chromosomal aberrations, autoimmune effects and the induction of anti-DNA antibodies. Today this discussion will be limited to integration into the host cell genome and the tissue distribution of plasmids after Intramuscular injection. Integration is of concern because by definition this produces insertional

mutagenesis and this in turn has the potential to activate oncogenes or inactivate tumor suppressor genes and could therefore play a role in tumorigenesis. A test system utilized for integration should have a sensitivity that permits comparisons to the spontaneous mutation rate in order to make risk evaluations. This can be achieved by electrophretic methods that can separate free plasmid from genomic DNA followed by PCR techniques to detect plasmid in the purified genomic DNA. This methodology is capable of detecting one plasmid integration in 150,000 nuclei. In studies of several different plasmids at 6 weeks and 6 months after injection, plasmids are detected at the site of injection. At time points of 1 to 7 days after injection plasmid is detected in small numbers in blood and many of the 14 tissues that are examined for tissue distribution. There has been no evidence of integration at the sensitivity of approximately one copy per microgram of DNA which, assuming that each integration would produce a significant mutation, is at least 3 orders of magnitude less than the sponteneous mutation frequency.

596 REGULATORY CONSIDERATIONS FOR GENE THERAPY CLINICAL TRIALS.

A. M. Pilaro. *FDA/CBER, Rockville, MD.* Sponsor: J. E. Sanders.

Gene therapy is one of the fastest growing areas in biotechnology, with over 300 clinical protocols currently active. Initially, gene therapy agents were used to "replace" defective genes in diseases such as cystic fibrosis, in which the underlying disease could contribute to the toxicity of the agent employed. Developing a toxicology program to determine the safety of these agents, therefore, requires a modified approach which encompasses both the pharmacology and toxicity of the gene product itself, as well as the vector system used for delivery, in the context of the application for the clinical trial. The issues involved in designing and developing appropriate preclinical safety testing for these agents, and the regulatory guidances currently in practice in the United States will be presented. An overview of the safety issues novel to gene therapies, and the strategies for designing preclinical studies to address these concerns will be discussed. The effects of the route of administration, frequency and duration of exposure, as well as the type of vector employed on the safety and clinical activity will be identified. Other safety considerations to be discussed include biodistribution, duration and degree of vector expression in target and other tissues, the effects of gene expression on organ pathology and/or histology, and interactions of the host immune system with the transduced cell population. Current recommendations for preclinical studies in support of future licensing of gene therapeutic agents will also be discussed.

597 ADVANCES IN THE USE OF MECHANISM-BASED BIOMARKERS IN RISK ASSESSMENT.

L. L. Smith[1], E. A. Lock[1] and J. A. Swenberg[2]. [1]*AstraZeneca CTL, Alderley Park, United Kingdom and* [2]*University of North Carolina, Chapel Hill, NC.*

Understanding the biochemical and molecular mechanisms whereby foreign compounds interact with and perturb biological systems is fundamental to toxicology. The use of molecular mechanistic data from whole animal studies, human exposure and *in vitro* studies with animal and human tissues is pivotal to assessing the likely risk to humans. Recent advances in molecular techniques and the use of biomarkers has enabled more accurate assessment of risk following exposure to chemicals. It is now well established that human susceptibility to tissue injury and cancer can in part be attributed to genetic polymorphisms. The symposium will focus on the application of molecular markers to determine exposure, early effects and susceptibility to benzene, butadiene, polycyclic aromatic and heterocyclic amines, nitrosamines, and polycyclic aromatic hydrocarbons found in cigarette smoke and polluted air. The utility of molecular markers causally related to the mechanism of toxicity of a novel herbicide will be presented. Emerging evidence on the role of gene environmental interaction associated with DNA damage, that may contribute to the etiology of human cancer will also be discussed.

598 MOLECULAR BIOMARKERS OF BENZENE EXPOSURE AND RISK.

M. T. Smith[1], L. Zhang[1], R. Hayes[2], G. L. Li[3] and N. Rothman[2]. [1]*University of California, Berkeley, CA,* [2]*National Cancer Institute, Bethesda, MD and* [3]*Chinese Academy Preventive Medicine, Beijing, China.*

Benzene is an established cause of leukemia at high doses, but the risk it poses at exposures of <1ppm in air is difficult to quantify. Molecular biomarkers may improve the accuracy of this risk assessment. We have therefore attempted to develop and validate biomarkers of exposure, early effect and susceptibilty to benzene. We have shown that NQO1 genotype and cytochrome P4502E1 phenotype are important biomarkers of susceptibility. Further, since chromosomal aberrations (CA) have been shown to be predictive of future cancer risk and specific CA are found in leukemias, we have proposed that specific CA may be excellent biomarkers of early effect. Leukemia-specific CA were found at elevated rates in the blood of 43 workers exposed to high levels of benzene (median, 31ppm). A larger study is planned for the year 2000 that will use these validated markers in biological samples from a substantially larger number of workers exposed to lower levels of benzene. The design of this new study will be presented together with a summary of data generated from our original cross-sectional study in highly exposed workers.

599 ADVANCES IN THE USE OF MECHANISM-BASED BIOMARKERS IN RISK ASSESSMENT.

J. Swenberg, H. Koc, A. Ranasinghe, N. Christove-Gueorguieva and P. Upton. *University of North Carolina, Chapel Hill, NC.*

Butadiene (BD) is a potent carcinogen in mice, a weak carcinogen in rats and a probable carcinogen in humans. Recent molecular dosimetry studies in these species have greatly increased our understanding of the metabolism of BD. It is clear that the major electrophile (>95%) in all three species is the epoxybutane diol (EBD). This is the result of metabolism of BD in the endoplasmic reticulum to BDO, then to BDO2, and finally to EBD. EBD is 1/200th as mutagenic as BDO2. BDO2 and EBD form trihydroxybutane adducts in DNA and hemoglobin. The DNA adducts of BDO show a linear response, while the trihydroxybutane adducts show a supralinear response due to saturation of metabolic activation. Molecular epidemiology studies on BD workers have shown clear evidence of exposure, based on hemoglobin adducts, but no evidence of genotoxicity following a battery of evaluations. Furthermore, lymphocytes from GSTT1 null individuals have been shown to to be at greater risk for SCE formation following *in vitro* exposure to BDO2, but no such increased susceptibility was observed for any biomarker in studies of exposed workers. Thus, the increased susceptibility of the GSTT1 null genotype appears to be associated with saturation of this detoxication pathway following high *in vitro* exposures.

600 TYROSINEMIA AS A MARKER OF EXPOSURE TO HPPD INHIBITORS.

W. M. Provan, J. Botham, J. E. Doe, R. W. Lewis and M. Robinson. *AstraZeneca, Macclesfield, United Kingdom.* Sponsor: E. A. Lock.

In mammals, the single mechanism of action of mesotrione, a selective herbicide, is inhibition of the hepatic enzyme, 4-hydroxyphenyl pyruvate dioxygenase (HPPD), a key enzyme in the catabolism of tyrosine. Prolonged inhibition of this enzyme increases plasma tyrosine levels, the extent of which is dependent on the efficiency of a second hepatic catabolic enzyme, tyrosine aminotransferase, (TAT). TAT activity is species and sex dependent in the rat, male rats having particularly little activity while humans and mice have high activity. Consequently, on prolonged exposure to mesotrione, rats develop a severe tyrosinemia with associated toxicological symptoms: ocular lesions, increased liver and kidney weight, reduced body weight and reduced pup survival. In contrast, high hepatic TAT activity in the mouse precludes extreme elevation of plasma tyrosine levels, yielding no significant toxicological effects in this species after exposure to mesotrione. The activity of TAT in mice and humans is similar. Thus, humans, like mice, are unlikely to develop the severe tyrosinemia and associated toxicity expressed in rats. This is confirmed by results in humans patients being treated for hereditary tyrosinemia type I with high doses of a more potent structurally related triketone. It is concluded that high levels of tyrosine mediate the species specific toxicity associated with exposure to mesotrione and tyrosine elevation is a function of the HPPD inhibition and innate TAT activity. Thus, the plasma concentration of tyrosine, and TAT activity levels are mechanism-based biomarkers which accurately reflect the species/sex-specific toxicity associated with exposure to mesotrione and are key to accurate human risk assessment.

601 RELATION BETWEEN GENETIC SUSCEPTIBILITY AND DNA ADDUCT FORMATION.

F. F. Kadlubar[1], P. H. Thompson[1], C. B. Ambrosone[1], M. Yang[1], B. F. Coles[1], N. P. Lang[2] and K. E. Anderson[3]. [1]*Division of Molecular Epidemiology, NCTR, FDA, Jefferson, AR,* [2]*Arkansas Cancer Research Center, Little Rock, AR and* [3]*University of Minnesota, Minneapolis, MN.* Sponsor: E. A. Lock.

Carcinogen exposure and carcinogen-DNA damage occur sequentially as necessary though not always sufficient events in the initiation of the cancer process. In humans, there is now unequivocal evidence that both exogenous and endogenous DNA adducts are present in carcinogen-target tissues. However, differences in human cancer susceptibility can also be attributed to genetic polymorphisms in carcinogen-metabolizing enzymes, in DNA repair mechanisms, and in genes that control cell growth and differentiation. Accordingly, a combined approach is now needed in order to the assess the relevance of these findings to cancer etiology, to identify high-risk individuals, and to provide better health monitoring, earlier diagnosis, and cancer prevention. Our recent efforts have focussed on aromatic and heterocyclic amines, nitrosamines, and polycyclic hydrocarbons found in cigarette smoke, polluted air, and in cooked foods and on the role of such gene-environmental interactions associated with DNA damage that may contribute to the etiology of human colon, pancreas, breast, prostate, and lung cancers.

602 THE ROLE OF ENDOTOXIN IN OCCUPATIONAL AND ENVIRONMENTAL LUNG DISEASE: EXPOSURE-RESPONSE RELATIONSHIPS AND SUSCEPTIBILITY FACTORS.

P. S. Thorne[1] and I. Glimour[2]. [1]*The University of Iowa, Iowa City, IA and* [2]*USEPA, Research Triangle Park, NC.*

Endotoxins (lipopolysaccharides, LPS) are integral components of the outer membrane of Gram-negative bacteria and are ubiquitous throughout our environment. They are comprised of a conserved lipid region (lipid A) which imparts toxicity, and a species-specific long chain polysaccharide moiety that binds host receptors such as LPS binding protein (LBP) and CD14. Inhalation exposures to endotoxin occur in complaint buildings, agricultural settings and in a variety of industrial operations. Epidemiologic studies consistently demonstrate that endotoxin exposure is a significant risk factor for the development of respiratory symptoms and decrements in lung function, and may be an important factor in the pathogenesis or progression of lung diseases such as byssinosis, organic dust toxics syndrome, asthma, and cystic fibrosis. After inhalation, endotoxin triggers the upregulation of an array of cytokines leading to neutrophil recruitment and lung injury. This symposium will present recent studies of the chemical and toxicological properties of endotoxin, report exposure concentrations from a variety of occupational environments, and describe current analytical methods for endotoxin measurement. Exposure-response data from epidemiology studies and recent inhalation experiments in humans and animals will then show the role of endotoxin in causing or promoting lung disease, and highlight differences in genetic susceptibility. Finally, the role of endotoxin exposure as a co-factor in asthma and its interaction with other forms of air-pollutant exposure will be described.

603 STRUCTURE AND FUNCTION OF ENDOTOXIN AND MOLECULAR RESPONSES IN THE LUNG.

M. I. Gilmour. *USEPA, Research Triangle Park, NC.*

Lipopolysaccharide (LPS) is the active component of endotoxin in the walls of all Gram-negative bacteria, and is capable of eliciting a febrile response and inflammation in a variety of tissues. LPS molecules comprise a conserved lipid region (lipid A), and a long chain polysaccharide moiety that displays high diversity across bacterial species. The polysaccharide component facilitates binding to serum proteins and cellular structures, while phosphorylated D–glucosamine residues, fatty acids, and acyl chains on the lipid A portion impart biological activity. Although almost any mammalian cell will respond to LPS, monocytes, leukocytes, and endothelial and epithelial cells are most often associated with its activation and subsequent effects. A number of different binding proteins have been shown to enhance the activational properties of LPS. LPS binding protein (LBP) is a serum factor that catalyzes the interaction between LPS and CD14 on the surface of monocytes, resulting in increased calcium flux, tyrosine phosphorylation and activation of nuclear transcription factors. In cells lacking CD14 receptors (e.g. smooth muscle and endothelial cells), LPS stimulation may occur through other receptors (CD11 and CD18). Following these various activational signals, cells produce an array of potent endogenous mediators including IL-1α, IL-6, IL-8, TNFα, IFN-γ; and prostaglandins. Depending on the concentration of LPS exposure, symptoms may range from a mild fever and myalgia to severe leukopenia, tachycardia, hypotension, and multi–organ failure (septic shock syndrome). While mild symptoms will resolve within hours, more severe illness can be treated with receptor antagonists of the various mediators or by competitive inhibition with less toxic forms of LPS. (This abstract does not reflect EPA policy.)

604 ENDOTOXIN AS A CAUSATIVE AGENT FOR OCCUPATIONAL AND ENVIRONMENTAL LUNG DISEASE.

P. S. Thorne. *College of Public Health, The University of Iowa, Iowa City, IA.*

Inhaled endotoxins are bioactive components of aerosols found in both occupational and non-occupational settings. Exposures to airborne endotoxins are generally quantified from air samples using a bioactivity assay, the Limulus amebocyte lysate (LAL) assay. Assay validation studies in our laboratory and others have demonstrated acceptable within-lab reproducibility but generally poor between-lab reproducibility. Research is underway to improve or replace the LAL assay. We have used the LAL assay to assess endotoxin exposures in a variety of occupational and domestic environments. Exposures to endotoxins occur at high levels in agricultural settings such as livestock barns and during crop harvesting, transfer and processing. Exposures in agriculture can exceed 35,000 EU/m3 of air but generally range from 100 to 10,000 EU/m3. Endotoxin exposures usually occur at lower levels (1-2,500 EU/m3) in industrial settings such as metals machining plants, composting facilities, and sawmills. Our analyses of dust samples from homes across the U.S. indicate dust concentrations ranging from 102 to 106 EU/m2 of vacuumed surface with presence of carpeting, geographic location, and indoor storage of kitchen waste as important determinants. Epidemiologic studies have linked endotoxin exposure with a variety of pulmonary conditions including chronic nose and throat irritation, organic dust toxics syndrome, grain fever, asthma-like syndromes and exacerbation of asthma. Inhalation toxicology studies using susceptible mouse strains have demonstrated production of pro-inflammatory cytokines (e.g. IL-1, IL-6, TNFα, and MIP-2) and neutrophil recruitment to the airways leading to airway remodeling. Recognition of endotoxin-induced pulmonary toxicity has led to proposals to establish occupational exposure limits, although currently none exist in the U.S.

605 THE GENETICS OF ENDOTOXIN-INDUCED AIRWAY DISEASE.

D. A. Schwartz. *University of Iowa, Iowa City, IA.* Sponsor: P. S. Thorne.

Despite the tremendous inter-individual variability in the response to inhaled toxins, we simply do not understand why certain people develop disease when challenged with environ-mental agents and others remain healthy. Although asthma is a complex disease, it appears to occur in genetically susceptible individuals who are primed to respond to a limited number of specific agents, such as endotoxin. Focusing on the airway response to inhaled endotoxin has permitted us to determine that humans and different strains of mice have a broad physio-logic and biologic response to this very common agent that is known to cause and exacerbate acute and chronic forms of airway disease. Moreover, our studies in humans indicate that a common missense mutation in the extracellular domain of the TLR4 receptor, a gene that has been shown to affect endotoxin responsiveness in mice, is associated with a significantly blunted response to inhaled endotoxin in humans. In vitro functional studies confirm the biological importance of this mutation in the TLR4 receptor. Moreover, the wild type allele of TLR4 rescues the endotoxin hyporesponsive phenotype in either primary airway epithelial cells or alveolar macrophages obtained from individuals with the TLR4 mutation. Our findings provide the first genetic evidence that a common mutation causes differences in endotoxin responsiveness, and demonstrate that gene sequence changes alter the ability of the host to respond to environmental stress.

606 INFLAMMATORY AND EPITHELIAL RESPONSES IN AIRWAYS EXPOSED TO ENDOTOXIN AND OZONE.

J. R. Harkema. *Michigan State University, East Lansing, MI.*

People are exposed daily to a combination of air pollutants (e.g. ozone) and airborne biogenic substances (e.g. bacterial, antigenic). Ozone is the principal oxidant pollutant in photochemical smog and a potent airway irritant. Millions of people live in urban environments (e.g. Mexico City) with high ambient

concentrations of both ozone and bacterial endotoxin. This lecture will focus on the results of studies designed to test the hypotheses that 1) co–exposure to ozone and endotoxin cause greater alterations to the airway epithelium than exposure to either toxicant alone and 2) that the magnitudes of these epithelial changes are dependent on the preceding or concurrent airway inflammatory response. F344 rats were exposed to 0 or 0.5 ppm ozone, 8 h/day, for 3 days and intranasally instilled with endotoxin (100μg) prior to each daily exposure. Nasal tissues were processed for light microscopy and image analysis, or for mucin–specific mRNA analysis. Ozone alone caused nasal airway alterations that included neutrophilic inflammation, increased mucin gene expression, and nasal epithelial proliferation (hyperplasia) and differentiation (mucous cell metaplasia). Endotoxin alone caused nasal inflammation, mucin–gene overexpression, and epithelial hyperplasia, but no mucous cell metaplasia. Rats exposed to both toxicants had greater inflammatory and epithelial responses than either toxicant alone, and endotoxin potentiated the ozone–induced mucous cell metaplasia. Subsequent studies have suggested that this potentiation is dependent on the neutrophilic inflammation. Our findings suggest that endotoxin exposure can magnify some, but not all of the epithelial lesions caused by ozone, and this augmentation may be mediated through inflammatory mechanisms. (Funded by NIH grant HL59391.)

607 THE ROLE OF ENDOTOXIN IN ASTHMA AND ALLERGY.

D. Peden. *UNC Chapel Hill, Chapel Hill, NC.* Sponsor: M. I. Gilmour.

Endotoxin is a commonly encountered air contaminant in a number of occupational settings, with high concentrations being reported in ambient air. Such levels are associated with acute reductions in lung function, respiratory symptoms and nasal inflammation in exposed persons. LPS levels in the range of 0.25 μg/m3 have been associated with increased reports of ocular and nasal symptoms by office workers, suggesting that even low levels of LPS may cause airway symptoms and inflammation. Furthermore, disease severity of mite-sensitive asthmatics has also been shown to correlate better with levels of endotoxin than mite allergen in samples of house dust collected from their homes. These observations suggest that lower levels of LPS may cause morbidity in sensitive subgroups, such as atopic asthmatics. In human challenge studies, allergic asthmatics have been reported to have increased responses (decreased lung function and increased non-specific airway reactivity) to LPS challenge than non-asthmatic subjects. Exposure of asthmatics to low levels of LPS enhances immediate and late phase responses to inhaled allergen. Likewise, nasal LPS challenge in atopic asthmatics induces eosinophil influx in asthmatics that have increased constitutive levels of GM-CSF. Allergen challenge of allergic asthmatics also causes increased levels of CD14 (the LPS receptor) in BAL fluid from these subjects, suggesting that allergen challenge could enhance inflammatory responses to LPS. Preliminary data from our group reveals that allergen challenge prior to LPS exposure yields greater PMN and eosinophil numbers in the nasal airway than either LPS or allergen alone. Taken together, these data support the hypothesis that asthmatics, especially atopic asthmatics, may be at increased risk for respiratory tract morbidity associated with low level LPS exposure.

608 FRITZ HABER AND HIS IMPACT ON TOXICOLOGY.

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Fritz Haber (1868-1934) was a German physical chemist, Nobel prizewinner and foreign member of the US Academy of Science. His greatest accomplishment in science was the development of a practical method to prepare nitrogen from air (nitrogen fixation or Haber-Bosch process). While working on the toxicity of war gases, he formulated "Haber's rule" [CxT = constant] in order to characterize the toxicity of a toxic inhalant. Between 1919 and 1933, he was one of the leading figures in revitalizing science in Germany. At his institute in Berlin, he worked with such luminaries as Albert Einstein, Lise Meitner and Otto Hahn. His last paper described what became know as the Haber-Weiss reaction. After his death he became for a long time forgotten by the Nazis because he was Jewish and after World War II by the Allies because they never forgave him for his work on war gases in World War I. And yet he was one of the truly great modern scientists, not only because of his science, but also because of his role in science politics and policies. Haber's rule can actually be rewritten as a special case of the family of power law curves and may be used in standard setting and risk assessment. Some newer experimenters show that the rule also applies to chronic toxicity, provided that exposure occurs under conditions of a steady state of exposure.

609 HABER'S RULE (CT=K): A SPECIAL CASE IN A FAMILY OF CURVES RELATING CONCENTRATION AND DURATION OF EXPOSURE TO A FIXED LEVEL OF RESPONSE.

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In the early 1900s, Haber expounded that the acute lethality of war gases could be assessed by the concentration of the gas in a cubic meter of air multiplied by the time in minutes that the animal had to breathe the air before death ensued (i.e., Ct=k). This concept was not original with Haber. In 1900, Warren used the equation T(X-8) = constant, where T was the time of killing and X was the strength of salt solution that killed Daphnia magna. Both Warren and Haber recognized that Ct=k was applicable only under certain conditions. However, many toxicologists have used this relationship to analyze experimental data whether or not their chemicals, biological endpoints, and exposure scenarios were suitable candidates for Haber's Rule. Since the relationship between C and t is linear on a log-log scale and was, therefore, relatively easy to solve by hand, Haber's Rule gained early acceptance among toxicologists. In 1940, Bliss provided an elegant treatment on the relationship between exposure time, concentration, and toxicity of insecticides. Bliss examined various situations involving the equation (C–C0)nt=k, where the constant, C0, represents a threshold concentration or physiologic zero for a given mode of toxic action. Haber's Rule has been applied to latency data from cancer studies, but enough exceptions were found that Ctn=k was proposed as a general rule for such data. Some argue that various aspects of concentration and duration of exposure leading to a fixed level of response have surfaced so frequently in toxicology that this relationship may be generalizable to the whole field. The family of power law curves represented by $(C-C_0)\alpha\ t^0=k$ allows for such a generalization and encompasses the full range of toxicokinetic and toxicodynamic conceptual considerations that will be discussed by others at this workshop.

610 THE USE OF HABER'S RULE IN STANDARD SETTING AND RISK ASSESSMENT.

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Haber's Rule simply states that the incidence and/or severity of a toxic effect depends on the total exposure, i.e., exposure concentration (c) rate times the duration time (t) of exposure (cxt). This rule, within constraints, often is used in setting exposure guidelines for toxic substances. Establishing reference doses (acceptable daily intakes) for long-term exposures when only the results of short-term studies are available requires the use of an uncertainty (safety) factor. The value of this uncertainty factor often approximates a value comparable to Haber's Rule for extrapolation from short-term to long-term exposure durations. As a default procedure, cancer risk estimates generally are based on the average lifetime daily dose which is derived from the total cumulative exposure, i.e., Haber's (CxT). This has been shown both theoretically and empirically to be valid within a factor of 10 for carcinogens. This provides some credence for the use of an additional safety factor of 10, in some instances, for exposures of children to carcinogens. Finally, a generalization of Haber's Rule, exposure concentration raised to a power times exposure duration, will be discussed.

611 THE HABER-WEISS REACTION AND MECHANISMS OF TOXICITY.

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The concept that the highly reactive hydroxyl radical (HO•) could be generated from an interaction between superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) was proposed (with Joseph Weiss) in Professor Haber's final paper published in 1934. Until it was recognized that free radicals are produced in biological systems, this finding seemed to have no relevance to biology. However, following the discovery that $O_2^{\bullet-}$ was a normal cellular metabolite, it was quickly recognized that the Haber-Weiss reaction ($O_2^{\bullet-} + H_2O_2 \rightarrow HO^{\bullet} + O_2 + HO^-$) might provide a means to generate more toxic radicals. Although the basic reaction has a second order rate constant of zero in aqueous solution and thus cannot occur in biological systems, the ability of iron salts to serve as catalysts was discussed by these authors. Because transition metal ions, particularly iron, are present at low levels in biological systems, this pathway (commonly referred to as the iron-catalyzed Haber-Weiss reaction) has been widely postulated to account for the *in vivo* generation of the highly reactive

HO•. Recent data documenting the importance or redox regulation of various cellular signaling pathways makes it clear that free radicals are essential for normal cellular function. However, this also makes it obvious that disruptions of free radical production or defenses at many different levels can lead to adverse effects on cells. While the generation of HO•, which is by far the most reactive oxygen species, is generally indicative of an overtly toxic event, it is through studies at this level that we have reached a better understanding of free radicals as both signaling molecules and toxic species.

612 THE ROLE OF TIME IN TOXICOLOGY OR HABER'S CXT PRODUCT.

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It happened exactly 100 years ago that Warren established for the first time a quantitative link between dose and time while studying the toxicity of sodium chloride in Daphnia magna (Straus). During this century many toxicologists in different contexts returned to this idea, which has become known as Haber's Rule of inhalation toxicology. Every attempt to explore this relationship ended in frustration because of the supposed occurrence of exceptions. Thus, toxicologists concentrated on the quantitative relationship between dose and effect under mostly isotemporal conditions while time took a back seat and was assigned such arbitrary, semiquantitative designations as acute, subacute, subchronic and chronic. Time itself as a quantifiable variable of toxicity was seldom studied and when it was done, it was often not under isodosic (steady state) conditions as required by theory. A recent analysis of toxicological time indicated the impact of 3 independent time scales (toxicokinetic, toxicodynamic, exposure frequency) in toxicological studies, which interact with dose and effect to yield the enormous complexity known to every toxicologist. Based on prototypical examples when toxicokinetic (dioxins), toxicodynamic (nitrosamines, benzene) or exposure frequency (methylene chloride, chloroacetic acid, HgCl2, CdCl2 etc.) represent the critical time scale, the general validity of the cxt=k concept will be discussed as a starting point for a theory of toxicology. As endpoints of toxicity, (delayed) acute toxicity, blood dyscrasias and cancer will be used to illustrate the critical conditions needed to demonstrate the validity of this theory.

613 ETHANOL SIGNALING APOPTOSIS IN NORMAL HUMAN HEPATOCYTES, ROLE OF TUMOR NECROSIS FACTOR α, FAS LIGAND AND CASPASE-3 INHIBITOR.

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Aims: 1-To assess the mechanism by which ethanol (EtOH) is signaling for apoptosis in a normal human primary hepatocyte (NHPH) culture. 2-To delineate the role of tumor necrosis factor alpha (TNF) antibody (anti-TNF) and caspase-3 inhibitor (CAPI) in cell protection against apoptosis. **Methods:** In a culture of NHPH treated for 24 hours, either with 80 mM EtOH or 30 pg/mL TNF, apoptosis was assessed by ELISA, which quantitates histones associated with DNA cleavage at the internucleosomal linker region, and by transmission electron microscopy. In a second set of experiments, prior to the same treatment, cells were exposed to 5 nM CAPI or 30 pg/mL anti-TNF. For each set of experiments, apoptosis was assessed in 6 flasks (6000 cells/flask) and 30 wells. We also measured the cytochrome oxidase activity with the diaminobenzidine immunochemnistry technique. Fas(APO-1, CD95) molecules on the plasma membrane of hepatocytes increased and were represented immunohistochemically in cells without chromatin condensation. **Results:** ANOVA was used for statistical analysis and the values are given as mean % apoptotic cells or bodies ± SD. The value for control was 6±0.5; for EtOH, 26±1*; for TNF, 53±5*; for EtOH and anti-TNF, 16± 8#@; for TNF and anti-TNF, 4±2; and for EtOH and CAPI, 10±6@ (@p<0.05 lower than the EtOH value; #p<0.05; *p<0.001 higher than the control value). As shown by this data, apoptosis in TNF treated cells is abolished by anti-TNF. Hepatocytes are protected against EtOH-induced apoptosis by pre-treatment with anti-TNF and CAPI. In early stage of apoptosis was al rupture of the outer mitochondrial membrane. Parts of the inner membrane extended through the gap of the outer membrane, whereas the rest of the inner membrane still formed the cristae. This feature appeared in hepatocytes before chromatin condensation. Taking all the apoptotic hepatocytes we may characterized them by being apoptotic with condensed chromatin and high enzyme activity (10%), pre-apoptotic, without chromatin condensation but high enzyme activity (35%), and neither condensed chromatin nor a remarkable reaction product of cytochrome oxidase activity(55%) Fas(APO-1, CD95) molecules on the plasma membrane of hepatocytes increased in cells without chromatin condensation. **Conclusions:** EtOH-induced apoptosis is initiated by TNF and Fas ligand. Therefore, both anti-TNF and CAPI might inhibit hepatic apoptosis. Mitochondria play a pivotal role in pre-apoptotic stages.

614 ROLE OF TNFα- AND NFκB- MEDIATED CELLULAR SIGNALLING IN THE SUPPRESSION OF RAT HEPATOCYTE APOPTOSIS BY PEROXISOME PROLIFERATORS.

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Peroxisome proliferators (PPs) cause rodent liver enlargement and tumors. *In vitro*, PPs induce rat and mouse hepatocyte DNA synthesis and suppress both spontaneous apoptosis and that induced by the physiological negative liver growth regulator, transforming growth factor β1 (TGFβ1). Previously, we have shown that exogenous tumour necrosis factor α (TNFα) perturbs growth control in rat or mouse hepatocytes, implicating this cytokine in the mechanism of action of PPs. In addition, antibodies to TNFα receptor type 1 (TNFR1) abrogated completely the response of mouse hepatocytes to nafenopin whereas anti-TNFα receptor 2 blocking antibodies were without effect. Since TNFα signalling to the transcription factor nuclear factor κB (NFκB) is thought to be responsible for many of the effects elicited by this cytokine, we investigated activation of NFkB by nafenopin and by TNFα. Nafenopin shared the ability of TNFα to cause activation of NFκB as determined by electrophoretic mobility gel shift assay (EMSA). NFκB activation requires the successive action of NFκB inducing kinase (NIK), and the phosphorylation of NFκB inhibitory proteins (IκB) by an IkB kinase (IKK) complex. The IKK2 subunit of IkB kinase is thought to be essential for NFκB activation and prevention of apoptosis. To determine whether IKK2 played a role in the suppression of apoptosis by PPs, we expressed a dominant negative form of IKK2 (IKK2dn) in primary rat hepatocyte cultures. Expression of IKK2dn caused apoptosis in primary rat hepatocytes in the absence of addition of TNFα. Moreover, IKK2dn-induced apoptosis could not be rescued by addition of TNFα or nafenopin. These results demonstrate a requirement for TNFα signalling via TNFR1 and NFκB in the suppression of apoptosis by the PP class of hepatocarcinogens.

615 THE MITOCHONDRIAL PERMEABILITY TRANSITION STIMULATES LYSOSOMAL PROLIFERATION AFTER NUTRIENT WITHDRAWAL PLUS GLUCAGON IN RAT HEPATOCYTES.

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Autophagy rids cells of excess and dysfunctional organelles, liberates nutrients and may be involved in apoptosis. Mitochondrial depolarization indicates loss of function and suggests onset of the mitochondrial permeability transition (MPT). Previously, we showed that autophagic stimulation by nutrient withdrawal and glucagon causes depolarization of individual mitochondria, which then move into autophagic vacuoles. Cyclosporin A (CsA), a specific inhibitor of the MPT, blocks mitochondrial depolarization after autophagic stimulation. Here, we aimed to test the hypothesis that nutrient deprivation plus glucagon promotes proliferation of acidic lysosomal autophagic vacuoles in a CsA-dependent manner. Overnight cultured rat hepatocytes were co-loaded with MitoTracker Green-FM (MTG, 500 nM) and tetramethylrhodamine methylester (TMRM, 1 μM). Green-fluorescing MTG is retained covalently by mitochondria, whereas red-fluorescing TMRM is released after mitochondrial depolarization. TMRM quenches MTG fluorescence, and thus the recovery of green MTG fluorescence signifies individual mitochondrial depolarization. In other experiments, lysosomes were labeled with LysoTracker Red (200 nM). Switching from serum- and insulin containing growth medium to Krebs-Ringer-HEPES buffer containing 1 μM glucagon increased the number of individually depolarized mitochondria by196 and 358% after 20 and 40 min, respectively, and the number of lysosomes by 45, 208 and 430% after 20, 40 and 60 min. Lysosomal proliferation after autophagic stimulation lagged behind mitochondrial depolarization by 20 min. CsA (5 μM) blocked both depolarization and lysosomal proliferation. In conclusion, the MPT causes mitochondrial depolarization and promotes lysosomal proliferation after autophagic stimulation.

616 iNOS-DEPENDENT, p53-MEDIATED SUPPRESSION OF APOPTOSIS IN ALVEOLAR MACROPHAGES EXPOSED TO BLEOMYCIN *IN VIVO*.

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Chronic inflammation in the lung leading to the development of pulmonary fibrosis is a common response to environmental pollutants and cancer chemotherapeutics such as bleomycin. We speculate that pulmonary fibrosis may be initiated by bleomycin-induced apoptosis of alveolar macrophages resulting in impaired clearance of apoptotic cells and/or release of inflammatory cytokines. Here we report that bleomycin stimulated apoptosis in alveolar macrophages from C57/Bl6 mice exposed to bleomycin *in vivo* or *in vitro*. Cell death was associated with oligonucleosomal DNA fragmentation and was inhibited by the caspase inhibitor ZVAD-fmk. Analysis of p53 localization demonstrated translocation of wild-type p53 into the nucleus following exposure to bleomycin *in vivo* but not *in vitro*. Strikingly, alveolar macrophages from homozygous p53-deficient mice were 2 fold more sensitive to bleomycin than those obtained from wild-type mice following exposure *in vivo*. Similarly, TUNEL analysis demonstrated that lung epithelial cells were 4-5 fold more sensitive to bleomycin-induced apoptosis than were wild-type cells. However, there was no difference in levels of apoptosis in wild-type and p53-/- macrophages exposed to bleomycin *in vitro*. Furthermore, levels of apoptosis were significantly higher in iNOS-/- mice than wild-type mice treated with bleomycin *in vivo*. In addition, bleomycin upregulated iNOS expression in wild-type mice. Our results strongly suggest that nitric oxide activates p53 to suppress bleomycin-induced apoptosis within the lung.

617 TNF-α STIMULATES CARDIOMYOCYTE APOPTOSIS BY A p38 MAPK-DEPENDENT PATHWAY REQUIRING REACTIVE OXYGEN SPECIES.

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The molecular mechanisms of TNF-α (TNF) dependent apoptosis are incompletely understood. We have established a primary neonatal mouse cardiomyocyte culture system to study the mechanisms of cardiomyocyte apoptosis. This system was used to culture cardiomyocytes from cardiac specific metallothionein (MT) overexpressing transgenic mice produced in our laboratory. MT is a potent antioxidant that scavenges many reactive oxygen species (ROS). Using this model, we addressed the hypothesis that TNF stimulates cardiomyocyte apoptosis by a p38 mitogen activated protein kinase (MAPK) dependent pathway. p38 MAPK activation was determined by immunofluorescent staining with anti-phospho-p38 MAPK antibody and visualization by confocal microscopy. Apoptosis was measured by Annexin V binding and TUNEL assay. TNF rapidly activated p38 MAPK and induced myocyte apoptosis. Pharmacologic p38 MAPK inhibition by SB203580 (an inhibitor of p38 MAPK alpha and beta, but not gamma and delta) blocked TNF-dependent apoptosis. Furthermore, MT cardiomyocytes were resistant to TNF-stimulated p38 MAPK activation and apoptosis. However, p38 MAPK is functional in MT-overexpressing cells as evidenced by the finding lipopolysaccharide (LPS) stimulated apoptosis and activated p38 MAPK in wild-type cardiomyocytes. SB203580 inhibited LPS-induced apoptosis. Interestingly, LPS activated p38 MAPK, but did not induce apoptosis in MT-overexpressing cardiomyocytes. These observations indicate that: 1.) Not all activated p38 MAPK isoforms cause apoptosis; 2.) TNF activates a specific p38 MAPK isoform that propogates the apoptotic pathway; 3.) ROS, through an unknown mechanism, activate a p38 MAPK isoform that is essential to apoptotic signalling.

618 ROLE OF FAS AND FAS LIGAND IN METHYLCHOLANTHRENE-INDUCED TUMORIGENESIS.

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Methylcholanthrene (MCA) is a member of the polycyclic aromatic hydrocarbons and a potent chemical carcinogen. In the current study, we investigated the role played Fas and Fas ligand(L) in the induction of tumors by MCA. Injection of MCA subcutaneously or intraperitoneally induced tumors more rapidly in two month-old C57BL6 lpr/lpr (Fas-deficient) and C57BL6 gld/gld (FasL-defective) mice when compared to age-matched C57BL6 +/+ mice. Also, in lpr and gld mice, the tumors grew at a faster rate than in wild-type mice. When the above mice were challenged with a LSA, a syngeneic tumor followed by treatment with BCNU, a nitrosourea, 100% of the mice could reject the tumor. However, upon rechallenge with the same tumor, 100% of the wild-type mice but not lpr or gld mice, were able to reject the LSA tumor. The lpr and gld mice but nor wild-type mice, exhibited increased levels of TGF-b and IL-10, particularly with increasing age. These studies demonstrate that lpr and gld mice are more susceptible to MCA-induced tumors because of the inability of the tumor cells to undergo Fas-based apoptosis and due to a defect in antitumor immunity. Recently, humans with defect in Fas have been identified and have been shown to develop lymphoproliferative disease. Our studies demonstrate that individuals with a defect in Fas and/or FasL may be more susceptible to tumors induced by chemical carcinogens (Supported in part by grants from NIH ES 09098, HL 058641 and AI 01392 and EPA.)

619 GLUTAMATE CYSTEINE LIGASE CATALYTIC SUBUNIT IS CLEAVED DURING APOPTOTIC CELL DEATH.

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Apoptosis is a highly regulated form of cell death which occurs following growth factor withdrawl and engagement of cell surface "death" receptors. Many chemical toxicants and chemotherapeutic agents exert their toxicity through the induction of apoptotic pathways. A common feature in numerous models of apoptosis is the disregulation of intracellular redox status. In particular, glutathione (GSH) levels often decline rapidly following apoptotic stimuli, and supplementation with GSH precursors has been shown to promote resistance to apoptosis in many models. The rate limiting step in GSH biosynthesis is catalyzed by glutamate cysteine ligase, (GLCL, aka. γ-glutamyl cysteine synthetase). We have found that during apoptosis the catalytic subunit (GLCL-C) is cleaved in a caspase-3 dependent manner. This cleavage occurs in a variety of cells undergoing receptor-mediated and chemically induced apoptosis, and also *in vivo*, in livers of mice treated with anti-Fas antibody. Purified recombinant GLCL-C treated *in vitro* with apoptotic cell lysates or with recombinant caspase-3 is cleaved to a stable, lower molecular weight product. In vitro cleavage is blocked by peptide inhibitors of caspase-3. GLCL-C cleavage is not observed in MCF-7 cells which do not express caspase-3; however MCF-7 cells transfected with caspase-3 do cleave GLCL-C during apoptosis. Furthermore, cleavage is specific to GLCL-C, and does not affect the molecular weight of the GLCL regulatory subunit. Recombinant mouse GLCL-C which has been mutated at the cleavage site is not cleaved *in vitro* or in transfected cells undergoing apoptosis. We are currently investigating the functional effects of GLCL-C cleavage on enzyme activity. (This work was supported by NIH grants CA75316, ES04696, and ES07033.)

620 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) INHIBITS GROWTH FACTOR WITHDRAWAL-INDUCED APOPTOSIS IN THE HUMAN MAMMARY EPITHELIAL CELL LINE, MCF-10A.

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Previous studies have demonstrated that under growth factor-restricted conditions 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) stimulates growth, the tyrosine phosphorylation of numerous effector molecules, and increases phosphatidylinositol 3-kinase (PI3K) activity in MCF-10A cells. In the present studies TCDD (3-30 nM) was found to increase cell recovery on days 2-6 by as much as 80% when insulin or epidermal growth factor (EGF) were removed from the media. In addition, TCDD inhibited apoptosis stimulated by EGF or EGF and insulin withdrawal by almost 80% as determined by annexin V binding and poly(ADP-ribose) polymerase (PARP) cleavage. However, withdrawal of insulin alone did not induce apoptosis even though TCDD did increase cell number in its absence. The phosphorylation status of Akt, a serine/threonine kinase that mediates PI3K-dependent inhibition of apoptosis, was examined. Immunoblot analysis revealed that TCDD treatment resulted in a transient increase in Akt phosphorylation that disappears within 18 hr. It appears that EGF stimulates an anti-apoptotic pathway, whereas insulin signals a pro-mitogenic pathway. TCDD seems to mimic both of these pathways. These data shed light on a potential mechanism whereby TCDD may act as a human breast carcinogen and a tumor-promoting agent. (NIEHS R01-ES-07259.)

621 DEXAMETHASONE INDUCES APOPTOSIS IN ALL SUBSETS OF T LYMPHOCYTES IN THE THYMUS AND PERIPHERY.

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It is well established that dexamethasone induces thymic atrophy by triggering apoptosis in immature CD4+ CD8+ T cells. In the current study, we investigated the effect of dexamethasone on induction of apoptosis in other T cell subsets in the thymus and spleen. C57BL/6 mice were injected with 100 mg/kg body weight of dexamethasone by the i.p. route. The control mice received the vehicle alone. Twelve hours later, the thymus and spleen were harvested and stained with antibodies against CD4 and CD8, and FITC-dUTP and analyzed flow cytometrically for triple color staining. The results demonstrated that dexamethasone induced apoptosis in all 4 subsets of T cells(CD4+, CD8+, CD4+CD8+ and CD4-CD8-). This was demonstrable both immediately and after *in vitro* culture. In the spleen, apoptosis was not detected immediately upon isolation, however, following *in vitro* culture, apoptosis could be detected in both CD4+ and CD8+ T cells as well as in B lymphocytes. Dexamethasone treatment induced upregulation of caspase-3 activity in the thymus but not in the spleen. The current study demonstrates that dexamethasone can not only induce apoptosis in immature cortical T cells in the thymus but also in mature lymphocyte subpopulations both in the thymus and periphery (Supported in part by grants from NIH ES 09098, HL 058641 and AI 01392 and EPA.)

622 TCDD-INDUCED APOPTOSIS IN THE THYMOCYTES OF PERINATALLY-EXPOSED NEONATES.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic environmental contaminant. The immune system is highly sensitive to TCDD and therefore this contaminant has raised significant health concerns. Recent studies from our laboratory demonstrated that TCDD treatment triggers apoptosis in thymocytes of adult C57BL/6 mice. Also, prenatal administration of TCDD has been shown to have profound immunotoxic effects. In the current study we tested the hypothesis that prenatal administration of TCDD results in immunotoxicity due to induction of apoptosis in thymocytes of the neonate. To this end, pregnant female C57BL/6 mice were given a single intraperitoneal injection on gestational day 14 with 0.1, 1, or 5mg/kg body weight TCDD or the vehicle, as a control. On postnatal days 2, 4, 7, 14, and 21, the thymocytes were isolated and studied for apoptosis using terminal deoxynucleotidyl transferase (TdT) and fluorescein isothiocynate (FITC-dUTP). We found that thymic cellularity and percent viability of the thymocytes was decreased in the pups exposed to the highest dose of TCDD, when compared to the control. On postnatal days 2 and 4, but not subsequently, thymocytes from TCDD-exposed pups showed increased apoptosis upon *in vitro* culture, when compared to the control. These results indicate that TCDD-induced toxicity in perinatally-exposed neonates is due to induction of apoptosis in the thymocytes (Supported in part by grants from NIH ES 09098, HL 058641 and AI 01392 and EPA.)

623 EFFECT OF ALACHLOR ON MOUSE SPLENOCYTE APOPTOSIS.

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Alachlor, a class II carcinogen, is widely used as a herbicide. The precise mechanism of carcinogenesis and an effective biomarker for monitoring alachlor exposure have not been defined. We studied the effects of alachlor on mouse splenocytes to assess programmed cell death (apoptosis). Splenocytes were treated with 0, 10, 50, 100, or 500 μM alachlor for 16hrs and a DNA ladder assay as a marker of cellular apoptosis and a flow cytometric TUNEL assay for quantitation of DNA breaks in single cells were utilized to assess apoptosis in these cells. The DNA ladder assay showed increased DNA breaks in cells treated with alachlor in a dose-dependent manner. A significant increase in laddering was observed exposing cells with as little as 10 μM alachlor. Treatment of cells with 100 μM alachlor resulted in a very intense DNA ladder with little intact genomic DNA remaining in the cell population. The results of TUNEL assay supported the results obtained from the DNA ladder assay. Thus, cells treated with 0, 10, 50, 100 and 500 μM alachlor showed 28, 56, 62, 74, and 93% FITC (an indicator of 3' DNA breaks) incorporation, respectively. These findings could be useful for the development of biomarkers to assess the safety of this chemical in the environment. (This work was supported in part by the US EPA under a cooperative agreement.)

624 FLUORIDE INDUCES APOPTOSIS BY CASPASE-3 ACTIVATION IN HL-60 CELLS.

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Fluoride is well known as a specific and effective caries prophylactic agent and its systemic or local application was therefore recommended during the past decades. However several problems resulting from fluoride toxicity like dental and skeletal fluorosis have been recognised. Fluoride toxicity is gaining increasing importance, but still very little is known on the mechanism of action of fluoride. In the present study the toxicity of fluoride on human leukemia (HL-60) cells were investigated. **Material and Methods:** HL-60 cells were cultured in RPMI 1640 medium containing 10% FBS at 37°C and 5% CO_2 atmosphere. The cells were exposed to fluoride and FITC-Annexin-V binding, DNA laddering, Western blotting using polyclonal anti-caspase-3 and PARP (poly ADP-ribose polymerase) activity were investigated. **Results and Discussion:** HL-60 cells underwent apoptosis on exposure to 2 mM fluoride. When fluoride treated cells were incubated with FITC-Annexin V, binding of FITC-Annexin to surface of cells were observed. Agarose gel of extracted DNA from fluoride treated cells showed DNA ladder which is a typical feature of apoptosis. Western blot reveals a 17 kd subunit of active caspase-3 in fluoride treated cells along with 32 kd unprocessed pro-caspase 3 seen in untreated cells suggesting that fluoride triggers apoptotic cell death by caspase-3 activation in HL-60 cells. The treated cells showed a cleaved 85 Kd fragment and complete loss of the intact 116 kd form of PARP as compared to untreated cells. The results clearly suggest that fluoride is toxic to HL 60 cells by triggering a apoptotic pathway,in the process activating caspase-3 which in turn cleaves PARP leading to DNA damage and ultimately cell death.

625 VARIABILITY IN HUMAN SENSITIVITY TO ARSENIC: AN INTERNATIONAL STUDY OF POPULATION RESPONSE TO ARSENIC IN DRINKING WATER.

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Estimating the nature and extent of human cancer risks due to arsenic (As) in drinking water is currently of great concern, since millions of persons worldwide are exposed to arsenic primarily through natural enrichment of drinking water drawn from deep wells. Humans metabolize and eliminate As through oxidative methylation and subsequent urinary excretion. Variations in arsenic metabolism may therefore affect individual risks of intoxication and carcinogenesis. Using data from three populations, from Mexico, China, and Chile, we have analyzed the distribution in urine of total arsenic and arsenic species (inorganic arsenic (InAs), monomethyl arsenic (MMA), and dimethyl arsenic (DMA)). Arsenic species were separated by ion exchange chromatography and measured by atomic absorption spectrophotometry. Data were analyzed in terms of total amounts of each species and by evaluating each species as a ratio to each species and to total As. In each population we examined male-female differences in urinary levels of the three forms of As within areas of high and low As exposure via drinking water. In all persons most of the urinary As was present as DMA. Male-female differences were discernible in both high and low exposure groups from all three populations, but the gender differences varied by population. The data also indicated bimodal distributions in the ratios of DMA to InAs and to MMA. While the gene or genes responsible for arsenic methylation are still unknown, the results of our studies among the ethnic groups in this study are consistent with the presence of functionally genetic polymorphisms in arsenic methylation leading to measurable differences in toxicity. The metabolic differences among these populations are consistent with reported differences in population risks of arsenic-induced cancer. These studies highlight the need for continuing research on the health effects of As in humans using molecular epidemiologic methods.

626 CYTOTOXICITY AND MITOGENICITY OF ARSENICALS ON RAT AND HUMAN UROTHELIAL CELLS.

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Dimethylarsinic acid (DMA) chronically fed to F344 rats produced urothelial tumors. We showed that it leads to urothelial cytotoxicity with consequent regeneration. Various arsenicals are present in the urine of DMA-treated rats [9.9 μM AsIII and 78.8 μM DMA, (Wanibuchi et al., Carcinogenesis, 1996)]. To evaluate the cytotoxicity and mitogenicity of arsenic compounds, we added them to cultured non-tumorigenic rat bladder urothelial cell lines S6 and MYP3. The cells were treated with sodium arsenite (AsIII), sodium arsenate (AsV), DMA, and monomethylarsonic acid (MMA) for 7 days and then cell viability was evaluated using Trypan blue. The LC50 was 0.5 μM AsIII and 5 μM AsV in both S6 and MYP3 cells, but was 0.2 mM for DMA and 1 mM for MMA in S6 cells and 0.5 mM for DMA and 5 mM for MMA in MYP3 cells. The effect of DMA on the cell cycle of MYP3 cells was examined by flow cytometry. After 7 days treatment with 1 mM DMA, most cells arrested in the G2 phase and many multinucleated cells were present. These results indicate that urothelial cell lines are more sensitive to arsenic compounds compared with other cell lines previously reported. However, as with other cell lines, inorganic arsenicals are considerably more cytotoxic than organic arsenicals. Cytotoxicity *in vitro* of AsIII occurred at concentrations comparable to those produced in urine of rats fed cytotoxic and carcinogenic doses (100 ppm of the diet or drinking water) of DMA.

627 GENETIC EVENTS ASSOCIATED WITH ARSENITE-INDUCED MALIGNANT TRANSFORMATION: APPLICATION OF cDNA MICROARRAY TECHNOLOGY.

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Arsenic is a human carcinogen. Epidemiological studies link arsenic exposure to cancers of the skin, lung, liver and other internal. However, inorganic arsenic has never been unequivocally shown to be carcinogenic in animals, and its mechanism remains unknown. Our previous showed that chronic (>18 weeks), low level exposure (125 to 500 nM) to arsenite induces malignant transformation in a liver epithelial cell line (PNAS 94: 10907, 1997). DNA methylation is significantly reduced and aberrant expression of the oncogene, c-*myc*, is increased with transformation. To further examine aberrant gene expression associated with arsenic carcinogenesis, the Clontech cDNA expression microarray was used. Our results show that the expression of more than 50 genes were altered in arsenite-transformed cells. For example, many oncogenes (c-*myc*, c-*jun* and c-*met*, etc.) were increased, while the tumor suppressor gene P53 was decreased. IGFII and insulin receptor were downregulated. Expression of the cell cycle related genes CyclinD1, and PCNA were increased while CDK4 was decreased in transformed cells. Expression of GST gene and several transporter protein genes were increased. The results of Western Blot analysis of c-Myc, CyclinD1, PCNA, P53 and GST agree with the analysis of gene expression. These results revealed for the first time important aberrant gene expression patterns occurring in arsenite-induced malignant transformation. These initial studies could be critical in future studies designed to more fully elucidate the molecular mechanisms of arsenic carcinogenesis.

628 SODIUM ARSENITE INDUCES DNA-PROTEIN CROSSLINKS IN WRL HUMAN HEPATIC CELLS IN MOUSE LIVER.

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The induction of DNA-protein crosslinks (DPC) have been proposed as indicators of early biological effects due to the fact that known or suspected carcinogens induce an increased proportion of proteins tightly bound to DNA. Arsenic is a human carcinogen metabolized in liver cells through series of oxi-reduction reactions that could potentiate the formation of DNA-protein crosslinks. To investigate if arsenite could induce DPC we decided to use a human hepatic cell line (WRL-68). After treatment of confluent cells with different concentrations of As 3+, they were lysed and DPC precipitated and isolated with a solution of 2% SDS solution and KCl. Trivalent arsenic induced DNA-protein crosslinks linearly related with dose. Thus, we decided to test the induction of DPC in whole animals. 25-30 mg Balb C male mice, were treated with different concentrations of sodium arsenite i.p.(4.5, 9 and 12 mg/kg) and orally during 30 days (4, 8 and 10 ppm in drinking water). Mice were killed by cervical dislocation, livers where extracted and homogenized in PBS pH 7.4 three hours after i.p. treatments and on the 30th day in oral treatments. A significant increase of DPC was observed in mice receiving 12 mg/kg i.p. and in those receiving 8 ppm in drinking water. Since DPC are also induced by other known or suspected carcinogens, they could represent a early manifestation of liver damage by arsenite.

629 SODIUM ARSENITE-INDUCED DYSREGULATION OF PROTEINS INVOLVED IN PROLIFERATIVE SIGNALING.

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It is well accepted that arsenic is a human carcinogen, yet its mechanism of action is not defined. Arsenic cannot be classified as an initiating agent or as a promoter, although altered proliferative reponsiveness has been proposed as a mechanism by which arsenic exerts its carcinogenic effects. Based on the hypothesis that arsenic exposure results in modulation of both positive and negative regulators of cell proliferation, this study examined physiological and biochemical changes in the proliferative response of murine fibroblasts grown long-term in the maximum tolerated concentration of sodium arsenite. In response to EGF stimulation, DNA synthesis and the proportion of cells entering S phase of the cell cycle both were increased in cells grown long-term in arsenic as compared to arsenic-naive cells. Analysis of positive proliferative regulators revealed an increase in the expression of c-myc and E2F-1, thereby supporting the hypothesis that arsenic increases activity of positive growth modulators. In contrast, the activity of ERK-2 was decreased, and expression of EGF-receptor and mSOS were unchanged. When negative regulators of proliferation were examined, expression levels of MAP kinase phosphatase-1 and p27Kip1 were found to be lower in arsenic treated cells as compared to control cells; this result supports a model in which arsenic disinhibits normal regulation of cell proliferation. Taken together, these data indicate that long-term exposure to sodium arsenite creates conditions within the cell consistent with sensitization to mitogenic stimulation. It is further postulated that the observed changes in mitogenic signaling proteins contribute to the carcinogenic property of arsenic.

630 ARSENIC(III) ALTERS GLUCOCORTICOID RECEPTOR (GR) FUNCTION AND GR-DEPENDENT GENE REGULATION IN H4IIE CELLS.

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Chronic human exposure to non-overtly toxic doses of arsenic is associated with an increased risk of cancer. Although its carcinogenic mechanism is still unknown, arsenic is not directly genotoxic and is therefore thought to act principally as a tumor promoter. Previous studies in our laboratory demonstrated that low dose As(III) had profound effects on expression of several inducible genes, including the hormone-regulated phosphoenolpyruvate carboxykinase (PEPCK) gene, in whole animal and cell culture systems. The effects of As(III) on both basal and hormone-inducible PEPCK expression were strongly associated with the glucocorticoid receptor (GR)-mediated regulatory pathway. We therefore specifically examined the effects of As(III) on the biochemical function of GR in hormone-responsive cells. Non-cytotoxic As(III) treatments 1.0-3.3 μM) of rat hepatoma H4IIE cells significantly decreased both basal and dexamethasone (Dex)-inducible expression of transiently transfected luciferase constructs under the transcriptional control of either two tandem glucocorticoid response elements (GREs) or 600 bp of the proximal rat PEPCK promoter which contains two GREs. The decreases in Dex induction following As(III) were As(III) dose-dependent but not time-dependent, occurring even after simultaneous administration of As(III) and Dex. Gel mobility shift assays demonstrated no significant effect of As(III) treatment on basal GR-DNA binding in nuclear extracts from As(III)-treated cells. However, As(III) had little or no effect on cellular GR protein levels as measured by western blot assays. In addition, As(III) caused the translocation of a green fluorescent protein (GFP)-tagged GR fusion protein to the nucleus, as measured by confocal microscopy, whereas As(III) pre-treatment had no effect on Dex-induced translocation. These results suggest that As(III) is altering GR function and GR-dependent gene regulation principally by altering its function as a transcription factor. (NIH-NIEHS ES07373.)

631 ISOLATION OF P53-REPRESSOR PROTEINS INDUCED BY EXPOSURE OF HUMAN KERATINOCYTES TO LOW LEVELS OF ARSENITE.

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The skin is considered a major target of arsenic carcinogenesis. However, there is little known of the mechanism of arsenic carcinogenesis. We previously reported on the ability of environmentally-relevant levels of arsenite (As III) to modulate levels of p53 and mdm2 in human keratinocytes (HaCaT). The decrease in p53 levels was attributed to the concomitant increase in mdm2 levels. We investigated whether p53 modulation was due to factors other than mdm2 binding to p53 thereby increasing rate of degradation. We employed affinity chromatography by using a DNA sequence from the promoter region of the p53 gene to isolate proteins that might help modulate the levels of p53 due to arsenite exposure by binding to this region of the promoter. We isolated several proteins that were induced by arsenite treatment (0, 100, 500, 1000 nM) of human keratinocytes (HaCaT) for 14 days, and would tentatively act as repressors of the p53 gene by binding to its promoter region. We also report on using microarray technology to observe induction of a suite of genes representing a unique footprint for arsenic exposure. These genes might be useful for the screening of compounds for their potential effects that are related to those of arsenic.

632 ELEVATED LEVELS OF INDUCIBLE HEAT SHOCK PROTEIN (HSP70-1) PROTECT MCF-7 CELLS FROM ARSENITE TOXICITY.

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Elevated HSPs protect against cellular damage from heat but it is unclear whether HSP induction alters the damaging effects of chemical contaminants. This study was designed to investigate the effects of sodium arsenite (As) in MCF-7 cells that overexpress inducible HSP70. A tetracycline-controlled expression system was developed to regulate HSP70 levels. Clone14 (C14) exhibited the highest induction of HSP70 in the absence of doxycycline ("ON") and HSP70 could be down-regulated to control levels in the presence of doxycycline ("OFF"). In order to examine the cytotoxic and genotoxic effects of sodium arsenite, C14ON and C14OFF cells were exposed to either 0 (control), 5 μM or 10μM As. For cytotoxicity studies, cells were exposed for 24 hours and assayed for viability and apoptosis using acridine orange and ethidium bromide staining. Both C14ON and C14OFF cells showed a dose-dependent decrease in cell viability and increase in apoptosis in response to arsenite. Cells expressing elevated HSP70 were significantly less affected (i.e. higher viability, lower apoptosis) than cells that did not overexpress HSP70. The genotoxicity of arsenite was evaluated using a cytokinesis-block micronucleus (MN) assay. MN were induced in both C14ON and C14OFF cells in response to As. However, induction levels of MN were significantly lower in HSP70 overexpressing cells as compared to cells that expressed control levels of HSP70 ($p< 0.05$). Collectively, these data demonstrate a protective effect of HSP70 expression on the cytotoxic and genotoxic effects of arsenite in MCF-7 cells. (This abstract does not necessarily reflect EPA policy.)

633 CARCINOGENICITY AND CO-CARCINOGENICITY OF SODIUMARSENITE IN p53+/- MALE MICE.

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Arsenic is a human carcinogen for which there are presently no rodent model systems. Methylation is the proposed mechanism for thedetoxification of arsenicals. Hepatic methyl donor status in rodents can be decreased by maintenance on a choline deficient (CD) diet compared to a choline sufficient (CS) diet. This study evaluated the carcinogenicity and co-carcinogenicity of arsenic in male p53+/- mice as a function of hepatic methyl donor status. Co-carcinogenicity for bladder and skin, two human tumor sites for arsenic, was tested usingp-cresidine and 4-vinyl-1-cyclohexene diepoxide (VCD), respectively. Over a 26-week period, groups of =20 male p53+/- mice were exposed tosodium arsenite (0.005%) in drinking water, p-cresidine (0.2%) in the diet, VCD (25 mg/week) administered dermally, both sodium arseniteand p-cresidine, or both sodium arsenite and VCD. At the completion of the 26-week treatment period, mice treated with VCD or VCD and arsenic were maintained for an additional 22 weeks on arsenic alone. In terms of hepatic methyl donor status, levels of s-adenoysl-methionine and s-adenoysl-homocysteine in liver were independent of diet while CD mice exhibited reduced hepatic levels of phosphocholine. CD mice on arsenic excreted lower levels of arsenicals in urine. Treatment with arsenic alone did not induce a significant tumor response in mice on either diet. The incidence of skin tumors was increased in CS but not CD mice treated with VCD; the incidence was not modulated by exposure to arsenic. Both the CD diet and arsenic enhanced the extent of bladder hyperplasia and the incidence of carcinomas in p-cresidine-exposed mice. Under these conditions, arsenic was not carcinogenic inmale p53+/- mice. However, concomitant exposure to arsenic appeared to increase the incidence of bladder carcinomas in CD mice exposed top-cresidine. (This work was supported by AWWARF.)

634 ROLE OF MAP KINASES (MAPKs) AND APOPTOSIS IN ARSENIC-INDUCED MALIGNANT TRANSFORMATION AND SELF-TOLERANCE.

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Arsenic is a human carcinogen. Our prior work showed that chronic arsenite (As^{+3}) exposure induced malignant transformation in a rat liver epithelial cell line (TRL 1215) and that self tolerance to As^{+3} occurs concurrently with transformation in these cells. This study examined the role of signal transduction in malignant transformation induced by As^{+3} and in the concurrent development of self-tolerance to acute As^{+3} toxicity. Transformation was induced in TRL 1215 cells by continuous exposure to 0.5 μM As^{+3} for 18 or more weeks, and these cells were then compared to passage matched control cells. Other TRL 1215 cells were treated subchronically (2-3 weeks) with As^{+3} to define events occurring prior to transformation. The various treatment groups and controls were subjected to an acute high dose As^{+3} exposure and levels of phosphorylated MAPKs, ERK1/2, JNK and P38, were determined by Western blot analysis. After acute As^{+3} exposure, all three MAPKs were phosphorylated in a time-dependent and dose-dependent fashion in all treatment groups. However, the levels of phosphorylated ERK1/2 and JNK were decreased in the As- transformed cells compared to control, while the levels of phosphorylated ERK1/2, JNK and P38 remained the same as control in cells treated subchronically with As^{+3}. Flow cytometry analysis using Annexin/FITC revealed that As^{+3}-induced apoptosis was markedly reduced in As-transformed cells compared to control cells, while As^{+3}-induced apoptosis was increased in cells exposed subchronically to As^{+3} compared to control. These results indicate that As^{+3} induces apoptosis in the early stages of exposure prior to transformation but that after As^{+3}-induced transformation has occurred, cells show resistance to As^{+3}-induced apoptosis, a finding which might be due to down-regulation of MAPKs pathways. This resistance to apoptosis may play a critical role in As^{+3}-induced self tolerance and, if generalized, has potential implications for As-induced malignancies.

635 FURTHER TRANSFORMATION OF IMMORTALIZED HUMAN KERATINOCYTE FOLLOWING TREATMENT OF MNNG, ARSENIC, OR AN ARSENIC-CONTAINING MIXTURE.

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As an initial phase of a project exploring the carcinogenic potential of arsenic (As) and an As-containing metal mixture in human keratinocytes, cell transformation studies were conducted. To generate a positive control for chemical transformation of immortalized human keratinocytes, we chose the RHEK-1 cell line and a known human carcinogen, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG). In parallel, RHEK-1 cells were exposed to MNNG (one 24hr exp.), or to low doses (0.12 - 0.18μM) of As or to an As, Cr, Cd, Pb mixture; the latter two treatments were carried out chronically for 6 mos. At passage 13 after treatment with 0.1μg/ml MNNG, the exposed cells (OM3) gained the ability to grow in methylcellulose (MC); at passage 25 the culture tested highly positive for tumorigenicity in immunocompromised mice. Interestingly and in contrast to what we observed with OM3, we saw no conversion of RHEK-1 to the anchorage independent phenotype at any dose of As tested. As-treated cultures had an altered morphology from control cells, showed altered growth characteristics in MC, and exhibited decreased differentiation when confluent. Our findings support the idea that As is not capable of transforming immortalized keratinocytes when administered alone, but does have substantial effects on cell growth and differentiation. Cultures treated with the 4-metal mixture showed very similar behavior to As-treated cultures. We are

currently analyzing the tumorigenicity of both the As- and mixture-treated cultures in nude mice. Establishment of a malignant cell line (OM3) is crucial for all of our carcinogenesis studies, as it will enable us to identify distinct transformation-associated end points in human keratinocytes. (This study was supported by ATSDR [Cooperative Agreement U61/ATU881475], and NIEHS Superfund Basic Research Program [P42 ES05949].)

636 ARSENIC, ALONE AND IN CHEMICAL MIXTURES, ANTAGONIZES THE DEVELOPMENT OF GLUTATHIONE S-TRANSFERASE π (GST-P) POSITIVE FOCI IN THE RAT LIVER.

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Using Ito's Medium-Term Liver Foci Bioassay, we implemented a "top-down" approach to further investigate the antagonistic effects of a 4-chemical mixture (Pott *et al.*, *Cancer Letters* 133:185-190, 1998) containing arsenic (As), 1,2-dichloroethane (DCE), trichloroethylene (TCE), and vinyl chloride (VC) on the development of glutathione S-transferase π (GST-P) positive hepatic foci in the rat. On day 1, adult male Fischer 344 rats were administered diethylnitrosamine (DEN; 200 mg/kg intraperitoneally) or saline as a negative control. During weeks 3-8, the 4-chemical mixture (75 ppm As, 300 ppm DCE, 1900 ppm TCE, 15 ppm VC), or a 3- (As, DCE, TCE) or 2-chemical submixture (As, DCE), or As alone was orally administered to rats. An additional treatment group received the 4-chemical mixture with a higher dose level of As (300 ppm weeks 3-4, 150 ppm weeks 5-8). Arsenic and DCE were administered in the drinking water, TCE and VC via once daily corn oil gavage, 5 days/week. DEN positive controls received deionized water and corn oil gavage. Rats underwent partial hepatectomy on day 22 and were sacrificed at the end of week 8. Preliminary morphometric analysis revealed a significant decrease in the total area of GST-P positive foci associated with exposure to As alone (75 ppm) compared to DEN positive controls ($p < 0.05$). Exposure to the 4-chemical mixture (300/150 ppm As) significantly decreased the total area of GST-P positive foci and the number of large foci (> 0.2 mm in diameter) compared to DEN positive controls ($p < 0.05$). These results suggest that As is responsible for the observed antagonistic effect. Experiments to further validate these findings and enhance comparisons among treatment groups are currently underway. (This project is funded by the ATSDR [Cooperative Agreement No. U61/ATU881475], NIEHS Superfund Basic Research Program [P42 ES05949], and NCI [K08 CA72396-03].)

637 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON SP1 ABUNDANCE IN THE NEOCORTEX AND CEREBELLUM IN RATS.

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Gestational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been strongly implicated to produce fetotoxic responses that consist of thymic hypoplasia, decreased fetal growth, prenatal and postnatal mortality along with developmental toxicity in male and female reproductive systems, in laboratory mammals. We have studied the effects of three single low dose exposures of TCDD in timed-pregnant rats on the developmental expression profile of Sp1 in different brain regions. Differential gene expression is partially regulated by transcription factors which may be targets for perturbations by TCDD. Single oral doses of 0.25, 0.5 and 1.0 μg TCDD/kg, respectively, were given on gestational day 15 to three groups of Harlan Sprague Dawley rats. Electrophoretic mobility shift analysis using nuclear extracts from the cerebral cortex and cerebellum of the offspring on postnatal days (PND) 3, 5, 10, 15, 20 and 30 revealed a dose dependent leftward shift in the developmental expression profile of Sp1 as compared to controls. There were little no statistically significant differences observed with regard to birthrate, body weight gain, and gross brain weight over the PND period. These observations suggest that *in utero* and lactational exposure to TCDD alters the developmental regulation of gene expression in the rat cortex and cerebellum through the modulation of the transcription factor Sp1. (Supported by NIH G12 RRO 3032 and NIEHS ES00287.)

637A GESTATIONAL EXPOSURE OF LONG EVANS RATS TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) LEADS TO STUNTED MAMMARY EPITHELIAL DEVELOPMENT IN FEMALE OFFSPRING.

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The effects of exposure of pregnant rats to TCDD on mammary gland development of resulting offspring was examined. Time-pregnant Long Evans rats were exposed to 1μg/kg TCDD or vehicle on gestation day 15. On postnatal day (PND) 4, litters were culled to 8 pups each. Female offspring were sacrificed on PND4, 33, 37, 45, 68 and 110 to examine mammary gland morphology. Treated animaals from all early time points were significantly smaller than control litter mates and displayed delayed vaginal opening. TCDD exposed offspring displayed vaginal threads when examined on PND33, 37, and 45. Mammary glands taken from treated animals on PND4 had severely stunted epithelial development. They displayed fewer primary branches (2.5 vs 3.5 for control), and dramatically fewer terminal end structures per gland (45 vs. 107 for control). This phenomenon was subsequently observed in glands of 33 and 37 day old females. Furthermore, unlike fully developed glands of control 45 day old females, all of those previously exposed to TCDD retained prominent terminal end buds. Although end buds were differentiated in glands of 68 and 110 day old animals, the spindly nature of the glands from treated dams was still prominent. In mammary transplantation studies, involving the addition of epithelium taken from the nipple area of a donor animal into the cleared fat pad of the recipient, the epithelium taken from a TCDD exposed 25 day old female grew and appeared normal in the fat pad of control animals after 5 weeks. However, the epithelium of control animals transplanted into TCDD exposed fat pads grew at a slow rate and appeared underdeveloped. We conclude from these studies that prenatal exposure to TCDD adversely alters mammary gland development in resulting female offspring and that the hormonal milieu or mammary stroma may play a critical role in this development. (This abstract does not reflect EPA policy.)

638 TRANSFER OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN RATS THROUGHOUT PREGNANCY AND LACTATION.

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The transplacental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during developmental stage of fetuses causes reproductive disorders. We have shown that a relatively low dose of TCDD caused a significant decrease of anogenital distance in male rats. To determine the exact level of fetal body burden, we utilized high-resolution GC/MS (HP6890, Hewlett Packard, US/JMS-700, JEOL, Japan) and measured the amounts of TCDD. Pregnant Holtzman rats were given a single oral dose of 0, 200, 800 or 1600 ng TCDD/kg body weight on Gestation Day (GD) 15. Dams were killed on GD 16 and GD 20, and fetuses and placentas were removed. On Day 21 after birth, the other dams and pups were sacrificed, and liver, fat and serum were collected. On GD 16 at an 800 ng TCDD/kg dose, 145, 135 and 111 pg TCDD/g wet-tissue were found in maternal serum, placenta and fetus, respectively. On GD 20, 221, 114 and 59.6 pg TCDD/g wet-tissue were found in maternal serum, placenta and fetus, respectively. Whereas the TCDD concentration in fetus was declined, the TCDD amount transferred from dam to fetuses was increased 0.8 ng/litter (GD 16) to 2.6 ng/litter (GD 20). At weaning, each individual pup had 4.1-4.5% of the administrated TCDD. The present results support the earlier findings in which the TCDD exposure level via placenta was lower than that via lactation.

639 ABRUPTION OF PLACENTAL MATURATION BY MID-PREGNANT EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN RAT.

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The haemochorial placenta is unique in that the fetal-derived trophoblast cells contact directly to maternal blood. Transplacental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes disorder of fetal and postnatal development, but nearly no reports on the possible involvement of TCDD in placental growth and development are available. In the present study, pregnant

Holtzman rats (CR) were given a single oral dose of 0, 800 or 1600 ng TCDD/kg body weight on gestation Day (GD) 15 and placental tissue was collected on GD 20. TCDD treatment resulted in intrauterine fetal death in a dose-dependent manner by GD 20. The weight of placenta showed no significant difference between control and TCDD-treated rats. However, in TCDD-treated rats, the junctional zone that consists of glycogen cell, spongiotrophoblast cell and tropholbast giant cell was found disorganized and vacuolized at spongiotrophoblast cells layer. In addition, northern blot analysis of placental total RNA showed down-regulation of spongiotrophoblast cell marker proteins such as prolactin-like protein-D and spongiotrophoblast cell-specific protein, which was consistent with the histological observation. The present results suggest that TCDD affects the fetal development by altering placental growth and function.

640 HEMATOPOIETIC TOXICITY OF TCDD IN DEVELOPING ZEBRA FISH.

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The initial signs of toxicity exhibited by zebrafish larvae exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as newly fertilized eggs are cardiovascular. To determine whether TCDD affected vascularization, expression of the endothelial cell marker flk-1 was analyzed by *in situ* hybridization. The spatial-temporal expression of flk-1 in TCDD-treated embryos did not vary from control embryos suggesting that the vascular network was initiated correctly. TCDD-induced reduction in blood flow was analyzed by differential interference contrast microscopy and quantified using transgenic embryos expressing GFP from the GATA-1 promoter specifically in hematopoietic cells. As early as 60 hours post fertilization (hpf) the blood flow rate was reduced 20% in TCDD exposed embryos and continued to decline thereafter until no flow was observed. A new observation was that the number of circulating blood cells was also reduced by TCDD treatment. While TCDD did not appear to alter the number of hematopoietic progenitor cells present at 22-26hpf before the onset of circulation, the number of cells circulating in the blood of TCDD-treated embryos was less than controls as early as 48hpf and continued to decline until blood flow was undetectable. GFP protein levels from whole embryo extracts was analyzed by Western blot, and confirmed the microscopic observations that TCDD-treated embryos had fewer GFP-labeled blood cells than control embryos. These preliminary data suggest that a primary embryotoxic effect of TCDD is on hematopoiesis. Ongoing studies are addressing whether TCDD treatment alters the differentiation and/or the proliferation of hematopoietic cells using a variety of histologic and molecular analyses. (Supported by NIEHS F32-ES05862-02 to CDB and UW SeaGrant R/BT-12.)

641 DEVELOPMENTAL EXPRESSION OF THE ARYL HYDROCARBON RECEPTOR (AHR) IN THE AVIAN EMBRYO.

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To elucidate tissues in which the AhR may play a role during embryogenesis, we investigated AhR expression in the chick embryo by immunohistochemistry. On incubation days 1.7-5, chick embryos were preserved in Bouin's fixative, dehydrated, paraffin-embedded, and sectioned. Sections were stained with rabbit anti-mouse AhR polyclonal antibody (3 μg/ml), washed, incubated with goat anti-rabbit IgG-biotin, washed, incubated with streptavidin-B-galactosidase, and developed using X-gal. Western blot analysis of the *in vitro* expressed chicken AhR cDNA confirmed that the AhR antibody specifically recognized the chicken AhR. AhR expression was not detected on incubation day 1.7. On day 2.2, AhR expression was detected in ectoderm: skin, diencephalon, otic vesicle, myelencephalon, and mandibular arch mesenchyme; mesoderm: somitic myotome and myocardium; and endoderm: pharyngeal and foregut epithelium. From incubation days 3-5, AhR expression became more restricted in the brain and expanded in neural-crest cell derivatives including to the dorsal root, sympathetic, and cranial ganglia. AhR expression was also present in the smooth muscle of dorsal aorta and carotid arteries, in the dorsal mesentery, limb muscle masses, and epithelium of the mesonephric ducts, while expression was also detected in a variety of endodermal derivatives such as thyroid rudiment, Rathke's pouch, liver, lung bud epithelium, pancreatic rudiment, and the entire gastrointestinal epithelium. In general, the AhR tended to be expressed in the epithelial component, or responding tissue, at many sites of tissue induction. The role of the AhR in the epithelium at these sites of tissue interaction is not known; however, the AhR may function to maintain epithelial competence to respond to the mesenchymal signals or may participate in the epithelial response to the inductive signals. (Supported, in part, by NCI IRG #192, NIH #BH953016, and AHA WI # 96-GB-77.)

642 AH RECEPTOR (AHR) IN MOUSE PROSTATE GROWTH AND DEVELOPMENT: PHYSIOLOGICAL ROLE AND ROLE IN MEDIATING TCDD EFFECTS.

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To determine if inhibitory effects of TCDD on prostate development are AhR-dependent and if AhR might play a physiological role in regulating prostate development, heterozygous ($Ahr^{\pm}$) male and female mice (provided by Dr. Chris Bradfield) were mated and pregnant females treated with 5 μg TCDD/kg or vehicle on Gestation Day (GD) 13. Pups were weaned on postnatal day (PND) 21. Tissue was obtained from GD 18 fetuses and PND 10-16 pups for genotypic analysis by PCR. *In utero* and lactational TCDD exposure of wild-type ($Ahr^{+/+}$) males inhibited ventral prostate (VP) growth to such an extent that on PND 21 the VP resembled that typically seen in control fetuses on GD 18. Ductal development was impaired and only 50% of the luminal epithelial cells expressed androgen receptor (AR) compared to almost 100% of luminal epithelial cells in control. By PND 35 VP, anterior prostate (AP), dorsal lateral prostate (DLP), and seminal vesicle (SV) weights were 12, 28, 47, and 50% of control, respectively. By PND 90, DLP and SV weights had recovered somewhat (to 68 and 71% of control values) but VP and AP weights were still only 15 and 27% of control. Significantly, TCDD failed to reduce prostate or SV weights in null mutant ($Ahr^{-/-}$) males, demonstrating that the above effects of TCDD were AhR-dependent. The inhibitory effect of TCDD was also detected in the GD 18 fetus by a complete blockade of ventral prostatic bud formation, the initial step in ventral prostate development. Scanning EM examination of urogenital complexes on GD 18 revealed agenesis of ventral prostate buds in TCDD-exposed $Ahr^{+/-}$ fetuses but no effect in their $Ahr^{-/-}$ littermates. To determine if AhR has a physiological role in male accessory sex organ growth, VP, DLP, AP, and SV weights were compared on PND 35 and PND 90 for males with different AhR genotypes from the same litter. There were 13-26% reductions in the weights of these organs in vehicle-exposed $Ahr^{-/-}$ males compared to $Ahr^{+/+}$ males, suggesting that AhR signaling may be involved, physiologically, in modulating prostate growth. (Supported by NIH Grant ES01332.)

643 EFFECTS OF *IN UTERO* AND LACTATIONAL TCDD EXPOSURE ON DIHYDROTESTOSTERONE (DHT) FORMING ENZYMES IN RAT VENTRAL PROSTATE.

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In utero and lactational (IUL) exposure of rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits prostate growth without reducing fetal and postnatal plasma androgen concentrations. However, it is not known if TCDD affects formation of the most biologically active androgen, dihydrotestosterone (DHT), in rat prostate. Since a reduction in prostatic DHT formation could impair prostate growth and development, we determined effects of IUL TCDD exposure on DHT-forming enzymes and on the expression of prostatic binding protein subunit C3 (PBP-C3) mRNA, which is androgen-regulated. The activities of 3α-hydroxysteroid dehydrogenase (3α-HSD) and 5α-reductase type 2 (5α-R) were evaluated in ventral prostate homogenates after exposure to a single maternal dose of vehicle or 1 μg TCDD/kg on gestation day 15. During the postnatal period (PNDs 14, 21, and 32) 3α-HSD activity in the ventral prostate was 70-200 times greater than 5α-R activity in vehicle-exposed rats. TCDD exposure reduced 3α-HSD activity to 38 and 82% of control on postnatal days (PNDs) 14 and 21, respectively, but did not affect it on PND 32. On the other hand, the activity of 5α-R was *increased* 2.5-, 3.6-, and 5.1-fold, respectively, on these same PNDs. In a separate experiment androgen responsiveness was assessed in ventral prostates removed from TCDD- and vehicle-exposed males on PND 11 and 18. These organs were cultured for 1 day without androgen exposure, then for 2 days with graded concentrations of testosterone, DHT, or 5α-androstane-3α,17ß-diol, and harvested at the equivalent of PND 14 and 21. Androgen-induced increases in PBP-C3 mRNA abundance were significantly inhibited by TCDD exposure. In addition, immunohistochemical PBP-C3 and androgen receptor protein expression were reduced in TCDD-exposed prostates. Thus, IUL TCDD exposure caused substantial effects on androgen responsiveness of prostate luminal epithelial cells with far less effect on androgen metabolism. One possible interpretation for these results is that IUL TCDD exposure reduces androgen responsiveness by inhibiting luminal epithelial cell development. (Supported by NIH Grant ES01332.)

644 THE MECHANISM OF RETARDATION OF JAW GROWTH BY TCDD IN EARLY ZEBRA FISH EMBRYOS.

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Naturally fertilized zebrafish embryos were exposed to various concentrations of TCDD (2,3,7,8-tetrachloro-p-dibenzodioxin, 0.1-1ppb) dissolved in water solution from 30% epiboly to the end of experiments (usually 84 hrs after fertilization) at 28.5 degrees centigrade. TCDD caused a dose-dependent inhibition of lower jaw development other than edema or fish death, although it little affected the total body length. Movement of neural crest cells into jaw primordia did not seem involved in these actions. Delay in blood circulation became prominent just after 60 hrs after fertilization, when lower jaw was developing. Beta-Naphthoflavone, another AhR agonist and CYP1A (one of cytochrome P450) inducer, caused toxic effects on zebrafish embryos very similar to TCDD. These toxic effects by AhR agonists were effectively recovered by proadifen, general inhibitor for P450. Expressions of mRNA like goosecoid, bone morphogenetic proteins (2 and 4) or distal-less homeoboxes (2-4) at jaw primordia were not markedly decreased by TCDD before or at the beginning of jaw growth, as revealed by in situ hybridization. These results suggest the correlation between circulation failure and jaw growth retardation, and the involvement of P450 in TCDD-induced toxicity.

645 PHYSIOLOGICAL ROLE OF THE ARYL HYDROCARBON RECEPTOR (AhR) IN MURINE OVARIAN DEVELOPMENT.

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The AhR is a cytosolic ligand activated transcription factor that regulates the response to structurally related environmental contaminants such as 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). However, the physiological role of the AhR is not well understood. Thus, the purpose of this study was to investigate whether it played a role in murine ovarian development. In the mouse, ovarian development begins in prenatal life, continues through neonatal life, and results in formation of the primordial follicle pool. This pool is required for ovarian function throughout the reproductive life span. By embryonic day 18 (E 18), germ cells are present in the developing ovary. By postnatal days 2-3 (P 2-3), most germ cells are surrounded by granulosa cells to form primordial follicles. By postnatal day 8 (P 8), all germ cells are surrounded by granulosa cells, resulting in complete formation of the primordial follicle pool. Thus, to determine the effect of the AhR during ovarian development, ovaries were harvested from AhR knockout (AhRKO) and wildtype (WT) mice on E 18, P 2-3, and P 8, and complete serial sections were prepared for histological evaluation of germ cell and follicle numbers. At E 18, there were no significant differences in germ cell numbers between WT and AhRKO mice, suggesting that the AhR is not required for germ cell development. At P 2-3, WT ovaries had fewer fully formed primordial follicles than AhRKO mice (21,120±2688 vs. 38,440±3632, n=4-7, p=0.008). WT ovaries also had more naked germ cells than AhRKO mice (18,160±720 vs. 12,696±1192, n=3, p=0.05). At P 8, WT and AhRKO ovaries had similar numbers of primordial follicles (39,960±4400 vs. 33,504±3000, n=8-9, p=0.25). These results suggest that the AhR slows formation of primordial follicles during neonatal life. (Supported by the Heinz Family Foundation and the Veteran's Administration [JF] and NIH grant ES01332 [RP].)

646 *IN UTERO* AND LACTATIONAL TCDD EXPOSURE ALTERS ESTROGEN RECEPTOR-ALPHA EXPRESSION IN THE RAT MAMMARY GLAND.

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In the mammary gland, estrogen triggers ductal cell proliferation. Intact estrogen signaling pathways are required for normal ductal elongation and development. This study was initiated to determine whether *in utero* and lactational (IUL) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure alters estrogen receptor-alpha (ERα) expression and response in the rat mammary gland. Pregnant Holtzman rats were administered a single oral dose of 1Fg/kg TCDD, or vehicle, on gestation day 15 and offspring were weaned on postnatal day 21. TCDD exposed and control non-exposed rats were oophorectomized at 9 weeks of age. Two weeks later both the TCDD and control rats were divided into three groups, receiving either placebo, 0.025, or 0.1 mg 17b-estradiol pellet implants. After 48 hours all rats were euthanized and the mammary tissue removed for analysis. Whole mount studies revealed baseline morphological abnormalities as well as enhanced ductal growth in TCDD exposed rats as compared to control rats. Ki67 immunohistochemistry demonstrated a higher rate of ductal cell proliferation at the 0.025 mg 17b-estradiol dose in the TCDD treated groups as compared to the non-exposed control groups. In the absence of exogenous estrogen, both Northern and Western blot analysis showed an increase in ER-α expression levels in the TCDD exposed group as compared to the control. RT-PCR of progesterone receptor (PR) mRNA demonstrated little PR expression in either control or TCDD baseline groups, indicating that the ERα was not activating PR transcription despite its higher expression in the TCDD treated group. PR expression was induced by estrogen exposure in both groups. These results demonstrate that *in utero* and lactational TCDD exposure increases steady-state levels of ER-α in the rat mammary gland.

647 INFLAMMATORY MEDIATORS IN NASAL LAVAGE FLUID OF NON-SYMPTOMATIC VOLUNTEERS: ONE-YEAR FOLLOW-UP STUDY.

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There has been intense interest in developing noninvasive methods to detect airway inflammation in different respiratory disorders induced by exposure to bioaerosols. The measurement of inflammatory mediators in nasal lavage (NAL) fluid represents a direct method to investigate upper airway inflammation. We aimed to confirm the validity of this technique using one-year follow-up study with 9 non-symptomatic volunteers. NAL was performed every other week altogether 27 times. Production of inflammatory mediators in the nasal lavage fluid was studied ie. interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF) by immunoassay, and nitric oxide (NO) as nitrite by Griess reaction. After NAL subjects filled in a one-page questionnaire concerning their health and use of medicine during the preceding week. The highest 5% of the measurements were excluded as outlayers. Most of these peak values can be explained with the questionnaire data. The mean value of nitrite concentration for the remaining 95% of the measurements was 1,502±0,109μM. The respective figures for TNF were 4,117±0,306 pg/ml, for IL-1 28,365±2,150 pg/ml and for IL-6 9,160±0,683 pg/ml. Individual and seasonal variations were low. These results suggest that nasal lavage technique is a valid method for the assessment of upper of airway inflammation.

648 ENVIRONMENTAL MYCOBACTERIA INDUCE INFLAMMATORY RESPONSES IN HUMAN 28SC MACROPHAGES.

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Excess moisture in building structures enables the growth of various fungi and bacteria. Some of these bioaerosols may be able to cause respiratory tract disorders and other inflammatory symptoms, typically associated with exposure to indoor air of moldy houses. The identification of most potent microbes and mechanisms of diseases are still under investigation. In the present study the ability of four different environmental mycobacteria strains to induce the production of inflammatory mediators and cause cytotoxicity in human 28SC macrophage cell line was investigated. The macrophages were primed with IFN-gamma (1 ng/ml) for 24 h and exposed for 24 h (exp5, exp6, exp7 spores/ml) to two strains of Mycobacterium avium and Mycobacterium terrae which were isolated from a mouldy building. Production of inflammatory mediators were measured from the culture medium; cytokines IL-6 and TNF-alpha immunochemically, nitric oxide (NO) as nitrite by Griess reaction and Western Blot analysis. In addition, the induced cytotoxicity in the macrophages was measured with MTT-test. The nitrite and IL-6 concentrations in the culture medium of the control cells were 1,6 μM and 0,2 pg/ml, respectively. Mycobacteria exposure induced dose dependent production of nitrite (up to 2,4 μM), and IL-6 (up to 3,6 pg/ml) in human macrophages, whereas no TNF-alpha production was detected. However, the potency of the mycobacteria to induce these responses differed between the two tested species. Apathogenic environmental Mycobacteria terrae was more effective than potentially pathogenic Mycobacterium avium. Moreover, mycobacteria exposure did not cause cytotoxicity in human 28SC macrophages.

649 INFLAMMATORY RESPONSES AFTER INTRATRACHEAL INSTILLATION OF SPORES OF *STREPTOMYCES CALIFORNICUS* IN MICE.

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Moisture related microbial growth in buildings is associated with respiratory symptoms and inflammatory reactions, but the mechanisms of the effects leading to clinical manifestations are not known. We studied the effects of a single dose of intratracheally instilled spores of Streptomyces californicus (1,1x10E8 spores/animal), isolated from a moldy building, on inflammatory mediators i.e., interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF alpha) in bronchoalveolar lavage fluid (BALF) of male NIH/S mice. Moreover, the total cell numbers, total protein, cell differentials and expression of inducible nitric oxide synthase (iNOS) in BALF cells were analysed. The mice were exsanguinated by cardiac puncture under pentobarbital anesthesia at time points 3 h, 6 h, 24 h, 3 days or 7 days. The tracheas were cannulated and the lungs were lavaged with HBSS. The cell suspensions were spun for cell differentials. IL-6 and TNF alpha concentrations were analysed by ELISA and total protein by modified Lowry method from the BALF supernatant. Total cell numbers were defined from cell pellets resuspended to medium by Trypan blue exclusion method. Expression of iNOS were analysed from the combined cell pellets by Western Blotting. The highest concentrations of TNF alpha and IL-6 in BALF were reached at 6 hours of exposure but only IL-6 was significantly increased at 24 hrs. Expression of iNOS was seen 24 hrs after exposure. The maximum levels of total protein concentrations in BALF were measured at 24 hrs. Total cell number was increased after 6 hours and reached the maximum after 24 hrs. Neutrophil number reached the highest level after 24 hrs. The highest number of macrophages was detected after 3 days of exposure and that of lymphocytes after 7 days of exposure. Altogether, these results show clearly that the spores of S. californicus from moldy houses caused an acute inflammation in mice lungs. The acute phase was over in 3 days after a single dose but abnormal numbers of macrophages and lymphocytes were detectable in airways even after 7 days.

650 INFLAMMATORY RESPONSES AND CYTOTOXICITY INDUCED BY *STREPTOMYCES ANULATUS* IN MICE MACROPHAGES ARE DEPENDENT ON NUTRIENTS AND PH LEVEL IN THE GROWTH MEDIA.

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Epidemiological data indicate that living or working in a moldy building is associated with increased risk of respiratory diseases related to inflammatory reactions, but the specific causes and the cellular mechanisms of the illnesses are not clear. The effect of pH level and nutrients in the growth media on the ability of Streptomyces anulatus, to induce inflammatory responses and to cause cell death in RAW264.7 macrophages was studied. The macrophages were exposed 24 h (10exp5, 10exp6, 10exp7 spores/10exp6 cells) to S. anulatus, which was isolated from a mouldy building and grown on six media at pH 4, 5.5, 7, 8.5, 10 or 11.5. Production of inflammatory mediators i.e., IL-6 and TNFalpha by immunoassay, nitric oxide (NO) by Griess reaction and Western Blot analysis and reactive oxygen species (ROS) by luminometric method in RAW264.7 cells were assayed. The cell viability was measured by MTT-test. Significant dose dependent NO (up to 30.4 µM), TNFalpha (up to 2900 pg/ml) and IL-6 (up to 4800 pg/ml) production was induced in RAW264.7 cells by the spores of S.anulatus. However, the potency of the spores to induce these responses was dependent on both nutrients and pH in the growth media. The spores grown on media containing glycerol were the most cytotoxic (up to 96%) whereas the percentage of dead cells was from 52 to 3% when the spores were grown on the other media. Only the spores grown on media with glycerol also significantly increased ROS production (up to 320% of controls). These results suggest that both pH and nutrients play an important role in the ability of S.anulatus to induce inflammatory responses and cytotoxicity in mammalian cells.

651 COMPARISON OF THE INFLAMMATORY POTENCY OF BACTERIA AND THEIR CELL-WALL COMPONENTS IN THE LUNG.

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Bacteria are components of aerosols found at high concentrations in a variety of occupational settings, especially agriculture. Inhaled non-pathogenic organisms may induce potent inflammatory responses in the lung. We have previously shown that pulmonary exposures to certain components of bacteria (e.g., lipopolysaccharides {LPS}) and fungi (e.g., glucans) induce production of inflammatory cytokines (e.g., IL-6, TNFalpha, MIP-2) and monocyte and neutrophil recruitment to the airways. In recent studies, we explored the hypothesis that inhaled Gram-negative (Gm-) bacteria and their cell-wall components are more potent inducers of inflammation than Gram-positive (Gm+) organisms and their cell-wall components. BALB/cJ mice were exposed acutely to inhaled bacteria (Gm-: Enterobacter agglomerans, Pseudomonas aeruginosa; Gm+: Micrococcus luteus, Bacillus megaterium) in post log-phase growth; to LPS, peptidoglycan, or lipoteichoic acid by inhalation or instillation; or to inhaled or instilled sterile, pyrogen-free saline (sham exposure). Exposure-response relationships for inflammation were studied at 5 and 24 hours post exposure. For both time points, inhalation of Gm- bacteria resulted in a dose-dependent increase in inflammatory cells and cytokines when exposure was quantified by either concentration of bacteria or concentration of LPS associated with the bacteria. Exposure to Gm+ organisms, peptidoglycan, or lipoteichoic acid resulted in significantly lower inflammatory responses at both 5 and 24 hr. Our studies add evidence to the hypothesis that organic dust-induced acute lung inflammation is primarily associated with Gm- bacteria and less so with Gm+ organisms or their cell-wall components. (Supported by CDC/NIOSH U07CCU706145.)

652 DEVELOPMENT OF A RAT MODEL TO INVESTIGATE INFLAMMATORY REACTIONS OF THE LUNG.

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Inflammatory reactions of the lung play an important role in clinics under conditions like e. g. ARDS or hemorrhagic shock. These reactions are mainly associated with bacterial infections. But inflammatory reactions of the lung can also be induced by by other agents like particles or fibres which are deposited in the lung in aereas with high environmental polution. Irrespective of the causative agent the underlying mechanisms are very similar. After an activation of macrophages and epithelial cells these cells produce inflammatory mediators with cell activating, cytotoxic, and chemotactic potential. In a second step infiltrating neutrophils contribute to the reaction with the production and release of different mediators finally leading to the damage of lung tissue. Because there is a need for animal models which can be used to study inflammatory reactions of the lung we treated Spraque Dawley rats intratracheally with two concentrations of LPS and monitored the course of the reaction from 3 to 48 h. We investigated changes in the differential cell counts in the lung and measured the concentration of different cytokines like interleukin-1, interleukin-6, and tumor-necrosis factor-a in the bronchoalveolar lavage fluid (BAL). The tissue and cell damage was evaluated by measuring the concentration of total protein, ß-glucuronidase, and g-glutamyltransferase (g-GT) in the BAL. We found an increase in the neutrophils and total cell number and elevated levels of the soluble mediators in the BAL. These changes were time and concentration dependent with a maximum between 6 and 18 h and a decline at 48 h. Our data show that this model displays the characteristic features of an inflammatory reaction in the lung. It can be used to investigate the effect of drugs on the inflammatory reaction of the lung and may therefore be usefull to study new approaches in the treatment of the inflammatory reaction.

653 TIME COURSE AND KINETICS OF EOSINOPHILIC INFLAMMATION AND CORRESPONDING LUNG TOXICITY EFFECTS IN AN ALLERGIC ASTHMA MODEL IN BROWN NORWAY RATS.

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Allergic Asthma is a pulmonary disease characterized by antigen-induced pulmonary eosinophilia, airway hyperresponsiveness, antigen-specific IgE anti-

body responses and bronchoconstriction. In attempting to elucidate mechanisms associated with the pathogenesis of this disease, a number of animal models have been developed. The current studies were undertaken to develop a model of allergic asthma model in Brown Norway rats. Unlike the neutrophilic inflammatory response to inhaled particles in most strains of rats, inhalation of antigens in sensitized Brown Norway rats results in a complex cellular response which is characterized by a variety of inflammatory cell-types, and is dependent on the time course of inflammatory cell recruitment. In characterizing this ovalbumin-challenged model of allergic asthma, it was important to assess the time course of pulmonary inflammation, cell proliferation and apoptosis. Male Brown Norway rats were sensitized and boosted with intraperitoneal injections of ovalbumin in aluminum hydroxide on experimental days 1 and 8. On days 15-17, rats were challenged by an inhalation exposure to 5% ovalbumin, and evaluated by bronchoalveolar lavage and BrdU cell proliferation assessments at 24 or 48 hrs postexposure. Control rats were similarly treated to ovalbumin aerosol exposures, however, these animals had been sensitized and boosted with aluminum hydroxide (minus the ovalbumin). Cell differential evaluations demonstrated that the rats exposed for 3 days/24 hrs postexposure and for 2 days/48 hrs postexposure produced the greatest numbers of BAL eosinophils and corresponding indicators of pulmonary toxicity. It was interesting to note that earlier exposure time periods (i.e., 1 day/24 hrs) generated a predominantly neutrophilic inflammatory response, while longer exposure/postexposure time periods (i.e., 3 day/48 hrs) produced a predominant mononuclear inflammatory reponse. Subsequent studies demonstrated that the 2 day/48 hr protocol produced the optimum eosinophilic, cytotoxic, cell proliferative and apoptotic response. Histopathological evaluations demonstrated a chronically active alveolitis and bronchiolitis, characterized by epithelial cell proliferation in the airways and inflammatory cell proliferation in the alveoli. Studies are ongoing to assess the cell-types undergoing apoptosis in both the airway and parenchymal regions to fully characterize this model in order to assess it relevance/utility for humans.

654 SUBSTANCE P AND OTHER MEDIATORS IN A RABBIT MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS).

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Mortality due to acute respiratory distress syndrome (ARDS) remains unacceptably high at greater than 50% because there is still much to be learned concerning its primary pathogenesis. Little information is known about the mechanisms of neurogenic inflammation relating to ARDS. It is our objective to determine that afferent neural response was involved in the pathogenesis of acute smoke-induced ARDS. New Zealand rabbits were exposed to a 60 tidal volume of diesel fuel-polycarbonate plastic smoke and ventilated for 30 minute post-smoke. Smoke exposure induced significant increases in bronchoalveolar lavage concentrations of substance P (SP), tumor necrosis factor-a (TNF-a), and 6-keto-prostaglandin F1-a, when compared to sham smoke control. Changes of SP were paralleled with alterations of lung morphological and gas exchange function. It is speculated that highly sustained SP levels may activate preinflammatory cytokines and/or -eicosanoids cross-talks due to affected affinity of neurokinin (NK) receptors and decreased activity of neural endopeptidase (NEP) following acute smoke inhalation. These data suggest that afferent neural response to smoke inhalation may be a key primary contributor to the cascade that establishes ARDS. (Supported by ALA of Arizona 12734 and DAMD 17-94-J4001.)

655 EFFECTS OF QUINOLINEDIONE DERIVATIVES ON THE EXPRESSION OF INDUCIBLE CYCLOOXYGENASE AND NITRIC OXIDE SYNTHASE IN LPS-ACTIVATED MACROPHAGES.

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Newly synthesized quinolinedione derivatives, 6-(4-fluorophenyl) amino-5,8-quinolinedione (OQ1) and 6-(2,3,4-trifluorophenyl) amino-5,8-quinolinedione (OQ21) have been tested for the ability as anti-inflammatory and antiseptic drugs using lipopolysaccharide (LPS)-stimulated macrophage cell line (RAW 264.7 cell). Both OQ1 and OQ21 suppressed *de novo* synthesis and cellular accumulation of prostaglandin E_2 in a dose-dependent manner, suggesting that these compounds blocked inducible cyclooxygenase (COX-2) activity. In addition, OQ1 and OQ21 inhibited the LPS-stimulated expression of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). In order to investigate the mechanism for this inhibition, LPS-stimulated protein tyrosine phosphorylation was determined. OQ1 and OQ21 suppressed tyrosine phosphorylation of mitogen-activated protein kinases (MAPKs) suggesting that OQ1 and OQ21 inhibited LPS-induced MAPK activation resulting in the decrease of COX-2 and iNOS expression. These results suggest the possibility that quinolinedione derivatives, OQ1 and OQ21, may be effective therapeutic agents for septic shock and other inflammatory diseases.

656 POLYCHLORINATED BIPHENYLS INCREASE PRO-INFLAMMATORY CYTOKINE PRODUCTION AND MODIFY THE INFLAMMATORY RESPONSE TO LIPOPOLYSACCHARIDE.

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Polychlorinated biphenyls (PCBs) alter immune and nervous system functions while lipopolysaccharide (LPS) stimulates both systems. The objectives of this research were to assess whether PCBs alter tissue and circulating pro-inflammatory cytokine production either alone or after LPS challenge. Mice were fed 0, 25, 50, or 100ppm of Aroclor 1254 for 2 or 6wk. Following PCB exposure, mice received either 1 mg/kg/BW LPS or saline, and were analyzed 3h later. LPS-induced plasma cytokine levels were not altered by PCBs. However, PCBs elevated splenic IL-1β levels at 2wk (100ppm) and at 6wk (50 and 100ppm), but did not alter the LPS-induced splenic IL-1β increase. At 6wk, but not at 2wk, PCBs enhanced LPS-stimulated levels of splenic IL-6. In the brain, 50ppm PCBs enhanced the LPS-induced IL-1β increase at 2 wk, but the effect was lost with 100ppm. Interestingly, brain IL-6 levels were increased by 50ppm PCBs (2 and 6wk) with the magnitude of the increase being lower at 6wk than at 2wk. As with brain IL-1β, brain IL-6 levels were not affected by 100ppm PCBs; this lack of a response at 100ppm was not due to toxicity since the LPS-induced brain IL-6 was not altered. It is concluded that PCBs induce pro-inflammatory cytokines in the brain and the spleen and modify the inflammatory response to LPS. Therefore, PCB-induced inflammation in the CNS and periphery could potentially be a causative factor for previously reorted PCB-related neurotransmitter and behavioral alterations. (Supported by NIEHS ES07251 and ES05879.)

657 HEPARIN OR NEUTROPHIL DEPLETION ATTENUATES ENDOTOXIN-INDUCED POTENTIATION OF ALLYL ALCOHOL HEPATOTOXICITY.

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Noninjurious doses of endotoxin (LPS) enhance liver damage in rats from a variety of chemicals including allyl alcohol. The potentiation of allyl alcohol hepatotoxicity could not be reproduced in the isolated, buffer-perfused liver, suggesting that extrahepatic factors are necessary. The coagulation system, and particularly thrombin, is a critical factor in liver injury resulting from large doses of LPS (10^8 EU/kg). Accordingly, the purpose of these studies was to test the hypothesis that thrombin is involved in the potentiation of allyl alcohol hepatotoxicity by LPS. Rats were treated with heparin (2000 U/kg body weight, i.v.), an inhibitor of thrombin, one hour before treatment with LPS (1.2×10^5 EU/kg body weight, i.v.). Two hours later, allyl alcohol (30 mg/kg body weight, i.p.) was administered. Liver and blood samples were taken at three, four, and nine hours after heparin. Four hours after cotreatment with LPS/allyl alcohol, plasma fibrinogen concentration was decreased, suggesting activation of coagulation, and plasma concentration of tumor necrosis factor α (TNF-α) was increased. At nine hours, liver injury in rats cotreated with LPS/allyl alcohol was evident from elevations in plasma activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Heparin attenuated the changes in TNF-α, fibrinogen, ALT, and AST induced by treament with LPS/allyl alcohol. Evaluation of tissue sections stained immunohistochemically for neutrophils revealed increased staining in livers from rats treated with LPS. The contribution of neutrophils to hepatotoxicity was tested in rats treated with an anti-neutrophil immunoglobulin G fraction and then given the LPS/allyl alcohol cotreatment described above. Depletion of circulating neutrophils decreased the accumulation of neutrophils in the liver and reduced liver injury in the LPS/allyl alcohol cotreatment group. In summary, both the coagulation system and neutrophils are involved in allyl alcohol liver injury potentiated by endotoxin. (Supported by ES08789.)

658 ENDOTOXIN POTENTIATES AFLATOXIN B_1-INDUCED HEPATOTOXICITY THROUGH A TN-α-DEPENDENT MECHANISM.

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Exposure to a small, nontoxic dose of bacterial endotoxin (LPS) potentiates the hepatotoxicity of aflatoxin B_1 (AFB_1), characterized by periportal necrosis, inflammation and cholestasis. Some of the pathophysiological effects associated with LPS are mediated through tumor necrosis factor-α (TNF-α). This study was conducted to explore the role of TNF-α in the AFB_1/LPS model. Male, Sprague-Dawley rats (250-300 g) were treated with either 1 mg AFB_1/kg, ip, or its vehicle (0.5% DMSO/water) and with either E. coli LPS (7.4 X 10^6 EU/kg, iv) or its saline vehicle 4 hours later. Serum TNF-α was measured by ELISA at 6, 12, 24, 48, 72 and 96 hr after AFB_1 administration. LPS administration resulted in a marked rise in TNF-α at 6 hr that preceded the onset of liver injury. TNF-α mRNA in liver was measured by semi-quantitative RT-PCR and was increased by LPS. The mRNA of receptors (R1 and R2) for TNF-α was also examined. R1 mRNA levels were not altered; however, R2 mRNA was increased by either AFB_1 or LPS. To determine if TNF-α plays a causal role in the development of liver injury, the increase in TNF-α was attenuated by administration of either pentoxifylline or anti-TNF-α serum, and liver injury was assessed from alterations in the concentration of bile acids and the activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase and 5'-nucleotidase in serum and from histopathologic examination of liver. Administration of either of these TNF-α inhibitors resulted in protection. These data support the hypothesis that LPS-induction of TNF-α plays a causal role in the potentiation of AFB_1-induced hepatotoxicity. (Supported by NIH grant ES04139.)

659 ACUTE ENDOTOXEMIA IS ASSOCIATED WITH INCREASED STAT1 AND NFKB NUCLEAR BINDING ACTIVITY IN HEPATIC MACROPHAGES AND ENDOTHELIAL CELLS.

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Acute endotoxemia induces the accumulation and activation of macrophages in the liver. This is associated with priming of these cells to produce increased quantities of nitric oxide (NO) and prostaglandin E2 (PGE2) in response to inflammatory mediators such as lipopolysaccharide (LPS) and g-interferon (g-IFN). In the present studies we analyzed mechanisms regulating increased mediator production by macrophages and endothelial cells during acute endotoxemia. The promotor regions of the inducible nitric oxide synthase (NOSII) and cyclooxygenase-2 (COX-2) genes which regulate NO and PGE2 production respectively, contain binding sites for several transcription factors including signal transducers and activators of transcription-1 (STAT1) and nuclear factor kB (NFkB). Whereas, STAT1 is activated by g-IFN, NFkB activation occurs in response to LPS. We speculated that increased NO and PGE2 production in response to g-IFN and LPS during acute endotoxemia is due to altered transcription factor activity. Acute endotoxemia was induced by injecting rats with Escherichia coli LPS (5 mg/kg i.v.). We found that macrophages and endothelial cells from control rats expressed low levels of activated STAT1 as determined by electrophoretic mobility shift assays and Western blotting. Treatment of the cells with g-IFN for 30 min resulted in a marked increase in STAT1 binding activity and phosphorylated STAT1 protein. Induction of acute endotoxemia caused a time-dependent increase in STAT1 binding to the g-IFN activation site (GAS) on DNA and an increase in phosphorylated STAT1 protein. These effects were most pronounced in cells isolated 24 hours after endotoxin administration to animals. The addition of g-IFN to hepatic macrophage and endothelial cell cultures further increased STAT1 binding activity and phosphorylated STAT1 levels when compared to cells from control animals. In contrast to STAT1, constitutive NFkB binding activity was not observed in macrophages and endothelial cells from control animals. However, these cells were responsive to *in vivo* endotoxin. Thus, treatment of rats with endotoxin resulted in a time dependent increase in NFkB binding activity. Taken together these data suggest that increased mediator production by liver nonparenchymal cells during acute endotoxemia may be due to increased activity of STAT1 and NFkB. (Supported by NIH GM34310, ES06897 and The Burroughs Wellcome Fund.)

660 HEPATOTOXICITY FROM COADMINISTRATION OF SMALL, SYNERGISTIC DOSES OF MONOCROTALINE AND BACTERIAL ENDOTOXIN IS ATTENUATED BY HEPARIN.

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Coadministration of small, noninjurious doses of the food-borne toxin monocrotaline (MCT) and bacterial endotoxin (LPS) to male, Sprague-Dawley rats results in pronounced hepatotoxicity. Histopathologic analysis of livers demonstrated both midzonal (i.e., LPS-like) and centrilobular (i.e., MCT-like) lesions. Endothelial cell injury in central veins and decreased plasma fibrinogen concentration observed in these animals suggested that the coagulation system might play a causative role in the hepatocellular injury. Accordingly, we treated animals with the anticoagulant, heparin (HEP), to determine if the coagulation system might be involved in liver injury in this model. MCT (100 mg/kg, i.p.) was given to rats 4 hours before LPS (7.4 x 10^6 EU/kg, i.v.), and then HEP (2000U/kg; i.v.) or saline (SAL) vehicle was administered 5.5 hours after MCT. A significant decrease in hepatocellular injury, as marked by plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and by histopathologic examination, was observed in animals that received the MCT/LPS/HEP combination in comparison to animals that received MCT/LPS/SAL. Liver histopathological analysis revealed that lesion size and frequency were less in animals that received HEP, but the qualitative nature of the lesions remained unchanged. These results are consistent with a causative role for the coagulation system in this MCT/LPS model of liver injury. (Supported by NIH Grants ES 04139 and ES08789.)

661 LIVER INFLAMMATION AFTER ACETAMINOPHEN OVERDOSE: ROLE OF NEUTROPHILS.

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Acetaminophen overdose induces severe liver injury and hepatic failure. There is evidence that inflammatory cells may be involved in the pathophysiology. Thus, the aim of this investigation was to characterize the neutrophilic inflammatory response after treatment of C3Heb/FeJ mice with 300 mg/kg acetaminophen. A time course study showed that neutrophils accumulate in the liver parallel to or slightly after the development of liver injury. The number of neutrophils in the liver was substantial (209±64 PMN/50 high power fields at 12 h) compared to baseline levels (7±1). Serum levels of TNF-α and the C-X-C chemokines KC and MIP-2 increased by 28-, 14-, and 295-fold, respectively, over levels found in controls during the injury process. In addition, mRNA expression of MIP-2 and KC were upregulated in livers of acetaminophen-treated animals as determined by Ribonuclease Protection Assay. However, none of these mediators was generated in large enough quantities to account for neutrophil sequestration in the liver. There was no upregulation of Mac-1 (CD11b/CD18) or shedding of L-selectin on circulating neutrophils. Moreover, an anti-CD18 antibody had no protective effect against acetaminophen overdose during the first 24 h. These results indicate that there is a local inflammatory response after acetaminophen overdose including a substantial accumulation of neutrophils in the liver. However, neutrophils do not contribute to the injury. The inflammation observed after acetaminophen overdose may be characteristic for a response sufficient to recruit neutrophils for the purpose of removing necrotic cells but is not severe enough to cause additional damage.

662 MECHANISMS OF MUTUAL ANTAGONISM BETWEEN NF-κB AND THE GLUCOCORTICOID RECEPTOR.

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The transcription factors NF-κ B (NF-κB) and glucocorticoid receptor (GR) have diametricially opposed roles in the modulation of immune/inflammatory responses. NF-κB induces the expression of pro-inflammatory genes, while GR suppresses immune function in part by suppressing expression of these same genes. We have demonstrated that physiological antagonism between NF-κB and GR is due to a mutual transcriptional antagonism requiring the presence of specific domains of each transcription factor. By *in vitro* coimmunoprecipitation (co-IP), we show that the p65 subunit of NF-κB physically interacts with GR, and that this interaction is enhanced by the transcriptional cointegrator CREB Binding Protein (CBP). To clarify the mechanism

by which p65 and GR interact to antagonize each other's function we employed p65 RHD, a dominant negative regulator of p65 transactivation, in transient cotransfection assays. Interestingly, p65 RHD efficiently blocks p65 transactivation of an NF-κB responsive reporter, yet cannot interfere with p65 mediated repression of GR transactivation and has no direct effect on GR transactivation (despite the fact that GR and p65 RHD co-IP). This indicates that the transactivation domain of p65 is required for repression of GR transactivation, but that NF-κB need not be transcriptionally active to repress GR. Cotransfection studies in which CBP is overexpressed indicate that both GR-mediated repression of p65 transactivation and p65 mediated repression of GR transactivation are dose-dependently enhanced by CBP, suggesting that the mechanism of p65-GR antagonism is not a simple competition for a limiting pool of CBP, but rather that CBP might serve as an integrator for p65 and GR which facilitates physical interaction between the DNA-bound transcription factors. Studies using the glucocorticoid antagonist RU 486 indicate that antagonist-bound GR, though transcriptionally inactive, retains the ability to interact with p65 and repress its transactivation. However, antagonist bound GR does not co-IP with CBP. Consequently, CBP cannot enhance the physical interaction of GR with, or the repressive effect of antagonist-bound GR on, NF-κB.

663 PROTECTIVE EFFECT OF RECOMBINANT INTERLEUKIN-6 ON SKIN INFLAMMATION CAUSED BY 7,12-DIMETHYLBENZ[A]ANTHRACENE IN INTERLEUKIN-6 NULL MICE.

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Interleukin-6 (IL-6) is a multifunctional cytokine, which regulates essential physiological functions such as acute phase reaction, immune response, hematopoiesis and bone metabolism. In order to examine the protective effect of IL-6 on skin damage caused by 7,12-dimethylbenz[*a*]anthracene (DMBA), we have conducted administration of IL-6 extracorporeally to the IL-6 null (IL-6-/-) mice. Female IL-6-/- mice and wild type B6J129Sv mice were topically applied a single dose of DMBA (500μg /mouse) on dorsal skin. Osmotic pumps with a delivery rate of 0. 5 μl/h were filled with recombinant human IL-6 (rhIL-6) (10 or 25μg/day) and then implanted subcutaneously on the ventral side of the mice (IL-6-/- + rhIL-6 mice). In control mice, PBS was filled instead of rhIL-6. Tissue samples were collected 5 days after DMBA administration, and subjected to histopathological examination. A severe skin damage was observed in IL-6-/- mice characterized by lymphocyte infiltration and keratinocyte hyperproliferation, whereas only epidermal hyperplasia was observed in the wild-type mice. Recombinant hIL-6 treatment to DMBA-treated IL-6 null mice suppressed the ocurrence of the above-mentioned skin damage. The present results suggest that IL-6 plays an important role in defense mechanism for cutaneous inflammation caused by DMBA.

664 ELEVATED OXIDATIVE STRESS IN SKIN OF B6C3FL MICE AFFECTS DERMAL EXPOSURE TO MACHINE WORKING FLUID.

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Metal Working Fluids (MWFs) are widely used for metal cutting, drilling, shaping, lubricating and milling in the industrial world. Potential for dermal and inhalation exposure to MWFs exists in a large number of workers via aerosols, splashing during the machining process, and other industrial operations. Both females and males are involved in these industrial operations. It has been reported earlier that occupational exposure to MWFs causes health problems, e.g. allergic and irritant contact dermatitis. Previously, we have shown that dermal exposure of B6C3F1 mice (female and male) to 5% MWFs for 3 months resulted in an increase in skin histamine and mast cell counts. Topical exposure to MWF also resulted in depletion of two major water soluble antioxidants, e.g. ascorbate and glutathione in the liver of both genders. The level of lipid peroxidation (MDA) in the liver of both sexes and the testes in males was concomitantly increased. The goal of this study was to evaluate interaction between oxidative stress in the skin and topical application of MWF. To create acute skin inflammation, an H_2O_2 producing enzyme, glucose oxidase conjugated to polyethylene glycol (GOD-PEG), was injected intradermally. Intradermal administration of GOD-PEG to B6C3F1 mice (female and male, 8-12 weeks old) resulted in inflammation and muscle fiber necrosis in the skin. The level of GSH in skin was reduced after GOD-PEG treatment of B6C3F1 mice. To determine whether oxidative stress influences the dermal response to MWF, mice were injected with GOD-PEG 24 h prior to topical exposure to unused MWF (200 μl 30%). This resulted in an increased inflammatory response in skin, reduced GSH and protein thiols, and an increased level of peroxidative products. Further study is necessary to delineate the role of oxidative stress in enhancing contact/irritant dermatitis triggered by occupational exposures to MWF.

665 GLUTATHIONE (GSH) QUENCHES PEROXYNITRITE - MEDIATED NITRATION OF PROSTAGLANDIN H SYNTHASE FORM-2 (PGHS-2).

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Increased expression of both inducible nitric oxide (NO) synthase and PGHS-2 has been reported in intestinal inflammation. Overexpression of PGHS-2 induces cellular oxidative stress. NO reacts with superoxide produce under oxidative stress to form peroxynitrite, a potent toxic oxidant. To investigate whether, PGHS-2 isolated from sheep placenta was treated with peroxynitrite and nitration of PGHS-2 was analyzed by Western blot analysis using antibodies against nitrotyrosine. Nitrotyrosine levels of PGHS-2 dramatically increased after treatment with 300μM peroxynitrite. Surprisingly, addition of 30μM GSH to the reaction mixture prevented nitration of PGHS-2 by peroxynitrite. This result suggests that GSH prevents peroxynitritemediated nitration of PGHS-2 by formation of nitrosoglutathione and that nitration of GSH futher increases oxidative stress by lowering cellular GSH levels. (Sponsored by NIEHS SBIR contract ES95438(HK)and the Petroleum Research Fund 33957-GB4 (ESR-K).

666 DIGESTIBILITY OF PROTEINS IN SIMULATED GASTRIC FLUID.

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Previous investigators have reported that 16 purified food protein allergens exhibited markedly greater stability in simulated gastric fluid (SGF) *in vitro* than 9 common plant proteins, and have concluded that stability in SGF distinguishes food allergens from nonallergens (Astwood et al., Nature Biotechnology 14: 1269, 1996). In the present study, the stability of 17 food protein allergens in SGF (0.08% (w/v) pepsin, 0.2% NaCl, 0.7% HCl) at 37°C has been compared with the stability of 24 proteins not associated with food allergy (17 plant food proteins and 7 animal proteins). Protein stability was assessed by SDS-polyacrylamide gel electrophoresis, using a gel system that resolved polypeptides with molecular masses that exceeded approximately 2 kDa. 13 food protein allergens were partially or completely stable for at least 60 min and/or were cleaved to polypeptide fragments that persisted for at least 60 min. 2 food allergens were degraded to peptides that were stable for 15 min and 2 were degraded to peptides that were stable for 5 min. Of the 17 plant food proteins that were tested, 8 were degraded completely within 5 min, as was 1 of the animal proteins. In contrast, 3 plant proteins resisted digestion for at least 60 min (e.g. potato tuber carboxypeptidase inhibitor), while 3 yielded polypeptide fragments that persisted for at least 60min (e.g. turnip cytochrome f). Peptide fragments that persisted for at least 60 min were also observed with 4 of the animal proteins (e.g. horse heart myoglobin). These results confirm that stability in SGF is a general feature of many food protein allergens. However, it is clear that similar stability is exhibited by other plant and animal proteins not suspected to be allergens. The implication is that resistance of food proteins to digestion in SGF is not a defining characteristic of allergenic potential.

667 SOY DIETS CONTAINING GENISTEIN STIMULATE GROWTH OF ESTROGEN-DEPENDENT TUMORS IN A DOSE DEPENDENT MANNER.

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We have demonstrated that genistein and the glycoside form, genistin, stimulate estrogen (E)-dependent human breast cancer (MCF-7) cells *in vivo* (Cancer Research 58:3833, FASEB J. 13(4) A370). Genistin which is metabolized to genistein after consumption is the predominant isoflavone in soy protein commercially available. Depending on processing, soy isolates can contain different concentrations of genistein. We hypothesize that soy protein

isolates containing different concentrations of genistein will stimulate the growth of E-dependent cells *in vivo* in a dose dependent manner. We conducted experiments in which these soy protein isolates were fed to athymic mice implanted subcutaneously with E-dependent tumors. Genistein content of the soy isolate diets were 15ppm, 150ppm, or 300ppm. Positive (+E) and negative (-E) control groups received casein based diets. Tumor size was measured weekly. At completion of the study animals were killed and tumors collected for BrdU incorporation and pS2 expression. Incorporation of BrdU into cellular DNA was utilized as an indicator of cell proliferation whereas pS2 is an E-responsive gene. Soy protein diets containing genistein increased E-dependent tumor growth in a dose dependent manner. Cell proliferation was greatest in tumors of animals given E or dietary genistein (150 and 300ppm). Expression of pS2 was increased in tumors from animals consuming dietary genistein. (Supported by NIH grant (CA77355) to WGH.)

668 DEVELOPMENT OF A DOSE RESPONSE MODEL FOR EXPOSURE TO *LISTERIA MONOCYTOGENES* DURING PREGNANCY.

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Listeriosis is a foodborne illness resulting in an estimated 1850 cases and over 400 deaths per year in the U.S., about 1/4 of which are stillbirths, spontaneous abortions or neonatal deaths. Both human and non-human primates are susceptible to listeriosis when pregnant, with the same maternal and fetal reactions and pathological lesions. The objective of this study is to establish a dose response model for *Listeria monocytogenes* (*Lm*) using a the pregnant rhesus monkey as an animal surrogate for humans. The study was designed as a Phase I clinical trial using guidelines developed for testing anti-cancer drugs in humans. Initially, 3 animals were given a cocktail of 6 strains of *Lm* which were isolated from either human or primate outbreaks. Based on adverse pregnancy outcomes, two strains were identified for further testing. Nine rhesus monkeys were given oral doses ranging between 10^6 to 10^{11} cfu *Lm*/ml of whipping cream at approximately 110 days gestation. Animals were observed for outward signs of illness, and fecal and blood samples were collected and cultured to determine presence of *Lm*. Out of nine treated animals, four resulted in stillbirths. *Lm* was isolated from fetal tissue, and pathology indicated *Lm* as the cause of pregnancy loss. In pregnancies resulting in stillbirths, *Lm* was isolated from feces 3 or more days post-treatment indicating that *Lm* had survived and probably colonized the GI tract. (This work was supported by FDA grant FDU001622-01.)

669 DOES THE INCREASED CORN INTAKE BY MEXICAN-AMERICANS LOWER THEIR BLOOD FOLATE LEVELS?

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Several studies have explored potential associations between incidence of neural tube defects (NTD) and folate deficiency, maternal chemical exposures, including environmental pesticides and water disinfection by-products, or exposures to fumonisins in corn. A recent publication has made the assumption that an apparent NTD cluster among Mexican-Americans living in South Texas is related to the high intakes of corn based foods and to the potential contamination of these foods with fumonisin. We used data collected by the National Health and Nutrition Examination Survey III (NHANES III) and USDA's Continuing Survey of Food Intakes by Individuals (CSFII) and examined the frequency of intake of corn-based foods by the US population, specifically Mexican-Americans, as well as folic acid intakes and folate blood levels. The results of these analyses showed significantly higher frequency of consumption of corn-based foods as well as significantly lower blood folate levels among Mexican-Americans. However, no significant associations were detected between the frequency of consumption of corn-based foods among Mexican-Americans and their blood folate levels. In fact, blood folate levels of Mexican-Americans who reported not consuming corn-based foods in the previous months were similar to those observed among Mexican-Americans who consumed corn-based foods daily. Furthermore, blood folate levels of Mexican-Americans who reported not consuming corn-based foods were significantly lower than those observed among non-Hispanics who also reported not consuming corn-based foods. These results suggest that factors other than corn intake are related to the lower blood folate levels observed among Mexican-Americans and the NTD cluster observed in South Texas.

670 PRELIMINARY STUDIES ON THE FATE OF FUMONISINS DURING THE MANUFACTURE OF TORTILLA CHIPS.

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Fumonisins are mycotoxins produced by *Fusarium moniliforme* (= *F. verticillioides*) and *F. proliferatum*, fungi commonly found on corn. They cause fatal diseases in farm animals, are hepato- and nephrotoxic, and are rodent liver and kidney carcinogens. Human health effects of fumonisins are unclear, although survey data suggest that consumption of fumonisin contaminated corn as a dietary staple is correlated with high esophageal cancer rates in areas of southern Africa and China. Little information on how cooking affects fumonisins is available. Therefore, the fate of fumonisins during commercial scale production of fried tortilla chips, a popular snack food, was studied by determining the fumonisin concentrations of the corn, the process intermediates, and the chips using HPLC. Measurable fumonisin B_1 (FB_1) and B_2 (FB_2) concentrations in the chips varied, but were 40-80 percent lower than in the raw corn (n=4 batches) from which they were made. Cooking and steeping the corn in alkaline water, the first step in the process, was critical for lowering fumonisin concentrations. The intermediate steps of grinding the cooked corn into masa, baking, and frying the masa had little effect. Hydrolyzed FB_1 (HFB_1) was formed during cooking and steeping. HFB_1 concentrations in the fried chips were, however, relatively low compared to FB_1, suggesting that most HFB_1 remained in the steep liquid and other waste byproducts. These findings suggest that steeping and rinsing cooked corn in water effectively reduces fumonisin concentrations in food products manufactured from corn.

671 COMPARISON OF SPHINGOLIPID CHANGES IN TWO STRAINS OF MICE DOSED WITH FUMONISIN B1 (FB1) BY TWO DIFFERENT ROUTES.

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Fumonisins are fungal toxins produced by Fusarium moniliforme which occurs on corn worldwide.The fumonisins are potent inhibitors of ceramide synthase. Inhibition of ceramide synthase causes a rapid increase in free sphinganine. We determined the time course of changes (0 to 48 h) in levels of free sphingoid bases, complex sphingolipids, and ganglioside GM1 (based on Cholera toxin binding) in intestinal epithelial cells, liver, and kidney from male NIH Swiss mice and male BALB/C mice treated with a single gavage or subcutaneous dose, respectively, of FB1 (25 mg/kg body wt). The subcutaneous and gavage routes of exposure in male BALB/C mice and male NIH Swiss mice produced similar results: a rapid (maximal at 4 -12 h) and reversible (return to control at 24 -48 h) increase in free sphinganine in intestinal epithelial cells (~300 to 400%) and liver (~200 to 300%), and an equally rapid but more slowly reversible (return to control >48 h) increase in kidney (~2000 to 2500%). There was no change in concentration or expression of complex sphingolipids or GM1, respectively. Thus, strain and route of exposure had no effect on the time course of sphingoid base accumulation, complex sphingolipids, or expression of GM1. (Supported in part by NIH Grant # ES09403.)

672 THE EFFECT OF FUMONISIN PRODUCING *FUSARIUM MONILIFORME* ON SELECTED IMMUNE RESPONSES OF MICE TO *TRYPANOSOMA CRUZI*.

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Fumonisins, produced by *Fusarium moniliforme* (=*F. verticilloides*) and related fungi, occur naturally on corn. Although known to elicit various species- and organ-specific toxicities, fumonisins are largely unstudied with respect to effects on immune function. *Trypanosoma cruzi*, the causative agent of Chagas' disease in humans, has a complex life cycle that includes both bloodstream and tissue stages in the mammalian host. The host immune response to *T. cruzi* includes macrophages phagocytizing and killing the parasite (partially mediated by the production and release of nitric oxide) or serving as host cells for parasite replication. BALB/c mice (3 groups, 20/group) were fed diets containing 0, 50 or 150 ppm fumonisins (fumonisins B_1+B_2 provided by *F. moniliforme* culture material) for one week prior to intraperitoneal injection with *T. cruzi*. Blood parasites were counted every 5-6 days, and 5 mice/group were euthanized on days 0, 14, 26 and 36 post-infection. Although body and

organ weight effects were minimal, fumonisin doses did cause significant dose-related increases in tissue sphinganine concentrations, a biomarker of fumonisin exposure. No differences among groups in blood parasite counts were found on days 8, 14, 20 or 36. Parasite counts of the controls, but not the fumonisin-fed groups, increased sharply on day 26. Control parasite counts decreased again on day 31, but remained significantly ($p<0.05$) higher than those of the high dose group. Differences in nitric oxide (NO) production were not found among groups before day 14 or on day 36. NO production of high dose mice peaked and was significantly increased ($p<0.05$) compared to controls on day 14, whereas NO production in controls peaked on day 26, simultaneously with peak blood parasite counts. These findings suggest that fumonisins affected immune response to *T. cruzi* and that *T. cruzi*-infected mice may be a good *in vivo* model for further studying the immunotoxic effects of fumonisins.

673 APPLICATION OF AN *IN VITRO* GASTROINTESTINAL MODEL FOR THE PREDICTION OF MYCOTOXIN ENTEROSORBENT EFFICACY.

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The successful inclusion of NovaSil clay in diets for the prevention of aflatoxicosis in animals has created an interest in the investigation of other potential enterosorbents for mycotoxins. Since *in vitro* studies are not always predictive of *in vivo* results, our objectives in this study were to compare three rapid *in vitro* methods, i.e., single point adsorption (SPA), adsorption isotherm (Q_{max}), and chemisorption index (C_α) with a recently established gastrointestinal (GI) model. These four methods were used to delineate the efficacy of binding of aflatoxin B_1 (AfB_1) to five sorbents: NovaSil, sand, charcoal, clinoptilolite and organoclay. Additionally, a corn-based feed matrix was included in modified isotherm and GI studies to examine potential interactions. As expected, NovaSil proved efficacious in all test methods (SPA and GI = >99% AfB_1 bound; $C_\alpha = 0.89$; and $Q_{max} = 0.461$mol/kg). Clinoptilolite did not effectively bind AfB_1, but had a C_α equal to 0.54. Also, under GI conditions, this clay demonstrated catalytic activity that was not observed in the other methods. Binding of AfB_1 by charcoal was higher than, or comparable to, NovaSil (SPA and GI = >99% AfB_1, $C_\alpha = 0.78$; and $Q_{max} = 0.889$ mol/kg) but was hindered in the presence of a corn matrix as seen by a decrease in the distribution coefficient ($K_d = 1.19 \times 10^6$ vs 1.11×10^5). To further test the predictive power of the GI model, studies were conducted with zearalenone (ZEN) and fumonisin B_1 (FB_1) and compared with *in vivo* results. The GI model was shown to be an efficient method of screening potential mycotoxin sorbents that includes physiologically relevant parameters and allows a more confident prediction of *in vivo* efficacy (Supported by USDA 9703230, NIEHS P42-ES04917, NCI CA-74552 and TAES H6215.)

674 THIN FILM CLAY-BASED COMPOSITES AS AFFINITY PROBES FOR AFLATOXINS.

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The self-assembly of thin montmorillonite films on solid substrates has enabled the formation of advanced hybrid materials that possess unique properties. Stepwise film self-assembly is based on electrostatic attraction between oppositely charged components. It offers a novel strategy for building ordered organic/inorganic films on substrates while maintaining systematic control over film composition, structure and thickness. Our objectives were to utilize thin film technology to construct clay-quartz composites for the selective cleanup and analysis of aflatoxins from a variety of contaminated samples. In this study, a calcium montmorillonite clay (CMC), with high affinity and enthalpy of adsorption for aflatoxins, was electrostatically anchored onto the surface of quartz particles (212-300 μm) using poly(diallyldimethylammonium chloride) (P). Self-assembly of CMC films was accomplished by the sequential treatment of substrate in aqueous cationic solutions of P (1% w/v) and aqueous dispersions of CMC. Particle sizes of CMC and the number of deposition cycles of P and CMC on quartz were controlled experimentally. Composites (1.0 g) were tested for aflatoxin B_1 (AfB_1) capacity by eluting an aqueous AfB_1 solution (4 μg/ml) through prototypical glass affinity columns. Results showed that 50% breakthrough capacities for AfB_1 were highest with composites formulated with the largest CMC particle sizes and multilayer film depositions. Breakthrough capacities (50%) for AfB_1 ranged from 13-77 μg for monolayer composites and 15-140 μg for multilayer composites. Composites (0.5 g) were also tested for their ability to recover intact AfB_1 (12 μg) from aqueous solution. Results indicated that the highest AfB_1 recoveries were achieved using composites hybridized with the largest CMC particle sizes. Recoveries ranged from 1.7±0.1 to 7.5±0.4 μg AfB_1. Preliminary results suggest that thin film CMC composites may provide an alternative and cost-effective method for cleanup and analysis of aflatoxin contaminated media. (Supported by USDA 9703230, NIEHS P42-ES04917 and TAES H6215.)

675 HIGH AFFINITY SORPTION OF AFLATOXIN B1 BY HECTORITE CLAY.

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In the present study a variety of clays, differing in their chemical and structural characteristics, were tested for their ability to sorb aflatoxin B1 (AfB1) from aqueous solution. Results indicated maximal binding of AfB1to sorbents possessing high surface area, high cation exchange capacity (CEC) and intact interlayers. Based on these initial screening studies, hectorite (a trioctahedral smectite clay) was selected for further characterization and comparison with NovaSil (a dioctahedral smectite clay mineral). Equilibrium adsorption isotherms were run for each sorbent using methods previously established in our laboratory. The maximal capacities (Qmax) and affinity constants (Kd) for the adsorption of AfB1 were determined. At 25°C, the Qmax derived from a fitted Langmuir equation for hectorite was 0.919 ± 0.25 mol/kg vs 0.302 ± 0.01 mol/kg for NovaSil. The Kd was equal to 6.46 x 104 and 2.44 x 105 for hectorite and NovaSil,respectively. These findings suggest that the trioctahedral clay (hectorite) is an effective sorbent for AfB1 *in vitro*. A sensitive bioassaywas also used to confirm the detoxification of aflatoxins following sorption to hectorite. However, further *in vivo* studies in animals arerequired to determine the efficacy and safety of this clay before it can be used as a dietary enterosorbent. (Supported by USDA 9703230, NIEHSP42-ES04917 and TAES H6215.)

676 MODULATION OF BARRIER FUNCTION OF SMALL INTESTINAL EPITHELIAL CELLS BY TRICHOTHECENE MYCOTOXIN, DEOXYNIVALENOL.

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The trichothecenes are known immunomodulators in animals. In mice, repeated oral dosing of deoxynivalenol (DON) altered cell-mediated and humoral immune responses and thereby reduced the host resistance to infectious diseases. However, the effect of oral exposure to trichothecenes on intestinal structure and function is still unknown. We used the human intestinal cell line (Caco-2) to study the effects of DON on the structure and differentiation of the intestinal epithelium and the secretion of proinflammatory cytokines. When Caco-2 cells were exposed to DON at concentrations that did not inhibit protein synthesis, the structure of the apical surface was altered and permeability of mannitol increased. DON suppressed the development of brush border membrane associated enzymes. The secretion of IL-8 from intestinal epithelial cell, a proinflammatory cytokine, was enhanced by DON. These results show that DON can modulate intestinal epithelium structure and function. Therefore the DON modulation of barrier function *in vivo* may influence the susceptibility to enteropathogens.

677 MODULATION OF GLUTATHIONE *S*-TRANSFERASES BY DIETARY BUTYLATED HYDROXYTOLUENE IN TURKEYS.

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Poultry are the most susceptible food animal species to the toxic effects of the mycotoxin aflatoxin B_1 (AFB_1). We previously showed that the extreme sensitivity of turkeys to AFB_1 is due to a combination of efficient AFB_1 activation by cytochrome P450s (CYPs) 1A2 and 3A4 and deficient detoxification by glutathione *S*-transferases (GSTs). As a strategy to induce GSTs relevant to AFB_1-detoxication, we pretreated male turkey poults of varying ages with 0.01 and 0.04 % (w/w) butylated hydroxytoluene (BHT) in the diet for 10 d. Liver cytosol from these animals showed increased general GST activity in the high BHT groups of all ages. BHT also significantly induced μ class GST activity in all age groups. Western immunoblots showed that immunoreactive proteins corresponding to α, μ, π and σ-class GSTs were significantly increased by BHT treatment. There was, however, no measurable AFB_1-8,9-

epoxide conjugation activity *in vitro* using liver cytosol from either control or BHT-treated poults of any age. While turkeys appear to possess inducible elements for GST expression, BHT apparently did not induce a GST isoform specific to AFB_1 detoxification. (Supported by USDA-NRI grants 97-3081 and 98-3754.)

678 TOXICITY OF COPPER SULFATE SUPPLEMENT IN OHIO FEEDER LAMBS.

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Copper sulfate has wide use as an agricultural poison and as an algicide in water purification. It has been used as a growth promotant for food animals, although documentation for such use is minimal. An investigation was carried out subsequent to an outbreak of copper sulfate toxicity in a group of Ohio feeder lambs. Sixty lambs were orally exposed to as high daily dose of 1339 ppm of copper sulfate in feed. A miscalculation was made in the formulae by the owner. Thirteen percent of the exposed animals died within a few weeks of daily exposure. Clinically, the lambs exhibited signs of depression, weakness and anorexia prior to death. Gross postmortem examination revealed hepatomegaly and splenomegaly. Histopathologic examination of tissues was typical of ovine copper toxicosis. The liver showed diffuse hepatocellular degeneration with multifocal necrosis in association with an abundance of cytoplasmic brown pigment. The kidneys exhibited diffuse interstitial edema and luminal hemoglobin pigment in association with tubular epithelial necrosis and regeneration. The level of copper in the liver (298 +/-34.59 ppm) was 300 times higher than that of muscle and 75 times that found in the kidneys. The findings of this investigation indicate the need to establish regulatory limits of copper in order to limit producer usage and to ensure that the U.S. consumer is not exposed to meat and poultry products adulterated with metals of toxicological concern.

679 TOXICITY PROFILE OF DIFFERENT IRON COMPOUNDS IN THE DEVELOPING RAT.

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Iron is the most abundant metal in the human body and is essential for normal neurological development. Iron deficiency during development can cause behavioral alterations including deficits in learning and memory. A high level of iron in the cell is capable of producing reactive oxygen species such as hydroxyl radicals. The present study was designed to evaluate the toxicity of three different iron compounds in developing rats. One month old male Sprague-Dawley rats were dosed orally with an acute dose of ferrous sulfate ($FeSO_4$; 1 g/kg), carbonyl iron (Fe; 30 g/kg) or sodium iron ethylenediaminetetracetate (NaFeEDTA, 2 g/kg) and were humanely killed after 15 min, 30 min, 1 hr, 2 hr, 4 hr and 24 hr. Trunk blood was collected for serum iron determination and brain tissue was removed and quickly dissected into different regions for analysis of dopamine and its metabolites. Acute administration of all three-iron compounds increased blood iron concentration that reached peak level at 4 hrs. $FeSO_4$ produced a significant decrease in the concentration of dopamine and its metabolites DOPAC and HVA at 4 hrs. After the administration of Fe and NaFeEDTA, dopamine or its metabolites showed no significant alterations. Brain and liver iron concentrations were also evaluated by atomic absorption spectrophotometry after $FeSO_4$ administration. Iron levels increased significantly in the brain at 15 and 30 min, whereas the liver iron concentrations increased at 2 hr. At these doses, these data demonstrate that $FeSO_4$ is capable of altering dopamine levels when compared to Fe and NaFeEDTA.

680 PHENYLBUTAZONE: A NEW CHALLENGE TO THE SAFETY OF US MEAT.

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Phenylbutazone is a nonsteroidal anti-inflammatory drug. For humans the drug is usually prescribed as a last resort, because of serious idiosyncratic side effects. Blood dyscrasia and anaphylaxis are the main side effects, which are usually accompanied with difficulty in swallowing, gasping for breath, dizziness, or fainting. The drug could cause liver and kidney failure. Phenylbutazone is approved for relief of inflammatory conditions associated with the musculoskeletal system in dogs and horses (21 CFR 520.1720), but, because of its potential human health impacts, the Food and Drug Administration prohibits its use in all food animals. Most recently, residues of phenylbutazone were detected in the tissues of several food animals, including a "Grand Champion Steer" at an Ohio County Fair, and a horse. In the first case, both phenylbutazone and its metabolite oxyphenylbutazone were extracted from urine before being quantified and confirmed by gas chromatography/mass spectrometry. In the latter case, phenylbutazone was found in fat adjacent to muscle tissue. In the last two years, FSIS has found ten instances of illegal usage of phenylbutazone. These findings of phenylbutazone residues may warrant investigation of the compliance of food animal veterinarians and animal owners to Federal regulations as well as education regarding use of this drug in food animals. Use of this banned drug in food producing animals compromises the health of the American public and jeopardizes the safety of the American food supply. Any finding of phenylbutazone in a food producing animal renders that animal carcass, as well as all other carcasses that are "mixed" with that carcass, as "adulterated," leading to an economic loss to food animal producers. Cooperation between Federal Agencies and Veterinary Associations is required to prevent future occurrence of this problem.

681 SAFETY ASSESSMENT OF *BETA*-HYDROXY-*BETA*-METHYLBUTYRATE.

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Beta-Hydroxy-Beta-methylbutyrate (HMB), also called 3-hydroxy-3-methylbutyrate, or beta-hydroxyisovalerate, is a metabolite of the amino acid leucine, and is purported to enhance muscle development in humans and animals through a decrease in the breakdown of protein. Despite the fact that HMB is a normal component of human metabolism and is also obtained from dietary sources, the safety of this compound was assessed given that relatively high doses are ingested to provide the desired effects.A series of studies have been performed which have examined the potential for adverse effects of HMB in male and female human volunteers at doses of up to 3,000 mg HMB (as the calcium salt)/day (equivalent to 43 mg Ca-HMB/kg body weight/day or 17 mg of HMB free base/kg body weight/day in a 70 kg human) for up to 7 weeks, and up to 5,000 mg Ca-HMB/kg/day for 1 week in pigs or 200 mg Ca-HMB/kg/day for 4 weeks in hamsters. No adverse effects have been observed, although there were some decreases in serum cholesterol levels noted in both humans and animals. The lack of reported adverse effects of HMB ingestion in animals or humans, coupled with the endogenous role of HMB, supports the safety of ingesting doses of up to 3,000 mg per day by non-pregnant adults. There is no available evidence to suggest any increased risk of adverse effects from use of HMB, although the effects of long-term supplementation with high doses of HMB have not been investigated.

682 DEVELOPMENT OF MODELS TO PREDICT THE ALLERGENIC POTENTIAL OF FOOD PROTEINS.

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Introduction of novel proteins into the human diet following GM strategies has led to concerns of allergenicity. Although cross-reactivity of novel protein with existing allergens can be demonstrated, there are no techniques to determine the capacity of novel proteins to induce sensitization. We developed three interlinked models to assess inherent allergenic potential of food pro-

teins; these are based on the response of the BN rat (a high IgE responder strain) to (a) i.p. or (b) p.o. administration of protein together with the specific adjuvant carrageenan (1 mg/ml i.p.), and (c) analysis for allergenic fragments following *in vitro* digestion by immunoblot using PCA-positive sera from day 28 i.p. and day 42 p.o. The quantity of protein required to induce a 50% response (ED50) was calculated. We found that a protein of low allergenic potential, such as bovine serum albumin (BSA), produced an ED50 level of 10 μg i.p., whereas proteins of higher allergenic potential such as bovine lactoferrin (LF) or ovalbumin (OA) exhibited significantly lower ED50s of 50 ng and 6 μg respectively. For oral exposure, ED75s of 5.0, 50 and 100 mg/kg were observed for OA, LF and BSA respectively. These differences were reflected in the persistence of IgE binding epitopes following *in vitro* digestion. We have used these dose-response curves to rank food proteins in terms of their inherent allergenic potential both i.p. and p.o. and we are now integrating this data into a risk assessment analysis for novel proteins. (Supported by MAFF UK.)

683 ANTIBACTERIAL ACTIVITY OF IMMOBILIZED ORGANOCLAY FILTRATION BEDS.

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Previous research has shown that phyllosilicate clays modified with cationic surfactants cetylpyridinium (CP), hexadecyltrimethylammonium (HDTMA), and cetyldimethylethylammonium (CDEA) were effective in reducing *Salmonella enteritidis* (*SE*) colony counts in bulk antibacterial assays. In order to increase the hydraulic conductivity of these materials for use in filtration beds, the organoclays were immobilized onto sand. Various chemical agents were tested for adhesive ability, water insolubility and inherent antimicrobial activity. From the promising candidates a polyvinyl adhesive was chosen as the primary adhesive agent. Bulk antibacterial assays performed with 10 mg of the immobilized organoclays (IOC) showed that the these materials retained their antibacterial activity after processing. Reductions in plate colony counts ranged from 16.5% for CP-exchanged sodium montomorillonite (MS:CP*SWy-2) to 83.9% for CDEA-exchanged low pH montmorillonite (MS:CDEA*LpHM). The sand matrix (MS) and immobilized activated charcoal (MS:AC) failed to produce any significant decreases. Pilot studies with columns containing 1.0 g of IOC showed that maximal filtration efficiency was obtained at a filtration rate of 1.0 ml/min. Initial column studies with *SE* suspensions with concentrations of 3.0×10^8 CFU/ml produced reductions in colony plate counts varying from 28.5% for CP-exchanged calcium montmorillonite (MS:CP*STx-1) to 59.5% for MS:CDEA*LpHM. MS and MS:AC yielded much lower reductions (7.6% and 10.4%, respectively). Studies with *Escherichia coli* suspensions (3.0×10^7 CFU/ml) exhibited reductions varying from 59.3% for MS:CP*STx-1 to 99.9% for MS:CDEA*LpHM. These intial results suggest that these immobilized organoclays would be effective in reducting microbial numbers in wastewater. (Supported by NIH P42 ES04917, USDA 9703230, and TAES H6215.)

684 A RANDOM WALK MODEL OF STRATUM CORNEUM PENETRATION.

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Penetration of chemicals through skin is governed by its specific morphological structure and biochemical composition. In order to account for the complex "brick-and-mortar" structure of the stratum corneum, a mathematical model has been devised that explicitly incorporates this non-uniform structure. Diffusion is modeled as a random walk of particles through an asymmetrical 2-phase system of corneocytes bounded by continuous lipid bilayers. The model accounts for partitioning between the phases and relative diffusivity within both phases. Model results permit the calculation of effective diffusivity (D^*), effective path length (l^*), and lag time (τ). Thus results can be compared with experimentally determined permeation coefficients and lag times. Non-steady state permeation vs. time of methyl parathion was modeled, and the result corresponds closely (R^2=0.98) with measured data. Model results demonstrate the strong dependence of D^*, l^* and τ on both partitioning and relative diffusivity. In its current implementation, lipid-corneocyte partitioning and diffusivities within the phases are model input parameters. In future refinements, we will incorporate quantitative structure-activity relationships to model partitioning and diffusivity. This will permit individual chemicals to be used explicitly as inputs. Phenomena such as tissue adsorption and cutaneous metabolism can also be incorporated. Because it accounts for biophysical phenomena at the elementary molecular level, the model is comprehensive yet fairly simple.

685 PHOTOTOXICOLOGY: PHOTOCARCINOGENESIS HISTORICAL CONTROL DATA AS A KEY INTERPRETIVE ELEMENT.

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Studies have been conducted over the past two decades to assess the ability of compounds to modify skin tumor development elicited by exposure to simulated sunlight in Crl:SKH1-*hr*BR hairless mice (photocarcinogenesis). Low and high ultraviolet radiation dose calibration groups (i.e., mice exposed to 600 or 1200 Robertson Berger Units/week) have been included in these studies to permit determination of test article or vehicle effects. The skin tumor endpoints evaluated have been prevalence (i.e., the proportion of mice in a group exhibiting one or more qualifying tumors, as a function of time, and adjusted for the effects of competing mortality), median onset (i.e., the time at which one-half of the members of the groups have acquired one or more qualifying tumors) and yield (i.e., the number of tumors present, divided by the number of surviving mice). Examination of the prevalence and median data for seven studies conducted in the past four years revealed good interstudy reproducibility. Skin tumors tended to occur slightly earlier in female mice, as compared with male mice. Some topically administered vehicle products enhanced photocarcinogenesis, but oral (gavage) vehicles did not. Examination of the tumor yield data confirmed the gender difference and vehicle effect, but as compared with the prevalence and median data, more variability was seen with this endpoint. The inclusion of these calibration groups in photocarcinogenesis studies has provided useful information on the homogeneity of skin tumor responses in this test system and can provide valuable assistance with respect to assessment of product effects and detection of sources of variability.

686 INVESTIGATION OF SKIN PHOTOTOXICITY POTENTIAL USING AN *IN VITRO* HUMAN EPIDERMIS MODEL (SKINETHIC).

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The present study evaluated the suitability of the human reconstituted epidermis model SkinEthic® for the assessment of the *in vitro* phototoxic potential of topical products. The phototoxic compounds 8-Methoxypsoralen, Tetracycline, Promethazine and 6-methylcoumarine (weak phototoxicity), and the non-phototoxic compound Sodium lauryl sulphate were tested. The response of the tissue to the application of the test articles in the presence and absence of UV light, was analyzed in terms of viability (LDH release), pro-inflammatory activity (IL-8 and IL-1-alpha release and mRNA expression) and morphology (histopathological analysis). Two different prediction models were used: the Photo-Irritancy Factor (PIF) and the Mean Photo Effect (MPE). All the non-phototoxic and phototoxic products tested were correctly classified. Due to poor skin penetration, Tetracycline was identified as phototoxic only after application in the culture medium, and not via the topical route. These results indicate that the method is appropriate for the investigation of the phototoxic potential of products intended for topical administration. A simplified version based on the determination of LDH release is recommended for high throughput screening purposes (simple differentiation between phototoxic and non-phototoxic products). On the other hand, the complete version of the protocol, including all parameters, seems to be necessary for the evaluation of weak phototoxic products.

687 PREDICTIVE VALUE OF A HUMAN SKIN EQUIVALENT (APLIGRAF) FOR THE EVALUATION OF SKIN IRRITATION POTENTIAL.

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The present study was designed to investigate the response of the human skin equivalent Apligraf *in vitro* to the application of irritant substances, and its predictivity as a screening tool for skin irritant potential in humans. Sodium Lauryl Sulfate, Calcipotriol and Trans-Retinoic acid were applied to Apligraf *in vitro* for 24 hours. Cell viability (lactate dehydrogenase leakage, MTT mitochondrial reduction), release and mRNA expression of the pro-inflammatory cytokines IL-1-α and IL-8, and morphological changes were assessed. The same products were applied to 30 healthy volunteers in a double-blind, randomized, vehicle-controlled within-subject study. The skin reactions after

repeated 24-h applications over 3 weeks under Finn chamber patches were monitored by visual scoring and biophysical methods (TEWL, chromametry and blood flow). The sensitivity, specificity and predictivity of Apligraf for assessing skin irritation were evaluated. Sodium Lauryl Sulphate was cytotoxic to Apligraf, and increased the release and expression of cytokines at low (0.2%, 0.4%), but not at high (0.8%, 1%) concentrations. It induced severe irritancy *in vivo*. Trans-Retinoic acid increased the expression and release of cytokines with no detectable cytotoxicity, and showed moderate irritancy in humans. Calcipotriol did not affect cell viability, but it increased the production of cytokines, and was mild to moderate for healthy volunteers. Apligraf, used for *in vitro* testing showed a good predictive value for the substances under investigation. In conclusion, the irritation potential of the tested products could be predicted with Apligraf, by monitoring cytotoxicity, the profile of proinflammatory cytokines and morphological changes.

688 HIGHLY DIFFERENTIATED *IN VITRO* SKIN MODEL FOR HIGH THROUGHPUT SCREENING OF TOPICAL THERAPEUTICS.

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EpiDerm™ is an *in vitro* organotypic human skin model cultured at the air/liquid interface to produce a three-dimensional tissue with a stratum corneum possessing a barrier function similar to native epidermis. The model is suitable for topical application of creams or lotions, and has found use in applications including cytotoxicity, irritation, and/or efficacy screening of topical pharmaceutical or cosmetic products and ingredients. EpiDerm is presently produced on cell culture inserts, which can be handled and manipulated individually. The current abstract describes two experimental formats in which the tissues are cultured on one-piece 24-well or 96-well plates suitable for high-throughput applications. Histological evaluations show the experimental formats produce tissues which are indistinguishable from the standard format. To demonstrate the utility of the model, TNF-α secretion in response to topically applied phorbol ester (PMA) was measured by ELISA. PMA is a powerful tumor promoter which acts primarily via interaction with protein kinase C and has been shown to upregulate TNF-α expression. PMA treatment resulted in a 1.7-2.4 fold increase in TNF-α secretion but had no effect on tissue viability, as indicated by the MTT assay. In addition, high quality total RNA (absorbance ratio at 260/280 nm were 1.8 - 2.1; agarose gel electrophoresis ribosomal RNA bands were distinct and well defined) was extracted and purified from the tissues utilizing a procedure based on GSCN lysis and immobilization on glass-fiber filters. RNA yields averaged 46.2 $\mu g/cm^2$ tissue. These data indicate that the new experimental EpiDerm formats may find utility in high-throughput screening applications such as in monitoring mRNA levels or the effects of PKC inhibitors and/or similar pharmaceutically important cellular targets.

689 A CLINICAL SKIN SAMPLING APPROACH TO ASSESS SENSORY SKIN IRRITATION.

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We have developed a noninvasive Sebutape sampling procedure to adsorb and quantify molecular mediators of skin inflammation. This procedure has been used to distinguish inflamed from normal skin or mucosa, where the inflammation is caused by chemical irritants or existing dermatoses such as diaper dermatitis or gingivitis. Recently, we have applied this methodology to attempt to define quantitative markers for neurosensory skin irritation. Sensory skin irritation refers to the myriad of symptomatic complaints (including itch, sting, and burn) frequently associated with inflammatory skin conditions or skin intolerance to various products. Often, sensory skin irritation occurs in the absence of visual skin reactions. The use of our Sebutape procedure was introduced to provide potential means for quantitative assessment of these responses. Study subjects were first introduced to a clinical assessment scale (the labeled magnitude scale or LMS) used to evaluate chemosensory skin reactions to chemical agents. They were then treated with escalating concentrations of chemical stimuli (lactic acid, capsaicin) on the face or arm. After each treatment, they rated the intensity of the reaction on the LMS scale. Applications continued until a moderate degree of intensity was noted. At different times, Sebutape samples were collected from the treatment sites and frozen until analyzed. The clinical results showed a clear dose response pattern in the LMS to the chemical treatments, with most subjects achieving a moderate intensity of response. The Sebutape samples were extracted in buffer and analyzed (via chemical or immunoassay methods) for recoveries of various neuro and inflammatory mediators. Precise correlations between incidence and/or severity of sensory skin irritation must await large scale subject evaluations; however, clear indications of elevation or decline in specific mediator recoveries (e.g., interleukin-1α, interleukin-1 receptor antagonist, IL-1ra/IL-1α ratios, interleukin-8, nitric oxide) have been observed and continue to drive this research effort.

690 COMPARISON OF CHEMICAL-INDUCED SKIN IRRITATION RESPONSES BETWEEN CAUCASIAN AND ASIAN POPULATIONS.

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There is considerable speculation that certain human populations (particulary ethnic subpopulations) are more prone to developing adverse skin reactions than others. In particular, a heightened sensitivity among Asian subjects (vs. Caucasians) has been suggested. This has implications for the procedures used for skin safety assessment of new ingredients and products, especially those marketed globally. However, there is little published data to support the speculation (M.K. Robinson, Contact Dermatitis 41:65-79, 1999). The focus of this investigation was to directly compare skin irritation responses between Caucasian subjects and different subpopulations of Asian subjects (Chinese, Japanese) to determine if significant differences in response patterns would emerge. The studies used 2 different skin irritation test methods concurrently in the same subjects. The first was a cumulative response (14 day repeated occluded patch exposure) to several dilute concentrations of the anionic surfactant, sodium dodecyl sulfate (SDS). The second was an acute response (up to 4 hr occluded patch exposures) to several materials (20% SDS, 10% acetic acid, 100% octanoic acid, 100% decanol, and water) with diverse skin irritation potentials. In an initial study among Caucasian and Japanese subjects, the Japanese subjects responded faster than the Caucasian subjects in both the acute and cumulative irritation tests. A repeat of this exact study protocol was then conducted among Caucasian, Japanese, and Chinese subjects. In this second study, no differences were seen in the acute or cumulative irritation responses between the Japanese and Caucasian subjects. Minimal differences were seen between the Chinese subjects and the other populations. These divergent results underscore the difficulty in ascribing true population differences in skin reactivity based upon studies in limited subject populations. These findings may be indicative of the wide variation in skin responsiveness across human subjects in general.

691 HUMAN ACUTE SKIN IRRITATION POTENTIAL, *IN VIVO*: GLUCOWATCH® BIOGRAPHER.

D. R. Wilson and S. J. Fermi. *Cygnus, Inc., Redwood City, CA.* Sponsor: W. G. Reifenrath.

The GlucoWatch® biographer, developed by Cygnus, Inc., is a frequent, automatic, and non-invasive glucose monitor intended to provide diabetics with the information needed to control their glucose levels. Sponsored by Cygnus, Hill Top Research, Inc. (Miamiville, OH) performed a clinical trial evaluating the acute irritation potential of the GlucoWatch® biographer in 51 diabetic and 52 non-diabetic subjects following a single 15hr wear period. The irritation recovery process was also evaluated. Two test systems delivering iontophoretic current (up to $0.32 mA/cm^2$ for 6min during each 20min glucose measurement cycle) were placed on each forearm. Following removal, the skin sites were evaluated and scored on Day 1 (15min post removal) and on Days 2, 3, 4, 6, and 8 utilizing modified Draize erythema and edema scales (0 to 4). There were no serious or unexpected adverse events, and no subjects were discontinued from the study. Day 2 erythema scores (most sensitive evaluation uninfluenced by transient skin responses) for the 198 active devices applied to diabetics revealed these levels of irritation at glucose collection sites: 16% none, 72% mild, & 12% moderate; the 204 applied to non-diabetics produced: 14% none, 78% mild, & 8% moderate. Irritation responses began to resolve on Day 2; most resolved by Day 8; all were resolved by Day 15. No clinically meaningful differences existed in irritation rates between diabetic and non-diabetic subjects. Analyzing age and gender variables within the two groups, no clinically meaningful differences were observed.

692 A NOVEL USE OF AN *IN VITRO* PERCUTANEOUS ABSORPTION PROCEDURE TO ASSESS ABSORPTION POTENTIAL OF HUMAN VAGINAL TISSUE.

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Vaginal epithelium is permeable to a wide variety of compounds. Unfortunately, quantitative data on the extent of absorption are often lacking. The *in vitro* percutaneous absorption procedure is valuable in developmental stages of vaginal drug delivery systems. It allows for direct risk assessment and eliminates ethical and safety concerns associated with human *in vivo* testing. By using an *in vitro* technique, the absorption potential of polydimethylsiloxane (PDMS) was assessed through human vaginal tissue. In vitro percutaneous absorption experiments (96 hr) were performed with two PDMS samples (350 cSt and 10 cSt). Infinite doses (10 mg/cm2) were applied to the donor side of split thickness human abdominal skin sections (reference standard) and full-thickness human vaginal tissue mounted in Franz *in vitro* diffusion cells. In order to facilitate the measurement of percutaneous absorption, doses were spiked with the respective 14C-PDMS (350 cSt or 10 cSt) prior to the study. Based on 14C-PDMS, the dermal flux rate for the infinite dose of 350 cSt PDMS was 0.31 and 1.98 ng/cm2/hr for the abdominal skin and vaginal tissue respectively. For the 10 cSt PDMS, flux rates were 0.52 and 5.52 ng/cm2/hr for the abdominal skin and vaginal tissue respectively. Although vaginal absorption of 350 and 10 cSt PDMS is 6 and 10 times greater respectively, than through abdominal tissue, less than 0.01% of the PDMS was absorbed through the dermal barrier regardless of tissue or viscosity of PDMS. This novel *in vitro* approach allows for direct determination of vaginal bioavailability of drug products or vehicles either intended or not intended to be absorbed into the blood stream and is a promising tool for vaginal therapeutic drug development.

693 SKIN PENETRATION OF ORGANIC COMPOUNDS FROM SOILS.

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Nine C-14 labeled organic compounds were mixed with soils or acetone and applied to the epidermal surface of viable excised pig skin mounted in evaporation/penetration chambers. Skin penetration of the compounds was measured by assay of radiolabel in the dermis and receptor fluid. The compounds tested were 2,4-dinitrotoluene (2,4-DNT), 2,6-DNT, 2-amino-4,6-dinitrotoluene (2-ADNT), 4-ADNT, 2,4-diamino-6-nitrotoluene (2,4-DANT), 2,6-DANT, trinitrobenzene (TNB), thiodiglycol (TDG), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). A low carbon soil (Yolo) and a high carbon soil (Tinker) were used. Applications were made at a chemical dose of 10 µg/cm2 and a soil dose of 10 mg/cm2. Selected samples of dermis and receptor fluid were analyzed for radiochemical identity; non-parent compounds were detected for RDX, TDG, TNB and 2,4-DANT. Mean penetration from low carbon soil was generally higher than for high carbon soil; however, the effect was significant only for 2,4-DNT and 2,6-DNT (ANOVA, Newman-Keuls test, $p = 0.05$). Mean penetration from acetone solution was always higher than from soils; however, the effect was not significant ($p = 0.05$) for all combinations of compound and soil. These results demonstrated that incorporation of a compound into soil or incorporation into soil with a higher carbon content did not necessarily result in a significant reduction of skin absorption.

694 THE EFFECT OF SURFACE CONTACT ON DERMAL ABSORPTION OF PESTICIDES FROM HOUSE DUST.

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The effect of surface contact on the dermal absorption of pesticides was examined by comparing uptake in human skin *in vitro* using two application methods. House dust (<150 µm, 40% organic carbon) spiked with either 14C-pentachlorophenol (PCP, 260 ng/g) or 14C-2,4-dichlorophenoxyacetic acid (2,4-D, 435 ng/g) was deposited on or pressed into the skin under mild pressure (0.5 psi) in flow-through diffusion cells for 8 hrs. Applied dust loads for the two application methods ranged from 0.12 - 3.0 mg/cm2 skin. PCP uptakes, normalized for dust load, with the press and deposition methods were 1.04±0.88 and 0.65±0.47 fmol 14C/cm2, respectively. 2,4-D uptakes with the press and deposition methods were 0.31±0.49 and 0.43±0.67 fmol 14C/cm2, respectively. Normalized uptake did not vary with the applied dust load or the percent transferred to the skin for either chemical. When normalized by the applicator dust load, uptake increased markedly at applied dust loads below 0.2 mg/cm2, suggesting enhanced skin-dust contact at lower applicator loads. (Work performed under the auspices of the U.S. Environmental Protection Agency, IAG #DW89937390-01-0 and under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.)

695 EFFECT OF SOIL LOADING AND SOIL SEQUESTRATION ON DERMAL BIOAVAILABILITY OF POLYNUCLEAR AROMATIC COMPOUNDS.

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Literature reports show that sorption of polynuclear aromatic compounds (PAC) on soil can significantly impede their penetration through the skin. Studies conducted in our laboratory with PAC-contaminated soils from industrial sites show reductions in PAC dermal flux of 1-3 orders of magnitude compared to the neat PAC-containing contaminant. The dermal flux values are used to calculate site-specific dermally absorbed dose (DAD) values that are essential for estimating risk associated with human exposure. Two criticisms of the *in vitro* procedure used in our earlier studies are 1) the "infinite dose" soil loading used (10 mg/cm^2) is well above what some consider the actual soil adherence factor (AF) of 0.2 mg/cm^2 and, 2) the procedure does not account for the effect of contaminant aging or soil sequestration phenomena. To address these concerns, *in vitro* experiments were carried out using human skin sections and PAC-spiked soil at 10, 5, 2.5 and 1 mg/cm^2 to evaluate the effect of soil loading on dermal flux and percent of dose absorbed (PADA). Additionally, dermal experiments were conducted with the freshly spiked (PAC) soil (day 1) and with aliquots of the same soil following 45 and 110 days of laboratory aging. Dermal flux of benzo[a]pyrene (BaP) was measured using liquid scintillation counting and a newly developed HPLC-fluorescence assay for monitoring aged PAC in soils. Results from the soil loading study show that flux is not affected by loading above "monolayer" (~3 mg/cm^2) coverage and that flux decreases in proportion to soil loading below monolayer coverage. Further, the results show that PADA decreases proportionately with increased soil loading above monolayer and remains constant at sub-monolayer coverage. Results from the soil sequestration study show a 50% reduction in BaP dermal bioavailability following 110 days of aging.

696 EXHALED BREATH ANALYSIS AND PBPK MODELING OF THE DERMAL ABSORPTION OF TRICHLOROETHYLENE IN RATS.

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Due to the large surface area of the skin, the percutaneous absorption of chemicals from contaminated water or soil is potentially a problem. The amount of chemical absorbed through the skin is dependent upon the permeability coefficient (kp), exposure media, concentration gradient, surface area exposed, and the length of exposure. At steady state, dermal absorption is calculated using Fick's law. Physiologically based pharmacokinetic (PBPK) modeling was used to describe the kp of trichloroethylene (TCE) under non-steady state conditions based on exhaled breath data acquired from rats following dermal exposure to TCE in water or soil matrices. A patch (occluded or non-occluded) was applied to the clipper-shaved back of F-344 rats. Rats were placed in off-gassing chambers and the chamber air TCE concentration was quantitated for 4-5 hr using an ion trap mass spectrometer. Patch components and dosing solutions were analyzed to determine TCE concentrations before and after the exposures. Target water TCE concentrations were 0.05 and 0.15% (w/v). Target soil TCE concentrations were 0.5, 1.5, and 3%. The rate of loss of TCE (Kloss) from the non-occluded patch and kp were estimated for each individual animal by optimization of the least-squares fit of the model to the patch component and exhaled breath data using Simusolv™ (Mitchell & Gathier). The average kp for all water exposures was independent of concentration, at 0.28 ± 0.018 cm/hr. The uptake of TCE from a soil matrix was lower than from a water matrix, with the occluded soil exosures, the kp was estimated at 0.14 + 0.012 cm/hr and the non-occluded soil exposures kp was 0.053 ± 0.0041. The rate of loss from non-occluded systems was 0.74 ± 0.068 hr-1. The assessment of matrix effects on apparent kp will aid in addressing dermal risk assessments. (Supported by US DOE grant No. DE-FG07-97ER62509.)

697 HUMAN DERMAL ABSORPTION OF TRICHLOROETHYLENE FROM SOIL AND WATER.

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The study objective was to determine trichloroethylene (TCE) human dermal absorption in real time for risk assessment. The amount of chemical absorbed through the skin is dependent upon the permeability coefficient (kp), exposure media, concentration gradient, surface area exposed, and the length of exposure. At steady-state, dermal absorption can be calculated using Fick's law. For non steady-state conditions, physiologically based pharmacokinetic (PBPK) modeling is well-suited to determining kp. PBPK modeling was used to assess the percutaneous absorption parameters for TCE based on exhaled breath concentrations in human volunteers. Volunteers were exposed to 0.1% TCE in water or 0.5% TCE in soil either by placing a hand in 4 kg of soil or water or to 80 g of soil or water in patches placed on their forearm. Subjects were provided breathing air via a facemask to eliminate inhalation exposure. The subject's exhaled breath was analyzed using an ion trap mass spectrometer, which can quantitate TCE in the breath stream in the 1-5 ppb range. The pre- and post-exposure concentrations of TCE in the exposure media were analyzed to track changing exposure conditions. The percentage absorbed and kp were estimated for each individual subject by optimization of the least-squares fit of the PBPK model to the media concentrations and the exhaled breath data using Simusolv™ (Mitchell & Gathier). In the occluded water and soil patch studies, 4.0±0.20 and 0.90±0.32% of the total available TCE was absorbed over 8 hr, respectively. The kp for water exposures was 0.019±0.001 cm/hr for the patch system and 0.016±0.003 cm/hr for the hand immersion studies. The kp optimized from soil exposures was less than half that for dermal exposures to TCE in water. Determination of kp specific for exposure matrices will aid in the dermal risk assessments for real-life scenarios. (Supported by US DOE grant No. DE-FG07-97ER62509.)

698 PHARMACOKINETIC MODELING OF DERMAL EXPOSURE TO BROMODICHLOROMETHANE (BDCM) IN HUMAN VOLUNTEERS.

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Bromodichloromethane (BDCM) is a disinfection by-product commonly found in many U.S. finished water sources after chlorination of water that contains bromide and organic precursors. Routes of significant BDCM exposure include oral ingestion and absorption through the skin during showering and bathing activities. Epidemiological studies suggest a correlation between exposures to chlorinated drinking water and cancers of the urinary bladder and lower intestinal tracts. High-dose BDCM exposures produce tumors in the liver, kidneys and large intestines of rodents. The purpose of this research was to adapt a previously published physiologically based pharmacokinetic (PBPK) model for rats to predict the pharmacokinetics of BDCM in humans. Model compartments included alveolar space, venous and arterial blood, fat, liver, skin, and rapidly and slowly perfused tissues. A dermal absorption component was added to the model, that considered skin surface area exposed and thickness; where the skin mass transfer coefficient was assumed to be similar to chloroform, another trihalomethane. Tissue/blood partition coefficients were assumed to be constant across species. Average human organ volumes and blood flow rates to tissues were obtained from the literature. Metabolic parameters were allometrically scaled from rodents to humans. Healthy male subjects were exposed arm-only to Chapel Hill, NC drinking water that contained BDCM and other constituents. Blood was collected prior to each drinking water exposure and for predetermined time points until 24 hours following initiation of exposure for quantitation of BDCM. The scaled BDCM PBPK model simulations were in good agreement with experimental blood BDCM concentration-time data. Sensitive model parameters included skin permeability, hepatic metabolism, and blood flow to the liver and fat. The PBPK model will be valuable in evaluating target tissue BDCM dosimetry in humans after dermal exposure, and aid in quantitating the carcinogenic risk of BDCM in humans. (This abstract does not reflect official U.S. EPA policy.)

699 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR DERMAL ABSORPTION OF METHYL *TERTIARY*-BUTYL ETHER.

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Dermal exposure to the oxygenated fuel additive methyl tertiary-butyl ether (MTBE) occurs from contact with contaminated groundwater sources during daily activities such as bathing and showering. The purpose of this research was to develop a physiologically based pharmacokinetic model for dermal absorption of MTBE. Compartments in the model included alveolar space, arterial and venous blood, brain, fat, gastrointestinal tract, kidney, liver, rapidly perfused tissues, skin, and slowly perfused tissues. Metabolism of MTBE to tertiary butyl alcohol (TBA) was assumed to occur only in the liver, and elimination was assumed to occur via exhalation of MTBE and TBA and urinary elimination of TBA. Absorption of MTBE through skin was described by well-stirred compartments for exposure media, skin, and blood that perfused the skin, with equilibrium between the media and skin compartments and the skin and blood compartments. Predictions from the model were compared with data on blood and exhaled breath concentrations of MTBE and TBA in male subjects whose arms were submerged in water containing MTBE for one hour. The model overpredicted the absorption of MTBE from water into blood, shown by predictions of a more rapid increase in blood concentrations and higher peak concentrations of MTBE than experimentally observed values. The parameters to which the peak blood concentration of MTBE was most sensitive included permeability, thickness, and surface area of skin; blood flow to skin; and alveolar ventilation. In addition the predicted blood concentrations versus time indicated the compartmental model for dermal absorption is not appropriate for MTBE, and a distributed model may more accurately predict dermal absorption of MTBE into the blood. Ultimately the dermal model will be used to predict the relative contributions of various routes of exposure to total body burden of MTBE and TBA in humans exposed environmentally to MTBE. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

700 *IN VIVO* PERCUTANEOUS ABSORPTION OF ^{14}C-OCTAMETHYLCYCLOTETRASILOXANE IN FISCHER 344 RATS.

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The percutaneous absorption of neat ^{14}C-octamethylcyclotetrasiloxane (D_4) was evaluated in female Fischer 344 rats when applied topically at 10, 5 and 2 mg/cm^2 of skin. Each dose level was represented by twelve animals (4 rats/group) that were exposed for 1, 6, or 24 hr and two 24 hr controls. An additional 24 hr exposure group (N=4) was added to evaluate disposition of the residual D_4 following a soap and water wash. All rats were exposed in a semi-occluded manner using an aluminum skin depot with charcoal basket for collection of volatilized test material. During exposure, rats were housed in Roth-style metabolism cages to enable collection of urine, feces, and expired air. Rats in the wash group were removed from the cages after 24 hr of exposure, dose sites were washed, charcoal baskets were replaced, and the animals were returned to the cages for continued collection (168 hr) of excreta and expired air. At the termination of exposure or at 168 hr post exposure, blood was collected via cardiac puncture, the charcoal baskets were removed and extracted, skin was washed, tape stripped, excised and solubilized. Remaining carcasses were also solubilized. Radioactivity content in each sample was measured by liquid scintillation counting (LSC). In addition, six jugular vein cannulated females were exposed to the highest dose and blood samples were collected up to 10 hr after exposure. The samples were extracted and analyzed by LSC and GC/MS. Concentrations of D_4 in all blood samples were below limits of quantification. The percent dose absorbed was determined as the amount of radioactivity in expired air, carcasses, excreta, skin and cage rinses. The percent of absorption did not differ across the groups compared by dose levels ($p>0.05$). However, D_4 absorption in the wash group after 168 hr (0.350±0.009%, 0.494±0.015% and 0.513±0.084% dose absorbed at high, medium and low dose, respectively) was significantly lower ($p<0.01$) than that seen after 24 hr of exposure (0.609±0.086%, 0.599±0.056% and 0.756±0.081%, respectively). The percent of applied dose recovered from all the animals was >91%. Funded in part by the Silicones Environmental, Health and Safety Council.

701 *IN VITRO* ABSORPTION OF DECAMETHYLCYCLOPENTASILOXANE (D_5) IN HUMAN SKIN: A COMPARISON TO OCTAMETHYLCYCLOTETRASILOXANE (D_4).

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The absorption of ^{14}C-decamethylcyclopentasiloxane (D_5) was evaluated when applied to human skin using a flow-through diffusion cell. Human epidermis was prepared from intact abdominal skin. Skin discs from six donors were mounted in replicate in the flow-through chambers. A physiological receptor fluid was pumped underneath the skin samples for 24 hr and collected in a fraction collector. Barrier integrity of the skin was initially screened with ^3H-H_2O. Skin samples were evaluated on two separate days. On day one, skin samples from each of the three donors were dosed with neat D_5 and three were dosed with a generic anti-perspirant formulation containing D_5. On day two, new skin samples from the same six donors were dosed with the opposite test material. Immediately after dosing, charcoal baskets were placed above the skin and secured into a custom designed cap to capture volatilized material. At the end of 24 hr, the charcoal baskets were removed and extracted, skin was washed and solubilized, and the receptor fluid was collected. The radioactivity content in each sample was measured by liquid scintillation counting. The percent dose absorbed was determined as the amount of radioactivity in the receptor fluid combined with the amount remained in the skin after washing. This was compared with another siloxane octamethylcyclotetrasiloxane (D_4) which was evaluated under a similar protocol and reported previously. Data analysis indicated that 0.04±0.01% and 0.50±0.07% of the applied dose of neat D_5 and D_4, respectively was absorbed at the end of the assay and that this was not significantly different from that seen with formulated D_5 or D_4 (0.02±0.01% and 0.49±0.18%, respectively). The percent of applied dose recovered from all analyzed samples for neat D_5 and D_4 was >91%, and for D_5 and D_4 formulated in an antiperspirant was >98%. (Funded in part by the Silicones Environmental, Health and Safety Council.)

702 PARTICLE PENETRATION OF THE SKIN AS A ROUTE OF SENSITIZATION IN OCCUPATIONAL LUNG DISEASE.

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Chronic Beryllium Disease (CBD) is an occupationally-acquired lung disease that begins as a cell-mediated immune response to beryllium particulates that, over time, results in development of non-caseating granulomas. During the last ten years, the beryllium industry has made major improvements in respiratory protection and engineering control technology design, however, the rate of disease has not declined. We hypothesized that dermal exposure to beryllium particulates, coupled with joint motion, as at the wrist, would provide an alternative route for sensitization to beryllium. To test this hypothesis, 400 - 600 micron thick sections of human skin (n = 8) were applied to a flexing device with surgical glue. The integrity of the stratum corneum was verified with the NOVA DPM, and 100 µl of 0.5, 1 or 4 micron FITC-conjugated dextran beads were applied to the surface of the skin. The skin was subjected to repeated 30 degree flexure for 0, 15, 30 or 60 minutes. Control tissues were not flexed. Following treatment, tissues were fixed, cut in 20 micron thick sections, and mounted on slides in cross-section. Penetration of beads into the skin was evaluated at 1 micron intervals by laser scanning confocal microscopy. To verify that bead penetration occurred through the stratum corneum, we evaluated data between 5 and 15 microns only. We documented 0.5 and 1 micron bead penetration into the epidermis and the dermis in flexed samples, but not non-flexed tissues. Although not quantitative, only a very small percentage of beads was observed in the skin, and there was no clear time-dependence associated with penetration. Four micron beads did not penetrate the skin These data suggest that particle penetration of the skin is a potential route of exposure in dusty work environments.

703 PERCUTANEOUS ABSORPTION AND METABOLISM OF 7-(2H-NAPHTHO[1,2-D] TRIAZOL-2-YL)-3-PHENYLCOUMARIN IN HUMAN SKIN.

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The fluorescent brightening agent, 7-(2H-naphtho[1,2-d]triazol-2-yl)-3-phenylcoumarin (7NTPC), is a compound of moderately high production level (1 million pounds per year) that is used in several different industrial applications. Little toxicity data exist for 7NTPC and given the potential for occupational exposure, skin absorption and metabolism studies of 7NTPC were initiated in human skin. Confocal laser cytometry was used to visualize 7NTPC absorption across a section of human skin. Absorption was measured over 24 hr by using flow-through diffusion cells. The percent of total (receptor fluid + skin) applied dose absorbed was 16.2±2.08 for 10µg/cm²; and 5.4±0.40 for 50µg/cm². 7NTPC remaining in the skin after 24 hr was 14.7±1.67 percent of the applied dose absorbed for 10µg/cm²; and 5.1±0.41 for 50µg/cm². The upper stratum corneum (SC) was stripped with tape at the end of the study. Skin content was not reduced and indicates that 7NTPC remaining in skin was not on or near the skin surface. 7NTPC metabolism in skin was examined by HPLC, but no metabolites were found. Confocal laser cytometry revealed that fluorescence due to 7NTPC was present across the skin section in the SC, epidermis, and dermis. An intense fluorescence (a high concentration of 7NTPC) was associated with a section of a hair follicle present in the dermis. The majority of absorbed 7NTPC remains in the skin after 24 hr. Fluorescence studies show that 7NTPC diffuses from the SC down to the dermis to be absorbed. 7NTPC is a lipophilic chemical that probably forms a skin reservoir resulting in slow diffusion through skin. (Supported by Interagency Agreement No. Y1-ES-8056-01with NIEHS.)

704 PERCUTANEOUS ABSORPTION AND METABOLISM OF THE SELF-TANNING AGENT DIHYROXYACETONE IN HUMAN SKIN.

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The self-tanning agent which colors skin, dihydroxyacetone (DHA), is being increasingly used by the public because of the growing awareness about the dangers of sun exposure. Due to a lack of DHA absorption studies, we initiated skin absorption and metabolism studies of DHA in viable human skin. Absorption was measured over 24 hr by using flow-through diffusion cells. The percent of total (receptor fluid + skin) applied dose absorbed was 23.9±10.6 for 2.5% DHA in water; 21.7±9.9 for 5% DHA in water; and 22.3±5.7 for 5% DHA in an emulsion. DHA diffusing through the skin and into the receptor fluid was approximately 0.5% of the applied dose for both the water and emulsion dosing vehicles. The absorption of DHA into the receptor fluid was relatively slow with about 0.1% of the applied dose absorbed during each 6-hr collection fraction. Stratum corneum (SC) stripped with tape at the end of the study revealed that approximately 5% of the applied dose was found in each of the first five and the second five tape strips. No matter whether DHA was applied to skin in water or the emulsion, approximately 12% of the applied DHA dose was found in the viable epidermis and dermis. DHA metabolism in skin was examined by HPLC, but no metabolites were found. The majority of absorbed DHA remains in the skin after 24 hr. Preliminary covalent binding studies indicate that about 5% of the radioactivity found in the skin after 24 hr is covalently bound to protein. We conclude that even though DHA absorption is over 20%, little of the DHA passes through skin. (Supported by Interagency Agreement No. Y1-ES-8056-01 with NIEHS.)

705 COMPARATIVE *IN VITRO* PERCUTANEOUS ABSORPTION OF NONYLPHENOL AND NONYLPHENOL ETHOXYLATES (NPE-4 AND NPE-9) THROUGH HUMAN, PORCINE AND RAT SKIN.

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The purpose of this study was to assess the percutaneous absorption of 14C ring-labeled nonylphenol (NP) and the nonylphenol ethoxylates NPE-4 and NPE-9 in human, porcine and rat skin. In vitro studies were conducted for 8 hr in flow-through diffusion cells using topical solutions of 0.1, 1.0 and 10% in PEG-400 or 1% in water (NPE-9 only). NP absorption was assessed as a 1% solution in PEG-400. Perfusate fluxes over time and skin deposition at the end of perfusion was assessed. Absorption of NPE-4, NPE-9 and NP was similar across all species at less than 1% of the applied dose over 8 hr. Penetration (absorption + skin deposition) was generally below 5% of applied dose, the majority located in the stratum corneum. In all species and for both NPEs, the fraction of dose absorbed was highest for the lowest applied dose. Absorption expressed as actual mass absorbed over 8 hr was similar (approximately 0.3 mg/cm2) across all concentrations. Penetration, but not absorption, was

greater from a water vehicle compared to PEG-400, particularly in rat skin. These studies suggest that NP, NPE-4 and NPE-9 were minimally absorbed across skin from all three species. (Supported by the Alkylphenols and Ethoxylates Research Council.)

706 MIXTURE COMPONENT EFFECTS ON THE PERCUTANEOUS ABSORPTION OF TCB, PCB, AND PCP.

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Interactions between chemicals in a mixture and interactions of mixture components with the skin, can significantly alter the rate and extent of percutaneous absorption, as well as the cutaneous diposition of a xenobiotic. The predictive ability of dermal absorption models, and consequently, the dermal risk assessment process would be greatly improved through the elucidation and characterization of these interactions. As a first step, the effects of several generalized mixture components on the percutaneous absorption of 3,3',4,4',5-pentachloro biphenyl(PCB), 3,3',4,4'-tetrachloro biphenyl (3,3',4,4'-TCB), and pentachlorophenol (PCP) were examined using isolated perfused porcine skin flap (IPPSF) and porcine skin flow through (PSFT) diffusion cell systems. Mixtures containing combinations of the surfactant sodium lauryl sulfate (SLS), the vasodilator methyl nicotinate (MNA), ethanol, and water were studied. With all mixtures studied, PCB, and TCB absorption was negligible (~0.1% of the applied dose) as quantified using both radiolabel and an HPLC method. In contrast, the absorption of PCP occurred to a much greater extent (~14% of the applied dose), and showed significant mixture effects. Not only was the magnitude of PCP absorption altered, but the absorption profiles, and the disposition within the stratum corneum, dermis, epidermis, and subcutaneous fat were highly dependent on mixture components. The contrast of the results obtained for PCB and PCP, illustrate an area of interaction between the structural and chemical parameters that dictate the amount of chemical absorbed into the systemic circulation. (Supported by ATSDR U61/ATU484504.)

707 APPLICATION OF A HUMAN SKIN TISSUE CULTURE MODEL IN DERMAL ABSORPTION STUDIES OF 3,3',4,4'-TETRACHLOROBIPHENYL (TCB).

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TCB, one of the dioxin-like PCBs, demands much research and regulatory attention. To evaluate an *in vitro* generated human skin tissue culture model, cutaneous disposition of TCB under different exposure scenarios was investigated. Occlusive or non-occlusive doses of ^{14}C-TCB were applied at 4 or 40 μg/cm^2 in different vehicles including acetone, methylene chloride, a water-acetone mixture, and a soil-based mixture in flow-through diffusion cell studies (n=6~7/exposure condition). Significant exposure-dependent dermal absorption and disposition were observed. *In vitro* 8-hr absorption varied from 0.04% to 1.46% depending on vehicle, dosage, and occlusion. Much more TCB was absorbed into perfusate from soil than from liquid (organic or water-organic mixture) vehicles although the total penetration amount was less. Surprisingly, TCB dermal absorption/penetration ratios, which can reflect dermal absorption efficiency, were decreased by occlusion (soil dose, 0.45→0.13) or by adding water to the acetone vehicle (0.06→0.02). A lower (1/10) TCB dose in soil or in acetone showed a 3-5X higher fractional dose absorption, but a lower (1/3~1/2) transdermal flux (μg/cm^2/hr), than the higher dose in each vehicle. In conclusion, this human skin tissue culture model showed similar dermal absorption and disposition characteristics for TCB when compared to an *in vitro* porcine skin model. Dermal absorption data from liquid TCB doses might underestimate the risk of TCB from contaminated soil. Such observed exposure-dependent dermal disposition profiles need to be considered while assessing TCB dermal risk. (Supported by EPA-CR 824007.)

708 CHEMOMORPHIC ANALYSIS OF MALATHION IN SKIN LAYERS: IMPLICATIONS FOR THE USE OF DERMATOPHARMACOKINETIC (DPK) TAPE STRIPPING IN EXPOSURE ASSESSMENT TO PESTICIDES.

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The dermatopharmacokinetic (DPK) method of dermal tape stripping may prove to be a valuable addition to risk assessment protocols for toxic substances. To examine this possibility, the dermal penetration and absorption characteristics of [^{14}C]-malathion in the Sprague-Dawley rat was examined by three analytical techniques. [^{14}C]-malathion was applied in different vehicles for 30-minute and one-hour periods of exposure. Penetration into the stratum corneum (SC) was assessed by tape stripping followed by Instant Electronic Autoradiography (IEA). Also, the [^{14}C]-activity retained in three successive 16μm sections of the skin application site was determined by IEA and malathion was identified by Fourier Transform Infrared Microscopy (FTIR microscopy). Absorbed [^{14}C]-malathion was measured in selected tissues, organs, and the residual carcass by Liquid Scintillation Counting (LSC). Penetration into the SC followed a linear trend. The capacity of the SC reservoir for malathion amounted to approximately 1% of the dermal dose, while approximately 6% of the dose was absorbed. Results from this study support the view that LSC remains the method of choice to efficiently and reliably quantify absorption of a radiolabelled test substance. IEA offers the ability of the user to visualize the extent and profile of dermal absorption. When IEA is combined with FTIR microscopy, an effectual tool for studying the penetration of chemicals into layers of the skin emerges. The combined use of the three analytical techniques can be used to test the validity of the DPK method in hazard evaluation and exposure assessment of the organophosphorus insecticides. The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, participated in this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA.

709 ASSESSMENT OF SKIN ABSORPTION AND PENETRATION OF JP-8 JET FUEL AND ITS COMPONENTS.

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The dermal pharmacokinetics of jet fuels in general and JP-8 in particular is not well understood, even though the use by government and industry, worldwide, is over 59 billion gallons per year. JP-8, which is similar to kerosene, is composed of hundreds of hydrocarbon chemicals and their isomers. Exposures to JP-8 can occur from vapor, liquid or aerosol. Inhalation and dermal are the most prevalent routes of exposure. JP-8 may cause irritation when the skin is exposed repeatedly or for prolonged periods. The purpose of this investigation was to measure JP-8 and its major constituents in rodent skin and the flux through rodent skin to assess the potential for toxic effects with human exposures. Static diffusion cells containing dermatomed rodent skin were used in these 4-hour experiments. The absorption time course for six individual components (all aliphatic) of JP-8 was determined in the skin. All components appeared to be at a maximum by four hours. The chemical with the highest concentration in the skin was undecane (0.27 μg/mg skin) and the chemical with the lowest concentration was tetradecane (0.05 μg/mg skin). The penetration time course of thirteen individual components was determined from the receptor solution. The flux from this JP-8 fuel ranged from a high of 82.4 nanograms/cm^2/hr (the additive DIEGME) to a low of 0.5 nanograms/cm^2/hr (tridecane). The concentrations of chemicals in the skin suggest that it may be possible to determine which components are responsible for irritation. The fluxes suggest that JP-8 penetration through the skin will not cause systemic toxicity because fluxes are too low to cause significant body burden. (Supported by AFOSR 92HE05COR.)

710 CORRELATION APPROACHES FOR ESTIMATING SKIN PERMEABILITY OF HYDROCARBON COMPONENTS OF JP-8.

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The jet fuel, JP-8, consists of a complex mixture of hundreds of components. Component classes include straight chain alkanes, branched chain alkanes, cycloalkanes, diaromatics, n-alkanes and napthalenes. In a series of *in vitro* dermal penetration experiments using rat skin, dermal penetration coefficients

K_p (cm/h) were measured for the 13 most rapidly penetrating components. Several correlation approaches for predicting K_p for chemicals in water are found in the literature, based on molecular weight MW and the octanol/water partition coefficient $K_{o/w}$. Application of this approach for hydrocarbon components in JP-8 gave the following equation: Log K_p = -5.47 - 0.365 log $K_{o/w}$ + 0.00343 MW. The correlation coefficient (R^2) for this relationship is 0.876, which is higher than most of the published correlation approaches. (This value is further increased to 0.919 if the additive diethylene glycol is also included). Penetration into the skin was inversely related to log $K_{o/w}$, suggesting that the JP-8 vehicle is an even more lipophilic environment than the stratum corneum (insofar as it is adequately represented by octanol). In contrast to our study, correlations from studies in which water is the vehicle characteristically show a positive impact of $K_{o/w}$ and a negative impact of the molecular weight on the permeability coefficient. Since JP-8 is more lipid-like than the skin, as $K_{o/w}$ goes up the chemical would have a greater tendency to stay in the lipid vehicle and in the lipid layers of the skin, primarily stratum corneum, and not enter the receptor solution. Consistent with this, the log octanol/water partition coefficient has a much larger impact on the permeability than the molecular weight. Indeed, statistically log K_p alone appears to account for the variability in log K_p. Thus, for such lipophilic compounds, penetration through the skin is in this case likely limited by partitioning out of the lipid phase into the aqueous layers of the skin (and not by a molecular weight-dependent diffusion process). (Supported by AFOSR 92HE05COR.)

711 INFLUENCE OF JET FUEL MIXTURES ON DERMAL ABSORPTION OF AROMATIC AND ALIPHATIC COMPONENTS.

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Jet fuels are formulated with numerous aliphatic and aromatic components and performance additives. Jet fuel formulations can cause dermal irritation, but dermal absorption of these components in a jet fuel mixture is unknown. The purpose of this study is to evaluate the physicochemical properties of these mixtures in context of how they influence the absorption and permeability of an aromatic (14C-naphthalene) and aliphatic (3H-dodecane) marker in porcine skin and silastic membranes. In these 5-hour *in vitro* flow-through studies, JetA, JP-8, and JP-8(100) fuels, JP-8 evaporated (JP-8(puddle)), and JetA+DIEGME, JetA+8Q21, and JetA+Stadis450 were tested. These 3 additives are formulated with JetA to form JP-8. Naphthalene absorption (1.29 - 2.39%dose) was greater than dodecane absorption (0.1 - 0.28%dose). Stadis450 and JP-8(puddle) increased dodecane absorption, while DIEGME increased naphthalene absorption as well as diffusivity from JetA. These latter effects were not observed in silastic membranes suggesting that these interactions may involve the lipid pathway in skin. More dodecane was deposited in the stratum corneum (4.2 - 7.7%dose) at 5 hours than naphthalene (1.4 - 4.1%dose) and the 3 jet fuel additives enhanced deposition of naphthalene and not dodecane in JetA. These studies suggest that absorption and deposition of aromatic, aliphatic and jet fuel components can be influenced by specific performance additives and these interactions may be related to the unique chemistry of these components. (Supported by USAFOSR#FQ8671-98.)

712 CUTANEOUS IRRITATION OF TOPICALLY APPLIED JET FUELS IN THE PIG.

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Occupational exposure to jet fuel has recently become a source of concern. This study investigates the cutaneous exposure of 3 fuels by quantitating erythema, edema, transepidermal water loss (TEWL), and epidermal thickness of exposed pig skin. Yorkshire pigs were exposed to Jet A, JP8, and JP8-100 under occluded (Hill Top chamber or cotton fabric) and nonoccluded conditions for 5 hr, 24 hr, and 5 days. In the fabric sites, slight erythema was present within 5 hr, with moderate erythema present after 5 days. No erythema was noted in Hill Top sites after 5 days. Slight edema was observed in the fabric sites at 5 days. An increase in change in TEWL (DTEWL) was present in 5 day fabric sites, 24 hr and 5 day fabric-associated open sites, 24 hr and 5 day Hill Top sites, and 5 hr and 24 hr Hill Top-associated open sites. In general the mean DTEWL of the fabric and Hill Top sites was lower than the associated open sites. Epidermal thickness was greater in fabric than in open sites with little difference between the 5 day Hill Top and open sites. The epidermal peg thickness increased at 24 hr and 5 days with Jet A, JP8, and JP8-100 in the fabric sites. At 5 days the peg thickness was greater in the fabric than the Hill Top sites. In conclusion, the fabric treated with fuel caused the greatest increase in cutaneous erythema and epidermal thickness. (Supported by USAFOSR FQ8671.)

713 CYTOKINE RELEASE FROM KERATINOCYTES EXPOSED TO JET A, JP-8, AND JP8-100 JET FUELS.

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The toxicity of jet fuels has primarily centered on damage due to pulmonary exposure. Airway inflammation, immune disfunction, and memory disturbances are among the adverse effects associated with aerosolized fuels. However, there are few studies that have focused on the dermatotoxicity of jet fuels. These studies, primarily involving skin painting with middle distillates such as kerosene, have shown that they cause edema, erythema, blisters, and burning sensations indicative of a severe inflammatory response. With the recent advances in fuel mixtures that include an expanding number of compounds, increased concern is being given to the potential dermatotoxicology of jet fuels. The purpose of this study was to identify biomarkers of inflammation in normal human epidermal keratinocytes (NHEK) exposed to three jet fuel mixtures, Jet A, JP8, and JP8-100. Using an enzyme-linked immunosorbent assay (ELISA), NHEK were found to release the pro-inflammatory cytokines TNF-α and IL-8 in response to exposure to all three jet fuels within 8 hr and continuing to rise through 24 hr compared to controls. mRNA for both cytokines was also transcribed throughout exposure to the fuels which was detected via a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method as early as 15 min following exposure. These results demonstrate that jet fuels induce the production and release of proinflammatory cytokines in NHEK, and thus create the potential for chronic inflammation which may contribute to the development or progression of disease states in the skin. (Supported by USAFOSR FQ 8671.)

714 CUTANEOUS ENZYME HISTOCHEMISTRY OF TOPICALLY APPLIED JET FUELS IN THE PIG.

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The purpose of this study was to assess cutaneous enzyme activity in response to topical jet fuel exposure using enzyme histochemistry for alkaline phosphatase (ALK), acid phosphatase (ACP), and non-specific esterase (NSE) in the skin of pigs. Yorkshire pigs were exposed to Jet A, JP8, and JP8-100 for 5 hr, 24 hr, and 5 days under occluded fabric (335 ml), occluded Hill Top chamber (25 ml) and nonoccluded (25ml) conditions. The stratum basale layer (SB) showed a jet fuel treatment response and overall increase in ALK activity in the 5 day fabric sites. The SB layer had the greatest ACP staining in the 5 day Hill Top sites. With NSE, no differences were noted in the SB layer. In the stratum spinosum (SS) layer, ALK staining was the greatest in the 5 day fabric and 5 day Hill Top sites. Staining of ACP in the SS layer was highest in the 5 day Hill Top. NSE stained greatest in the 5 day fabric sites of the SS layer. In the stratum granulosum (SG) layer, the ALK staining was greatest at 24 hr in both the fabric and Hill Top sites. No differences were noted in the ACP of the SG layer. The intensity of NSE in the SG layer was greatest in the 5 day exposure fabric sites. In conclusion, the 5 day jet fuel treatments generally showed an increase in ALK, ACP, and NSE activity within each layer in response to JetA, JP8, and JP8-100. (Supported by USAFOSR FQ8671.)

715 USE OF METHYL SALICYLATE AS A SIMULANT TO PREDICT THE PERCUTANEOUS ABSORPTION OF SULFUR MUSTARD.

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Exposure to chemical vesicants such as sulfur mustard (HD) continue to be a threat to military forces which requires that protectant strategies to exposure

be evaluated. Methyl salicylate (MS) has historically been the simulant of choice to assess HD exposure. The purpose of this study was to compare the percutaneous absorption and skin deposition of MS to HD in the isolated perfused porcine skin flap (IPPSF). HD data were obtained from a previously published study in this model (Toxicol. Appl. Pharmacol 135: 25-34, 1995). 400 mg/cm 2 of 14C-MS or 14C-HD in ethanol were topically applied to 16 IPPSFs and experiments terminated at 2 hrs, 4 hrs or 8 hrs. Perfusate was collected at increasing time intervals throughout perfusion. Radioactivity was determined in perfusate and skin samples. Perfusate flux profiles were fitted to a bi-exponential model Y(t)=A (e-bt - e-dt) and AUC, peak flux and time to peak flux were determined. HD had more pronounced and rapid initial flux parameters ($p<0.05$). AUC determined from observed and model-predicted parameters were not statistically different, although mean HD AUC was 40 to 50% greater than MS. HD skin and fat levels were up to twice those seen with MS, but had lower stratum corneum and residual skin surface concentrations ($p<0.05$). Compared to other chemicals studied in this model, HD and MS cutaneous disposition were very similar, supporting the use of MS as a dermal simulant for HD exposure. (Supported by USACBDC #DAAD13-98-D-0014.)

716 EFFECTS OF LOW DOSE SULFUR MUSTARD ON GROWTH AND DNA DAMAGE IN HUMAN CELLS IN CULTURE.

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Sulfur mustard (HD) is a potent cytotoxic chemical agent with vesicating (blistering) properties when applied to the skin of humans. Development of medical countermeasures to protect individuals from injury following attack by this chemical warfare weapon requires an understanding of the mechanisms by which the pathology is generated. DNA is believed to be a major macromolecular target of HD in the epidermis. Using human epidermal keratinocytes (HEK) and human lymphocytes (PBL) in culture, we evaluated the effects of HD on DNA as assessed by the comet single cell electrophoresis assay. In addition we evaluated survival and growth through the measurement of viable cell number, metabolic fluorescence of alamar blue and membrane integrity assessment by calcein-AM at various times after exposure to low doses (0.01-5.0 μM) of HD. At 5 μM HD growth of HEK is retarded for 72 hours but then returns to control levels. Below 5 μM no inhibition of growth is detected. At 5 μM HD cross-links formed in PBL DNA inhibit the expression of H_2O_2 induced single strand breaks in the comet assay. Below 5 μM inhibition of single strand breaks is not observed. Most research with mustard toxicity is conducted at significantly higher dose ranges, around 100 μM, because these are believed to be equivalent to the vesicating doses on skin. At those high doses it is difficult to assess the genotoxicity or growth potential of HEK exposed to HD *in vitro*. These studies suggest that cells can be exposed to a concentration of HD (1-5 μM) that should generate significant DNA damage and survive the insult. Understanding the biochemical effects at this level may lead to medical countermeasures against the higher, injurious levels seen in battlefield conditions.

717 NEW INFORMATION DEBUNKS BLEACH FOR PATIENT DECONTAMINATION IN CHEMICAL/BIOLOGICAL WARFARE INCIDENTS.

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Threats of chemical and biological warfare and terrorism have spawned several national research and management initiatives, and the U.S. government is spending millions of dollars to educate first responders and cities on dealing with these critical issues. The United States is a recent arrival to this lethal field, as personnel in Israel, the Middle Eastern countries and the European Union have long been versed in prevention and treatment injuries and illnesses caused by these agents. However, there is a history of research conducted on these issues in the United States. The majority of this research was performed by, or for, the military, and most of it was classified and therefore unavailable to scientists, medical care givers, and emergency first responders. Recently (early 1999) several of these documents were declassified, revealing what appears to be new information. A 1953 study, classified as secret until early 1999, revealed that bleach solutions and slurries of sodium hypochlorite are more harmful to the skin of patients exposed to nerve agents, such as GB (sarin), than is the agent itself, and is less effective than washing with soap and water. This study compared the effectiveness of removal of the agents by bleach solutions, sodium hypochlorite, an ointment M5, and soap and water. This study evaluated the amount of damage to the skin caused by these agents in the presence of each method of decontamination and the subsequent lethality of the agent. Later reports on the topic seem to have been ignored by the military and the scientific community until the declassification of the above study. Additional studies have examined chemical warfare agents other than nerve agents and the effectiveness of various methods of decontamination, skin damage and lethality following the treatments. Bleach solutions, bleach slurries, and sodium hypochlorite may not be the "gold standards" as had been reported for the past forty years. In light of these declassified studies, it appears that first responders, city planners and medical personnel may need to reformulate their thinking, planning and protocols for dealing with these agents.

718 DIPHOTERINE® DECONTAMINATION OF C^{14}-SULFUR MUSTARD CONTAMINATED HUMAN SKIN FRAGMENTS *IN VITRO*.

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As about 70% of skin-deposited sulfur mustard is retained in the *stratum corneum*, various decontamination methods were studied *in vitro* on human skin *stratum corneum* fragments. Skin specimens were obtained from patients undergoing elective abdominoplasty who gave written consent after full disclosure. Skin fragments prepared according to a previously described method[1,2] were exposed to C^{14}-labelled sulfur mustard in methylene chloride solution with a specific activity of 1.66 GBq/mmol (CEN SACLAY, Molecules Marques, Gif sur Yvette, France). After a 5 minute exposure, skin fragments were immersed in one of 3 solutions: water+soap; physiologic saline; or Diphoterine® (Laboratoire Prevor, Valmondois, France - an hypertonic, amphoteric chelating agent). Three decontamination protocols were utilized: 3 minutes; 10 minutes; or 3 successive decontaminations of 10 minutes each. Residual decontamination solution radioactivity was measured as previously described[2]. With all 3 decontamination protocols, Diphoterine® resulted in more residual C^{14}-sulfur mustard in the decontamination solution, and this was significantly greater ($p<.05$) as compared to water+soap with either a single 10 minute or 3 successive 10 minute decontaminations. Diphoterine® deserves further study as a skin decontamination solution for sulfur mustard.

[1]Wepierre J, Marty J: Sci Techn Pharm 1979; 8:171-172; [2] Gerasimo P et al: Ann Pharma Fr 1997; 55:116-124.

719 POST EXPOSURE TREATMENT WITH IODINE PROTECTS AGAINST SULFUR MUSTARD-INDUCED SKIN LESIONS.

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Sulfur mustard (SM) is a powerful vesicant employed as a chemical warfare. The currently available antidotes against this blisterogen are of limited use. The present study demonstrates the protective effect of topical iodine preparation as post exposure treatment against SM (1.2mg net liquid) -induced lesions using the fur-covered guinea pig skin model. Iodine treatment 15 min after SM exposure resulted in statistically significant reduction of 48%, 50% and 55% in dermal acute inflammation, hemorrhage and necrosis, respectively, whereas the epidermal healing markers, hyperkerathosis and acanthosis were significantly elevated by 72% and 67%, respectively (skin analyzed 2 days after treatment). These healing parameters are the most reliable histological proof for the effectiveness of iodine. At longer interval of 30 min between SM exposure and iodine treatment there was significant degree of protection albeit to a lesser extent than that observed in the shorter interval. Although the epidermal healing markers were not elevated, the degenerative parameters such as epidermal microblisters and ulceration, and dermal acute inflammation, hemorrhage and necrosis were significantly reduced by 35%, 67%, 43%, 39% and 45%, respectively. At 45 min interval between exposure and treatment there was also certain degree of protection expressed by significant reduction in dermal acute inflammation and necrosis although the epidermal acanthosis and hyperkerathosis were reduced, indicating for low healing potential. At interval of 60 min between exposure and treatment, iodine was less efficient, nevertheless, significant reduction in epidermal microblisters and elevated acanthotic area were observed even at this long interval. Gross pathology analysis showed strong protective effect at intervals of 15 and 30 min and to a lesser extent at 45 and 60 min. The present findings suggest the iodine preparation as a potential antidote against skin lesions induced

by SM. (Supported by USAMRMC Cooperative Agreement No. DAMD17-98-2-8009.)

720 NONINVASIVE DETERMINATION OF SKIN SURFACE ASPARTIC PROTEINASE ACTIVITY IN THE LIVING ANIMAL-EFFECT OF NITROGEN MUSTARD.

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The present research demonstrates a method for *in situ* determination of stratum corneum aspartic proteinase in the living animal. A non-leaky well, containing [^{125}I]S-carboxymethylated insulin B-chain as a substrate, was constructed on the shaved back of anesthetized guinea pigs and rats. The enzymatic activity was determined by measuring the radiolabled trichloroacetic acid soluble material. We show pepstatin-sensitive proteinase activity bound to skin surface indicating the involvement of aspartic proteinase(s) such as cathepsin D and/or E. Aged rats had about 6 fold lower activity than young animal. The proteinase activity was inhibited by the alkylating agent mechlorethamine and by the cosmetic propylene glycol. Similar procedure was carried out with intact human skin pieces obtained from plastic surgeries. This activity was inhibited by anti human cathepsin D antibodies. Cathepsin D was immunohistochemically localized in the corneal and granular layers of the epidermis. Skin surface aspartic proteinase/cathepsin D activity may serve as a marker for skin toxicity or for certain skin disorders leading to new approach in their medical treatments.

721 COMPARISON OF THE EFFECT OF ALLERGEN AND IRRITANT TREATMENT ON PROLIFERATION AND SUBPOPULATION OF THE DRAINING LYMPH NODE CELLS IN MICE AND GUINEA PIGS.

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False positive results (Stimulation Index [SI]>3) have been reported for certain irritants in the murine local lymph node assay (LLNA). The purpose of this study was to distinguish allergenic responses from irritant responses using the murine LLNA and the guinea pig lymph node cell proliferation assay (GPLNA, R. Kashima et al., 1994). LLNA was conducted according to the conventional method. The GPLNA employed a single 24-hour occlusive patch, which causes well-defined erythema. Five days after application of occlusive patch, suspensions of superficial dorsal cervical lymph node cells (LNC) were individually prepared and the 3H-TdR incorporation was measured. In addition to LNC proliferative responses, we examined the phenotypic characteristics of LNC both in mice and in guinea pigs. In the LLNA, a maximum non-irritating concentration gave positive results for SLS (5%, SI=4.2) and Tween 80 (5%, SI=5.3). In GPLNA, 0.5% DNCB and 25% aniline gave positive responses. Conversely, negative results were obtained for the irritants (0.3% SLS and 50% Tween 80). Based on these results, the GPLNA was considered a useful method because no false positive results were generated. Phenotypic characterization of the LNC from mice following application of 0.25% DNCB showed a 30% decrease in the percentage of CD4+ cells and a 2.1-fold increase in the percentage of B220+ cells. Small changes in the opposite direction were observed with the irritants. Thus, these allergens and irritants could be distinguished by analyzing phenotypic characteristics of LNC in mice. Phenotypic characterization of the LNC from guinea pigs following 0.5% DNCB application gave similar changes to those observed in mice. These data suggest a possibility that analysis of surface markers may differentiate between allergen and irritant responses in guinea pigs.

722 COMPARISON OF THE GUINEA PIG MAXIMIZATION TEST (GPMT), THE MURINE LOCAL LYMPH NODE ASSAY (LLNA) AND STRUCTURE-ACTIVITY RELATIONSHIP MODELS TO PREDICT THE POTENTIAL OF CHEMICALS TO CAUSE OF ALLERGIC CONTACT DERMATITIS.

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Allergic contact dermatitis (ACD) is a delayed-type hypersensitivity response of the skin to a chemical. The prevalence of the disease in the general population is about 10%. Traditionally, guinea pigs have been used to test chemicals for their potential to cause ACD. We have developed a structure-activity relationship (SAR) model of ACD based upon the guinea pig data, and more recently have developed a SAR model based exclusively on human data. In the current study, we sought to compare the data from testing of the same chemicals in guinea pigs, humans and mice. We also examined the prediction of ACD activity by both SAR models with the test results reported for the LLNA. Based on 40 chemicals that were common to the LLNA and human ACD test sets, the sensitivity of the LLNA was 0.83, the specificity was 0.27. Comparison of data from the LLNA with that from the GPMT yielded a sensitivity of 0.83 and specificity of 0.57. The SAR model based on human data correctly predicted the activity of 65% of the LLNA-positive chemicals, and 40% of the inactive chemicals. The data are being examined to determine the ability of the models to predict strong, moderate and weak sensitizers as identified by the animal and clinical test results, as well as the ability of each model to identify structural features associated with activity. It is anticipated that such analyses will result in greater understanding of the appropriate use of animal tests and SAR models as well as greater understanding of the mechanism underlying ACD.

723 REGULATION BY VERAPAMIL OF DENDRITIC CELL MIGRATION IN MICE.

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It has been reported previously that p-glycoprotein (MDR-1), expressed by human Langerhans cells (LC), regulates the trafficking of LC from the epidermis. Thus, anti-MDR-1 antibodies or verapamil (a drug that antagonizes MDR-1 transport) was found to inhibit the migration of LC from the epidermis in human skin explant cultures. The interpretation was that MDR-1 function is necessary for the secretion by LC of a physiological substrate required for migration. To investigate the contribution of MDR-1 to LC migration *in vivo*, the influence of verapamil on contact allergen- and cytokine-induced dendritic cell (DC) migration in mice has been examined. Groups of mice (BALB/c strain) were exposed on the dorsum of both ears to 1% verapamil dissolved in methanol, or to methanol alone, 2 hours prior to application to the same site of the contact sensitizing chemical oxazolone (Ox; 0.5%). Under these conditions of exposure verapamil was found to inhibit, by approximately 80%, the accumulation of DC in draining lymph nodes measured 18 hours later. In subsequent experiments, the influence of verapamil on LC migration stimulated by intradermal administration of either tumor necrosis factor α (TNF-α) or interleukin 1β (IL-1β) was investigated. Prior exposure of mice to verapamil caused a marked inhibition (85%) of draining lymph node DC accumulation measured 4 hours following TNF-α injection. In contrast, verapamil was without effect on DC migration stimulated by exogenous IL-1β. Since Ox- and TNF-α-induced DC migration in mice are both IL-1β-dependent, it is proposed that one function of MDR-1 is to regulate the secretion by LC of IL-1β.

724 SUBACUTE INTRADERMAL CIS-UROCANIC ACID SUPPRESSES THYMIC CELLULARITY IN TWO STRAINS OF MICE.

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Urocanic acid predominates in the strateum corneum of mammals in the trans isomer form. Exposure to ultraviolet light causes isomerization to the cis isomer, which is then partially absorbed systemically. Excessive cis-urocanic acid (cis-UCA) has been suggested to be involved in sunlight-induced local and systemic immunosuppression. In this study, cis-UCA was administered intradermally in two strains of 6 week-old female mice (C3H-HEN and C57Bl/6) for one day, five consecutive days, or three times a week for four weeks. The following parameters were measured 48 hours after administra-

tion of the final dose of cis-UCA as indicators of alterations in immune function: thymic and splenic organ weights, total cellularity and leukocyte cellularity of the thymus and spleen, splenic macrophage phagocytosis, and splenic leukocyte production of hydrogen peroxide (chemiluminescence assay). Significant decreases in thymic leukocyte cellularity were seen in both strains of mice in the four-week dosing regime, supporting previous observations of thymic effects in mice exposed subacutely to cis-UCA. (Supported by NIH RO1-ES 09642-01.)

725 ROLE OF METABOLISM IN ARSENIC-INDUCED CYTOKINE PRODUCTION IN MURINE KERATINOCYTES.

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Inorganic arsenicals are skin carcinogens both in humans and rodents. It has recently been demonstrated that arsenic induces overexpression of keratinocyte-derived growth factors, which are likely to have a significant role in arsenic-induced skin hyperkeratoses and cancer. The mechanism(s) involved in this induction are, however, still elusive as the role of arsenic valence. The purposes of this study were to investigate the early intracellular events that follow *in vitro* treatment with sodium arsenate in a murine keratinocyte cell line (HEL30), which leads to interleukin-1α (IL-1α) production, and second, to characterize the role of the cell metabolism in arsenic toxicity. It has been known for some years that arsenic affects mitochondrial enzymes and impairs tissue respiration. We demonstrated by electron microscopy analysis that arseniate induced a dramatic alteration in keratinocyte mitochondria, that could be prevented by rotenone pretreatment, suggesting the possible involvement of mitochondria-derived reactive oxygen species (ROS). The first intracellular event following the binding of As to mitochondria is reactive oxygen species generation, followed by activation of transcription factors (NF-κB and AP-1), production of IL-1α and proliferation of keratinocytes. In mammals, the biotransformation of arsenic is based on several steps: arsenate species must be reduced to arsenite, through glutathione consumption. Arsenite is sequentially methylated, first to form monomethylarsonic acid and subsequently dimethylarsinic acid (DMA), which are less reactive and are rapidly excreted in urine. In our cells, DMA up to 1mM did not induce IL-1α production, confirming that the methylation of inorganic arsenic is indeed a detoxification mechanism. Regarding the effect of As valency on IL-1α production, we found that As(III) is five times more potent then As(V). However, this is due to a difference in cell uptake between As(III) and As(V), in particular, to a competition of phosphate anions with As(V) uptake. In medium without phosphates, As(V) shows the same potency of As(III). On the contrary the phosphates content does not influence the effect of As(III) on IL-1α production. Glutathione deprivation exacerbates both As(III) and As(V)-induced IL-1α production. Thus, it still remains to be elucitated the role of intracellular reduction of As(V), in order to identify the species responsible for IL-1α neosynthesis. (Acknowledgement: this study was partially supported by a grant from UNIPRO.)

726 QUENCHING OF CITRAL SENSITIZATION DEMONSTRATED IN A HUMAN REPEATED INSULT PATCH TEST.

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Quenching is the term used to describe the phenomenon of inhibition of sensitization. Citral is a fragrance ingredient that has a powerful lemon scent. It has been demonstrated that although citral produces sensitization reactions when applied alone, it does not produce any reactions in a human maximization test when combined with d-limonene (Opdyke, 1976). In this study, a solution of 4% citral and 1% d-limonene in diethyl phthalate was tested in a repeated insult patch test with a total of 118 human subjects. The test method was an adaptation of the Draize Patch Test that included an induction phase consisting of nine repetitive 24-hour occluded applications on the same skin site for three weeks. This was followed by a two-week rest period. The challenge phase consisted of a 24-hour occluded application to a naive site. No sensitization reactions were observed. In summary, quenching has been clearly demonstrated for citral with d-limonene in two different human sensitization tests (a human maximization test and a human repeated insult patch test).

727 SKIN PENETRATION BY THE NATURAL RUBBER LATEX PROTEINS, HEVEIN, AND RUBBER ELONGATION FACTOR.

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Differences in IgE antibody patterns have been demonstrated in latex allergic individuals who have undergone multiple surgeries and those with limited surgical exposure to natural rubber latex (NRL). Adult health care workers show a higher prevalence of antibody towards hevein (Hev b 6.02) whereas children requiring multiple surgeries have shown a higher prevalence of recognition of Rubber Elongation Factor (REF, Hev b 1). The mechanisms underlying the differences in protein recognition by these risk groups is presently not known. These studies were designed to examine the extent of dermal penetration of hevein and REF using hairless guinea pig skin in an *in vitro* flow through dermal penetration model. Proteins were radiolabeled with 125-I using a modified chloramine-T method and applied to dermatomed skin sections (250 μm) that were unaltered or abraded via tape stripping to remove the stratum corneum. In comparing the penetration of hevein and REF into intact and abraded skin samples (n=6), no significant differences were observed between the two proteins. For intact skin, 0.34% and 0.57% of the total applied radiolabeled proteins were recovered for hevein and REF, respectively. Dermal abrasion increased the penetration into the skin with 5.22% of hevein and 3.32% of REF being retained. For both hevein and REF, less than 2% of the proteins penetrated through the intact skin while greater than 20% was able to penetrate through abraded skin. Mass balance for all penetration studies was greater than 94%. Immunohistochemistry of skin sections revealed localization of proteins in the stratum corneum of intact samples with more intense staining in the viable layers of the epidermis in abraded samples. Based on the results of these studies, the differences in sera recognition of hevein and REF between HCW and multiple surgery patients does not appear to be related to the ability of the proteins at equal concentrations to penetrate the skin. (These studies were supported in part by the NIOSH/NIEHS interagency agreement #Y02ES10189.)

728 DERMAL AND SYSTEMIC TOLERABILITY OF TOPICALLY APPLIED ISIS 2105, A PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE (PS ODN), IN SPRAGUE-DAWLEY RATS.

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A topical formulation has been developed for dermal delivery of antisense ODNs. Local and systemic tolerability has been characterized using ISIS 2105, a representative 20-base P=S ODN, at concentrations of 0.2%, 0.5%, 2.0% and 10% (w/w). Vehicle absent ODN or formulated with ISIS 2105 was applied to the dorsal region of rats and an occlusion barrier placed over application sites for 6 hours per day. Local tolerability of ISIS 2105 was assessed following daily treatments for 14 days. Dermal tolerability was dose (conc)-dependent with moderate erythema and mild ulcerations at 10%; mild erythema and minimal ulcerations at 2.0%; minimal erythema at 0.5%; and no apparent effects at either 0.2% or in vehicle controls. Evidence of immune stimulation, e.g. a mononuclear cell infiltrate at the epidermal/dermal junction, was found at 10% and to a lesser extent at 2.0%. Immunohistochemical localization illustrated deposition of ODN in both dermis and epidermis. Quantitation of ODN in treated skin revealed non-linear, dose-dependent uptake; 5.8, 20.3, 51.1, and 362.0 μg/g in skin treated with 0.2%, 0.5%, 2.0% and 10%, respectively. Metabolism in skin was minimal, with 40% to 90% of total ODN measured as parent compound. Systemic exposure was low and dose-dependent. There was no evidence (hematology, serum chemistry, histology) of systemic toxicity. Most importantly, these studies verify epidermal and dermal uptake of ODN in intact skin. Dermal alterations were dose-dependent and correlated to significant local exposure. Systemic exposure was nominal, consistent with minimal toxicity. This suggests therapeutic dose regimens may be developed for safe/effective topical ODN delivery.

729 DERMAL TOLERABILITY AND IMMUNOSTIMULATORY EFFECTS OF PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES (PS ODN) FOLLOWING INTRAVENOUS, INTRADERMAL AND TOPICAL ADMINISTRATION IN RATS.

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ISIS 12450, ISIS 2105, and ISIS 12449 have low, medium, and high potential for inducing immune stimulation, respectively. The role of immune stimulation in inducing dermal irritation was assessed by comparing responses to these PS ODNs. Doses were given as 10 mg/kg i.v., 10 mg/kg i.d., or in a 10% (w/w) topical formulation. Topical and i.d. doses were given at the dorsal scapular region; dermal tolerability following all three routes was evaluated at this site. Following i.v. dosing, no dermal effects were noted; spleen weights were increased approx. 1.3, 1.6-, and 1.9-fold over control with ISIS 12450, ISIS 2105, and ISIS 12449. ID administration resulted in localized erythema, edema, and focal eschar. Mean severity scores (0 to 5 scale) were 2.0, 2.2, and 3.4 with ISIS 12450, ISIS 2105, and ISIS 12449. For ISIS 2105 and ISIS 12449 a minimal mononuclear cell infiltrate was noted at the epidermal/dermal junction. Spleen weights were increased approx. 1.1-, 1.4-, and 1.7-fold over control. Topical administration resulted in similar dermal alterations as noted above (mean scores 1.6, 2.8, and 3.8); a mononuclear cell infiltrate was noted for ISIS 12450 (minimal), ISIS 2105 (mild), and ISIS 12449 (mild to moderate); splenomegaly was minimal with all three compounds (1.0-, 1.1-, and 1.2-fold over control). Thus, relative potency for inducing immune stimulation correlated to relative potency for inducing dermal alterations. This suggests immune stimulation is likely to be a significant factor in the induction of dermal effects, particularly with routes of administration that provide extensive dermal exposure.

730 IMMUNE MODULATION BY AN ANTISENSE OLIGODEOXYNUCLEOTIDE DESIGNED TO INHIBIT ICAM-1 EXPRESSION.

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ISIS 2302 is a phosphorothioate oligonucleotide designed to inhibit human ICAM-1, and is intended for treatment of inflammatory diseases. Although this compound is able to inhibit inflammation, one of the potential toxicities associated with phosphorothioate oligodeoxynucleotides is immune stimulation. Immunotoxicity studies were performed in mice to fully understand the modulatory capability of ISIS 2302 and a murine analog, ISIS 3082. Effects of ISIS 3082 were limited to decreases in contact hypersensitivity and a 2-way MLR at doses of 1 to 20 mg/kg, consistent with anti-inflammatory properties. There was no other effect on cellular immunity, indicating selective modulation of immune function. These effects were consistent with selective inhibition of ICAM-1 expression. By comparison, immunostimulatory properties of ISIS 2302 were seen at doses ≥ 20 mg/kg. Dose-dependent increase in spleen weight was associated with increased number of B cells at 50 mg/kg. Mitogenic response to LPS was also increased, but there was no increase in total IgM or IgG, and no specific antibody response to the oligonucleotide. Increases in IL-6, IL-12, and MCP-1 were observed at 20 and 50 mg/kg, along with increased NK cell activity. There were no other changes in cellular immune function. Thus, anti-inflammatory activity occurs at doses 5 to 10-fold lower than required for toxicity. Effects on immune suppression or stimulation appear to be limited in mice. Furthermore, monkeys are less sensitive to immune stimulation than mice indicating even greater therapeutic margin. ISIS 2302 has a very low potency for immune stimulation relative to other compounds in this chemical class.

731 RECOVERY OF IMMUNE RESPONSE FOLLOWING ADMINISTRATION OF AN ANTI-CD154 MONOCLONAL ANTIBODY (HU5C8) TO CYNOMOLGUS MONKEYS.

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Hu5C8 is a humanized monoclonal antibody that binds CD40 ligand (CD154) on activated CD4+ T cells and inhibits its interaction with CD40. Inhibition of the CD40:CD40L interaction has been shown to block the development of specific antibodies, down-regulate the activation of T cells, and interfere with the maintenance of germinal centers (GC) within lymphoid follicles. Studies to examine the toxicity and pharmacologic activity of hu5C8 were conducted in cynomolgus monkeys. Pharmacologic activity was assessed by measuring the immune response to exogenous protein antigens including tetanus toxoid (TT) and Keyhole Limpet Hemocyanin (KLH). A dose-dependent inhibition of primary and secondary immune responses was observed following the administration of hu5C8. Test article-related changes observed at necropsy were limited to GC hypoplasia in the spleen and lymph nodes. The extent of these changes showed a clear dose-dependency and correlated with the extent of immune response following antigen challenge. The duration of treatment had a limited effect on the extent of GC effects. Animals that received similar doses of hu5C8 for 4 or 26 weeks displayed a similar extent of hypoplasia. Functional recovery, defined as the ability to achieve immune responses comparable to untreated animals, was observed in all hu5C8-treated animals. The time-period in which animals achieved functional recovery was dependent on the dose of hu5C8. Recovery at the tissue level was observed in all animals following the demonstration of functional immune recovery.

732 IMMUNOCHEMOTHERAPY ON TUMOR GROWTH AND METASTASIS BY POLYSACCHARIDES ISOLATED FROM *PHELLINUS LINTEUS.*

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It was previously reported that polysaccharides (PL) isolated from Phellinus linteus strongly stimulated cell-mediated and humoral immunity. This study was undertaken to investigate the immunochemotherapeutic activity of PL against tumor growth and metastasis. PL alone significantly prolonged the survival rate of B16F10-implanted mice, inhibited tumor growth in NCI-H23-implanted nude mice, and reduced the frequency of pulmonary metastasis of B16F10 melanoma. Adriamycin significantly inhibited tumor growth, but only slightly inhibited metastasis. The combination therapy with PL and adriamycin was more effective in inhibiting tumor growth, but not metastasis. PL did not induce direct toxicity in cancer cells, which is characteristic of immunotherapeutics. In conclusion, PL might be of use in immunochemotherapy of cancer because of its effective activities on tumor growth and metastasis through the immunopotentiation of the patients without toxicity.

733 IMMUNOTOXIC EFFECT OF INORGANIC LEAD (PB) ON HOST-RESISTANCE OF MICE ASSOCIATES WITH MOUSE SIDED-TURNING BEHAVIOR.

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Differential immune reactivities of mice were associated with behavioral sidedness; mice with left-turning preference had lower *in vivo* humoral and cell-mediated immune responses than mice with right-turning preference. The immune, endocrine, and nervous systems closely interact to maintain homeostasis, which is sensitive to environmental factors. In this study, we have investigated the influence of inorganic lead (Pb) on neuroimmune interactions. Pb reduced host-resistance of BALB/c mice to Listeria monocytogenes infection. Pb increased the susceptibility to Listeria in mice with both left- and right-turning preference; however, Pb did not alter host-resistance of mice with no turning preference. In contrast to mice with no turning preference, the spleens of Pb-exposed mice with turning preference were smaller than those of non-Pb-exposed mice 3 days after infection. The lower host-resistance of Pb-exposed mice with turning preference correlated with elevated serum gamma IFN and IL-6 levels, indicators of disease severity. Although the basal serum gamma IFN levels were lower in all groups after Pb exposure, the relative levels correlated with host-resistance. Reduction of basal serum gamma IFN levels by Pb weakened defense mechanisms early in infection and may inhibit effective proliferation of splenocytes. We suggest that the observed differential susceptibility to Listeria can be reordered by environmental stressors, in that the more resistant right-turners became more susceptible than no preference mice after Pb exposure. The differential effects of Pb based on behavioral differences suggest that neuroimmune circuitry may be involved in the processes described. (Funded by NIH ES03179.)

734 INVESTIGATIONS ON THE IMMUNOSTIMULATING POTENTIAL OF A COMBINATION OF OCTAMETHYLCYCLOTETRA- AND DECAMETHYLCYCLOPENTASILOXANE IN RATS AND MICE.

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Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) are low molecular weight cyclic polydimethylsiloxanes often used in combination e.g. in personal and health care product formulations. Previous studies with D4 did not indicate a suppressing/modulating effect on the immune system of Fischer 344 rats (Munson et al., The Toxicologist 36, 1997, 265). In B6C3F1 female mice subchronic oral exposure to D4 induced suppression of T-dependent humoral immune response (Le and Munson, The Toxicologist 36, 1997, 265). D5 had no immunosuppressing effects on Fischer 344 rats after inhalation exposure to concentrations up to 160 ppm (Burns-Naas et al., Toxicol. Sci. 43, 1998, 28-38). Our investigations aimed to study the combination of D4 (ca. 75%) and D5 (25%) with regard to a potential of an immunostimulating interaction with immunocompetent cells in NMRI mice and Wistar rats. As positive control, 2,4-dinitrofluoro-benzene (DNFB) was used. The D4/D5 mixture (in sesame oil) was injected subcutaneously into the pad of the left paw at doses of 3 and 10 mg/kg body weight (bw). DNFB was likewise administered at dose levels of 0.6 and 2 mg/kg bw. Popliteal lymph nodes were weighed and the cells counted, cells were stained with antibodies to mice and rat T- or B-cell surface markers and analyzed in a fluorescense-activated cell scanner (FACScan). Macrophage analyses was done after stimulation with 4,3-phorbol-12-myristate-13-acetate (PMA). Altogether there was no indication of a test substance specific activation of the cells of the immune system. DNFB, in contrast, led to a specific stimulation of lymphocytes (memory T-cell increase).

735 COMPARED EFFECTS OF MORPHINE AND NICKEL CHLORIDE ON NK CELL ACTIVITY IN RATS AND MONKEYS.

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Natural Killer (NK) cell activity is often recommended as a critical endpoint in the immunotoxicity evaluation of environmental chemicals. However, this assay is usually performed in rodents and rarely in monkeys so that no interspecies comparison of results is available. This study was conducted to compare the effects of *in vitro* exposure to 1, 10 and 100 µg nickel chloride and 0.01 nmol, 1 nmol and 1000 nmol morphine sulfate in rats and monkeys. NK cell activity was measured from ^{51}Cr release by YAC (rats) and K562 (monkeys) cells using three different ratios of target:effector cells, namely 1:25, 1:50 and 1:100. Nickel chloride and morphine sulfate induced a marked decrease in NK cell activity which was statistically significant only at the higher concentration. As a similar trend was noted in both species, these results suggest that similar results can be expected from NK cell measurement during immunotoxicity evaluation in monkeys and in rats. In addition, this assay prove useful for immunotoxicity evaluation of medicinal products.

736 IMMUNE, THYROID, AND HEMATOLOGICAL EVALUATION OF AMMONIUM PERCHLORATE IN B6C3F1 MICE.

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Studies were undertaken in female B6C3F1 mice to assess thyroid, hematological, and immunological effects of ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, and 30 mg/kg/day). Several immunological and hematological parameters were not significantly affected after exposure to AP for 14 or 90 days including: total white (WBC) and red blood cell number; hematocrit; hemoglobin; red blood cell indices; bone marrow cellularity and proliferative function; spleen and thymus weight and cellularity; kidney, liver, and body weights; cytotoxic T cell activity; thymic and splenic CD4/8 lymphocyte subpopulations; *in vivo* antibody responses to sheep red blood cells; macrophage nitrite production; and serum autoantibody production. However, an enhanced proliferative response to soluble listerial antigen was observed in a classical delayed type hypersensitivity assay; hypertrophy and/or colloid depletion were observed in thyroid tissue; and serum T4 levels were significantly decreased with no change in T3 or TSH. Decreased phagocytosis function by peritoneal macrophages was detected and this was reversible upon 30-day cessation of AP exposure. Despite decreases in phagocytic function of nearly 50% in some AP treatment groups, host resistance to Listeria monocytogenes challenge (2700 CFU) was normal. Increased natural killer cell activity was also detected but resistance to B16F10 tumor challenge was not correspondingly affected. In light of the normal host responses to tumor and listeria challenges after exposure to AP, it is apparent that the altered immunological, hematological, and thyroid parameters were not sufficient to alter host resistance under these experimental conditions. (This study was funded by the Department of Defense [DSWA01-97-1-0008].)

737 EFFECTS OF N,N,-DIETHYL-M-TOLUAMIDE (DEET) ON IMMUNE FUNCTION PARAMETERS IN B6C3F1 MICE.

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It has been suggested that DEET, in combination with a variety of environmental agents relevant to the Gulf War, may have contributed to the manifestations reported by veterans. Since some of the reported symptoms suggest a link to immune dysfunction, it is important to assess the immunological effects of DEET independently and in combination with other environmental agents. To determine the immune effects of DEET exposure singly, adult female B6C3F1 mice were injected subcutaneously for 14 days with DEET only at either 6.25,12.5, 25, or 50 mg/kg. Effects on lymphoproliferation, natural killer cell activity, thymus and spleen weight and cellularity, and thymic and splenic CD4/CD8 lymphocyte subpopulations were assessed 24 hours after the last dose. No effect was observed in lymphoproliferation, natural killer cell activity, thymic weight, splenic weight, thymic cellularity, or splenic cellularity. Significant decreases were, however, observed in the percentage of thymic CD4-/CD8- lymphocytes at the 12.5, 25, and 50 mg/kg treatment levels and in splenic CD4+ lymphocytes at the 50 mg/kg treatment level. These results suggest that DEET alone does not profoundly affect immune function. Future studies in our laboratory will investigate the immunological and autoimmune effects of DEET in combination with other agents to include JP-8 jet fuel, pyridostigmine bromide, and exercise stress.

738 DIETARY IODINE MODULATES AMMONIUM PERCHLORATE INDUCED IMMUNOTOXICITY.

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The use of ammonium perchlorate (AP) as an oxidant in solid propellants has resulted in groundwater contamination and the potential for human exposure via ingestion. Previous studies revealed that the administration of 30 mg/kg/day AP in drinking water to adult, female B6C3F1 mice resulted in the enhancement of natural killer cell activity and suppression of serum total T4 levels and phagocytic activity by peritoneal macrophages. Since AP alters thyroid function by competitively inhibiting the trapping mechanism that incorporates free iodine into T3 and T4, it was hypothesized that a reduction in dietary iodine would enhance the effects of AP consumption. AP (0 and 30 mg/kg/day) was administered to two groups of female B6C3F1 mice via drinking water. In addition to AP exposure, mice within each group were fed a diet containing 1.89 ppm or 0.15 ppm of iodine (Tek Lab Sterilizable Rodent Diet, formula no. 8656). After 14 days of AP exposure, the mice fed 1.89 ppm iodine had a 25% reduction in serum total T4 levels but no significant changes in peritoneal macrophage or splenic total cellularity, nitrite production by peritoneal macrophages, or natural killer cell activity. In contrast, mice fed 0.15 ppm iodine had a 50% reduction in total T4 levels, a 50% reduction in peritoneal macrophage cellularity, reduced nitrite production by peritoneal macrophages, and a 2-fold increase in natural killer cell activity. Thyroid hormone and immune parameters in control mice were not affected regardless of iodine diet. These findings suggest that dietary iodine may modulate the toxicity of AP. (This study was funded by MUSC internal research funds.)

739 EXPOSURE TO SODIUM BROMATE IN DRINKING WATER FOR 28 DAYS PRODUCED MINIMAL IMMUNOTOXIC EFFECTS IN FEMALE B6C3F1 MICE.

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Sodium bromate (SBM) is one of the disinfection by-products (DBPs) found

in drinking water. To evaluate the immunotoxic potential of SBM in female B6C3F1 mice, SBM (80-800 mg/l) was administered in the drinking water for 28 days. Exposure to SBM did not produce any signs of overt toxicity; no gross pathological lesions were observed. There were no significant differences in drinking water consumption, body weight, body weight gain, or weights of thymus, liver, kidneys and lung between animals exposed to SBM and controls. However, animals exposed to SBM had a significant increase in absolute (28%) and relative (26%) spleen weights. The erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, platelet count, total leukocyte count, and counts of differential leukocytes were unaffected by SBM. A dose-related increase in reticulocytes was observed following exposure to SBM with the greatest increase (78%) observed at the highest dose level. Overall there were no changes in the absolute number of total T cells, CD4+ T cells, CD8+ T cells, natural killer (NK) cells and macrophages after exposure to SBM. There was no alteration in the IgM antibody-forming cell response, mixed leukocyte reaction and NK activity while the activity of peritoneal macrophages was decreased after exposure to SBM. In conclusion, SBM produced minimal toxicological and immunotoxic effects in female B6C3F1 mice. (This work was supported in part by the EPA and NIEHS Contract ES55387. This abstract does not represent EPA policy.)

740 EXPOSURE TO DISINFECTION BY-PRODUCT DIBROMOACETIC ACID DOES NOT ALTER IMMUNE FUNCTION OR HOST RESISTANCE.

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Dibromoacetic acid (DBA) is a disinfection by-product contaminant found in drinking water. The purpose of these studies was to determine the potential effects of DBA on the immune system when administered in the drinking water. Female B6C3F1 mice were exposed daily, ad libitum, to DBA in the drinking water at dose levels of 250, 500, and 1000 mg/l for 28 days. Body weight and change in body weight were unaffected. While the high and middle doses produced a decrease in absolute thymus weight (19%), an increase was observed in absolute liver weight (25%) at the high dose. The mid and high doses produced significant increases in relative liver weight 11% and 23% respectively. Hematological parameters were not affected. No alteration was observed after exposure to DBA in the antibody forming cell response to sRBC. In the NK assay, both basal and augmented, no affect was demonstrated. No effect was observed on the mixed leukocyte response (MLR). Previous range finding studies showed modulation at the high dose of 2000 mg/L in several measured immune parameters. Hence, host resistance studies were conducted. No change was demonstrated in the B16F10 melanoma tumor model using 2 challenge levels, no alteration in immune susceptibility to S pneumoniae was seen with three challenge levels and no change was observed in the parasitemia with the Plasmodium yoelii challenge. These studies demonstrate that in female B6C3F1 mice the immune system is not a target for DBA at doses of 1000 mg/l or less. (This work was supported in part by the EPA and NIEHS Contract ES 55387. This abstract does not represent EPA policy.)

741 EXPOSURE TO SODIUM CHLORITE IN DRINKING WATER FOR 28 DAYS PRODUCED MINIMAL IMMUNOMODULATORY EFFECTS IN FEMALE B6C3F1 MICE WITH THE EXCEPTION OF INCREASED NK CELL ACTIVITY.

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Sodium chlorite (SCl) is one of many chlorination by-products found in drinking water. Its immunomodulatory properties were evaluated by exposing female B6C3F1 mice to SCl in their drinking water (0.1, 1, 5, 15, 30 mg/l) for 28 days. In addition to a routine toxicological evaluation of the mice, a panel of immune assays was used to assess immunomodulation. Other than a significant dose-related increase in the percent of reticulocytes, peaking at 34% in the 15 mg/l treatment group, no change in hematological or toxicological parameters was measured. A dose-related increase in splenic natural killer cell activity was observed in SCl-treated mice when data were expressed as lytic units/107 cells and lytic units/spleen. Peak NK activity was greater than 260% in the 30 mg/l treated mice for both parameters. Spleen antibody-forming cell response and mixed leukocyte response, and peritoneal macrophage activating factor were not affected by SCl exposure. Phenotypic analysis of the splenocytes revealed a 30% and 26% increase in the percent and absolute number of CD8+ cells in the 30 mg/l treatment group; no significant change in Ig+ B cells, CD3+ and CD4+ T cells, NK cells, and macrophages was detected. These results indicate that SCl enhances NK activity and slightly alters CD8+ spleen cell numbers. (This work was supported in part by the EPA and NIEHS Contract ES55387. This abstract does not represent EPA policy.)

742 EXAMINATION OF THE MECHANISM FOR RAPID LOSS OF MURINE NK CELL LYTIC FUNCTION IN CELL CULTURES.

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A number of immunotoxicants affect NK cells, and cell culture systems are often required to investigate the mechanisms responsible for these effects. Unfortunately, rodent NK cells rapidly lose their cytolytic function in culture. It is not known if preferential death of NK cells (as compared to other splenocytes) or loss of activity of viable NK cells is primarily responsible for the observed loss of lytic function. The present study addressed this matter using flow cytometry. Splenocytes were cultured for 12 hr in 48-well plates with an initial cell density of 10^8/ml. Cells were then washed and labeled with anti-NK1.1-phycoerythrin and 7-amino actinomycin D (7-AAD, which labels only dead cells). Gates were set using cells labeled with isotype control antibody or 7-AAD alone. Results from three experiments indicate that cells in control splenocyte cultures had an overall viability of 84±1% after 12 hr. In contrast to the total cell population, NK cell viability in control cultures was 36±3%. Cultures in which IL-2 (125 or 2000U/ml), polyinosinic polycytidylic acid (25μg/ml or 200μg/ml), or mouse growth hormone (5 ng/ml or 40 ng/ml) was added at the initiation of culture had significantly increased NK cell viability (78±2, 77±1, 56±3, 62±1, 48±1, and 49±3%, respectively). None of the agents added to these cultures significantly affected overall cell viability. This is not surprising, because NK cells constitute less than 5% of the cells in the spleen. These results suggest that part of the loss of NK cell activity is due to rapid and relatively selective loss of NK cell viability in culture systems. (Supported by NIEHS grant ES09158 and NIAAA grant AA09505.)

743 MERCURY: EFFECTS OF EXPOSURE ON *SCHISTOSOMA JAPONICUM* INFECTION IN MICE.

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Mercury (Hg) is a well described immunotoxin, with effects on both autoimmunity and host resistance to infection. We have previously reported that low level Hg exposure in mice can impair host resistance to murine malaria infection by *P yoelli*. In these studies, we extend our evaluation of Hg immunotoxicity to study the response of mice to infection by the trematode parasite *Schistosoma japonicum*. Schistosomiasis is a serious chronic parasitic disease that affects 150 to 200 million people worldwide. Cases of mercury poisoning have been reported in *S. japonicum* endemic areas in the Philippines. Mercury toxicity was induced in ICR mice by subcutaneous injections of $HgCl_2$ (100 μg/kg) every other day for 11 days. Following exposure to Hg, mice were infected with *S. japonicum* in two models: the acute infection model and the sensitized lung granuloma model, each with a control group. All infected mice developed hepatosplenomegaly. In both the sensitized lung granuloma model and the acute infection model, Hg exposure resulted in a significant reduction of granuloma area around *S. japonicum* eggs in either liver or lung tissue. These results are consistent with an inhibition of cell mediated immune response, as was described in our model of murine malaria infection. In the acute infection model we measured IgG antibody responses against soluble worm antigen preparations (SWAP) from *S. japonicum* using indirect ELISA. IgG antibodies to SWAP increased over the course of infection in all mice, but in male mice IgG levels were significantly lower in Hg treated mice as compared to controls. These results indicate that Hg exposure can also interfere with humoral immune responses in the schistosomal model. This is the first study of potential interactions between mercury and schistosomiasis infection. These findings have implications for the clinical treatment of schistosomiasis with praziquantel, acquired resistance to infection, and strategies for vaccine development. (Research supported in part by an NIH Fogarty ITREOH grant to EKS.)

744 MHC CLASS II EXPRESSION AND PROTEIN SYNTHESIS ARE NOT ALTERED IN EPIDERMAL LANGERHANS CELLS OF FEMALE BALB/C MICE FOLLOWING *IN VITRO* EXPOSURE TO 2-BUTOXYETHANOL.

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We have previously reported that topical exposure to 2-butoxyethanol (BE) significantly suppresses the local contact hypersensitivity response (CHR) in female BALB/c mice. As the primary antigen presenting cells in the skin, epidermal Langerhans cells (LC) play a key role in the CHR. Other studies have indicated that decreased MHC class II expression and/or protein synthesis in epidermal LC are ways in which chemical exposure can lead to suppression of the CHR. In order to uncover a potential mechanism for the observed immunosuppression following topical exposure to BE, we investigated the effects of BE exposure on MHC class II expression, protein synthesis and viability in epidermal LC. Freshly isolated epidermal cells were exposed *in vitro* to 10-10, 10-8 and 10-6 M BE for 48 hrs, then LC were assayed for MHC class II expression and viability using flow cytometric analysis. To study the effects of BE on protein synthesis, freshly isolated epidermal cells were exposed *in vitro* to 10-12, 10-10, 10-8, 10-6 and 10-4 M BE and pulsed with 1 microCi 3H-Leu/1 x 106 cells for 48 hrs. Uptake of 3H-Leu was then measured in a liquid scintillation counter. BE did not significantly alter viability, MHC class II expression or protein synthesis in epidermal LC of female BALB/c mice. We conclude that BE-induced immunosuppression of the CHR following topical exposure to BE is not due to reduced MHC class II expression, protein synthesis or viability of epidermal LC.

745 SUPPRESSION OF HUMAN CYTOKINE SECRETION BY CIGARETTE SMOKE.

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Although cigarette smoke is known to have detrimental effects on the immune system, the nature of this immune suppression is poorly understood. We have previously shown that two tar components, hydroquinone (HQ) and catechol, are potent inhibitors of lymphocyte proliferation. The purpose of the current study was to measure the effects of cigarette tar, HQ and catechol, on the production of proinflammatory cytokines. Cigarette smoke extracts were prepared by 'smoking' single cigarettes via a mechanically vacuum pump at 125 ml/min through 10 mls of RPMI1640. The levels of HQ and catechol in these extracts were quantitated by C18 reverse phase HPLC using an electrochemical detector set a 0.7 mV. Cytokine production was measured by pretreating 10^6 human lymphocytes/ml in RPMI1640 + 10% FBS with the tar extract, HQ or catechol, and then stimulating the cells with αCD3 + PMA for 24 hrs. Quantitation of the cytokines was performed by ELISA using standard curves with purified recombinant human cytokines. The levels of HQ and catechol were similar and directly proportion to the tar content of the individual cigarette; they ranged from 30-40μM (unfiltered *Camel*) to 15-20μM (*Marlboro*) to <1μM (*Carleton 100s*). Extracts from these three cigarettes blocked production of IL-1β, TNF-α, IL-2 and IFN-γ in a dose-dependent fashion, exhibiting >90% suppression even at 1:100 dilutions. HQ and catechol also blocked production of these cytokines by >90% at 40-50μM (IC_{50} ranged from 10-40μM for the different cytokines). Thus, level of HQ and catechol in 2-3 cigarettes is sufficient to induce near complete suppression of IL-1β, TNF-α, IL-2 and IFN-γ production. However, the potent inhibitory effects of the unfractionated cigarette tar cannot be accounted for by HQ and catechol alone. These experiments suggest that there may be additional immunosuppressive compounds present in tar that are even more potent than HQ and catechol, or there may be synergistic immunosuppression between several different compounds in cigarette tar. (Supported by NIH grants HL60538 and ES05673.)

746 AN ARYL HYDROCARBON RECEPTOR INDEPENDENT MECHANISM OF JP-8 JET FUEL IMMUNOTOXICITY IN TWO STRAINS OF MICE.

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JP-8 jet fuel is a kerosene-based, complex mixture of aliphatic and aromatic hydrocarbons with a high flash point and low vapor pressure. It is used extensively by both the military and commercial airlines and is the desired alternative to other fuel sources due to its resistance to crash-induced fires and evaporative losses. However, recent reports have shown that acute, relatively low dose exposures to aerosolized JP-8 vapors can overtly and persistently impair immune function in B6C3F1 mice. Thus far, no mechanism has been put forth to account for such immunotoxicity. Given the evidence that polycyclic aromatic hydrocarbons (PAHs) possess the ability to affect many aspects of the immune system through a putatively described mechanism which involves the aryl hydrocarbon receptor (AhR), it was hypothesized that JP-8 may exert toxicity through AhR-mediated signal transduction. To test this hypothesis, JP-8 was administered by oral gavage for 7-days to either a hydrocarbon 'responsive' strain of mice (B6C3F1) or a classically 'non-responsive' strain (DBA/2). The results show that each strain was equally sensitive at several endpoints when evaluated for spleen and thymus weight and cellularity, liver weight, B and T- cell lymphocyte proliferation, the plaque forming cell response, and western blots for CYP1A1/2 and AhR. Additionally, *in vitro* JP-8 administration in the murine Hepa – 1 and human THP-1 monocytic cell lines did not induce CYP1A1/2 or promote downregulation of the AhR protein. These results support a new hypothesis that JP-8 may exert its toxicity via an AhR independent mechanism.

747 HALOTHANE-INDUCED LIVER DAMAGE IN GUINEA PIGS: ROLE OF PROTEIN ADDUCTS AND ANTI-INFLAMMATORY CYTOKINES.

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The guinea pig model of halothane-induced liver injury resembles the mild acute toxicity produced by halothane in about 10 to 20% of humans and not the severe form of halothane hepatitis that appears to have an immunopathological basis. For example, in contrast to halothane hepatitis in humans, the severity of liver injury in susceptible guinea pigs does not increase after multiple exposures to halothane suggesting that immunopathological mechanisms are unlikely to be involved in the guinea pig model. The basis for susceptibility to halothane-induced hepatotoxicity and the mechanisms preventing immunological responses against trifluoroacetyl (TFA)-protein adducts were investigated. Male outbred Hartley guinea pigs were administered halothane (10 mmol/kg, i.p.) and livers and blood were collected after 48 and 72 hours. Immunoblot and immunohistochemistry studies indicated that the metabolism of halothane in livers of susceptible guinea pigs resulted in the formation of much higher levels of TFA-protein adducts than the resistant animals. Susceptible guinea pigs also had much higher levels of TFA-protein adducts in the sera than the resistant animals. These adducts were likely derived from damaged hepatocytes. At 48 hours and 72 hours after halothane treatment, we also found a high correlation between serum ALT levels and anti-inflammatory cytokines IL-10 and IL-4. These results indicate a role of TFA-protein adducts in the susceptibility of guinea pigs to halothane hepatotoxicity and suggest a pathway by which the liver TFA-protein adducts may come into contact with cells of the immune system to induce immune reactions. They also suggest that IL-10 and IL-4 may have a protective role in preventing immune reactions against TFA-protein adducts. Similar mechanisms may be important in the development of halothane hepatitis in humans.

748 TCDD SUPPRESSION OF IL-12 INHIBITS THE GENERATION OF THE TH1-MEDIATED IMMUNE RESPONSE TO OVALBUMIN.

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Interleukin (IL)-12 is a heterodimeric cytokine produced by antigen presenting cells (APC) which promotes the development of TH1-mediated immune responses. We have previously reported that TCDD exposure suppresses the TH1 response to ovalbumin (OVA) in the DO11.10 adoptive transfer model. TCDD suppressed the production of OVA-specific IgG2a antibodies and the production of IL-2 and IFN-from OVA-specific T cell receptor (TCR) transgenic (Tg) CD4+ T cells. These results led us to hypothesize that TCDD inhibits the generation of the TH1-mediated immune response to OVA via suppressed IL-12 production. To test this hypothesis, we measured splenic IL-12 production from adoptively-transferred mice following immunization with OVA. Results indicated that IL-12 levels from TCDD-treated mice were significantly suppressed over the course of the immune response to OVA when compared to vehicle-treated mice. Furthermore, the administration of recombinant IL-12 to TCDD exposed mice elevated anti-OVA IgG2a titers to levels normally detected in vehicle treated, OVA-immunized mice. Although adoptively-transferred OVA-specific T cells from TCDD-treated mice administered IL-12 produced greater amounts of IFN- not IL-2) upon restimulation, this effect did not appear significant enough to account for the restoration of anti-OVA IgG2a production in these animals. Recently, we have found that

TCDD enhances the deletion of OVA-specific TCR Tg+ CD4+ T cells in adoptively-transferred mice on days 4-7 post-immunization which could underlie the anti-OVA antibody suppression. However, IL-12 treatment of TCDD-exposed mice did not increase the frequency of OVA-specific CD4+ T cells when compared to vehicle treated mice suggesting that TCDD-induced deletion of CD4+ T cells is not responsible for the suppression of OVA-specific antibody production. These data indicate that TCDD may be inhibiting the generation of antigen-specific immunity by directly affecting APC functions such as IL-12 production. (This work was supported by NIEHS grants ES03966 and ES00040.)

749 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) EXPOSURE INCREASES A NOVEL CELL POPULATION IN THE SPLEEN OF P815 TUMOR INJECTED MICE: CHARACTERIZATION OF MAC1+GR-1+ CELLS.

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We have previously shown that TCDD caused a significant increase in Mac1+ cells in the spleen of C57Bl/6 mice in the P815 tumor allograft model. In the present studies, we report a similar increase in Mac1+ cells in the blood, and that these Mac1+ cells co-express Gr-1 antigen, a myeloid differentiation marker. Experiments were conducted to characterize the Mac1+Gr-1+ cells using flow-cytometric and immunohistochemical techniques. Mice were gavaged with vehicle or TCDD (15μg/kg), injected ip with P815 tumor cells and sacrificed on days 1 through 9 after P815 injection. The absolute number and percent of Mac1+Gr-1+ cells in the blood and the spleen gradually increased over time in both TCDD and vehicle groups, however, both the number and percent of the Mac1+Gr-1+ cells were consistently greater in TCDD-treated mice. For example, in the blood on day 9, we observed that 72% of the white blood cells were Mac1+Gr-1+ in the TCDD-treated mice, compared to 42% in the vehicle-treated mice. Likewise in the spleen, TCDD treatment resulted in 25% Mac1+Gr-1+ cells compared to 13% in vehicle-treated mice. Morphological analysis identified the Mac1+Gr-1+ cells in the blood as neutrophils. Immunohistochemical analysis showed that the Mac1+Gr-1+ cells were localized in the red pulp in the spleen. Flow cytometric analysis of the Mac1+Gr-1+ cells in the spleen on day 9 showed that these cells did not express the lymphocyte markers, CD4, CD8 or B220, or the dendritic cell marker, CD11c. Several costimulatory and adhesion molecules including ICAM, LFA-1, MHC Class I, CD40, F4/80 and B7-2 were expressed at various levels on the Mac1+Gr-1+ cells. TCDD exposure down-regulated the expression of B7-2 and ICAM on these cells. The possible immuno-regulatory role of Mac1+Gr-1+ cells in this model is currently under investigation. (Supported by NIEHS grant ES03966 and ES00210.)

750 CHARACTERIZATION OF MICE TRANSGENIC FOR A CONSTITUTIVELY ACTIVE MUTANT OF THE AH RECEPTOR SHOWS ALTERATIONS IN IMMUNE SYSTEM DEVELOPMENT.

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The Aryl Hydrocarbon Receptor (AhR) is generally assumed to mediate most toxic effects of dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and planar polychlorinated biphenyls (PCBs). This has been determined in several ways, most recently by using mice lacking a functional Ah receptor, which are resistant to the lethal wasting syndrome and cleft palate that is usually found after relatively high doses of TCDD. We have chosen an alternative transgenic strategy to study the toxic effects and mechanisms mediated by the AhR. A mutant form of the AhR which is constitutively active was defined and transgenic animals expressing this mutant AhR were created. The expression was targeted to the immune system where increased expression of CYP1A1 compared to wild type animals indicates that the mutant AhR is activated in the absence of exogenous AhR ligands. The immune system is affected in several ways in these mice. Relative thymus weights are decreased, which is considered to be a hallmark effect of dioxin exposure. The distribution of CD4/CD8-positive T-cells is not affected in the thymus but the CD4-positive T-cells are slightly increased in peripheral lymph nodes. The development of B-cells is skewed to a larger fraction of mature B-cells in both bone marrow and spleen. However, the most striking finding is the absence of a B-cell population in the peritoneum, belonging to the CD5-positive B1-cells. These transgenic mice clearly show that an activated Ah receptor can disturb the development of the immune system.

751 TCDD SUPPRESSES T CELL EXPANSION AND CYTOKINE PRODUCTION IN LYMPH NODES OF MICE INFECTED WITH INFLUENZA VIRUS.

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Halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are prevalent and highly toxic environmental contaminants. It is well established that TCDD-mediated immunosuppression includes impairment of antibody and cell-mediated immune responses. Suppression of immunity is attributed in part to the effects of TCDD on T cell function, although the underlying mechanism has not been determined. To examine the mechanisms of TCDD immunosuppression, we are using a murine model of human influenza A virus infection. During infection with influenza virus, antibody production and cell-mediated immunity depend on the activation and differentiation of T cells in the mediastinal lymph nodes (MLN). Specifically, viral clearance requires the differentiation of naive CD8+ T cells into cytotoxic T lymphocytes (CTL), which migrate to the lung and kill infected cells. We have previously shown that exposure to TCDD impairs antibody production and decreases recruitment of CTL to the lung during influenza virus infection. We hypothesize that these effects of TCDD are due to impaired T cell responses in the MLN. To test this, C57Bl/6 mice were gavaged with TCDD (10 μg/kg) one day prior to i.n. infection with influenza virus. After 1 to 9 days, mice were sacrificed and MLN cells were stained with fluorochrome-conjugated antibodies for flow cytometry. Our findings indicate that exposure to TCDD results in a two-fold decrease in CD4+ and CD8+ cell number in the MLN and a 50% decrease in the percentage of CD8+ T cells bearing the CTL phenotype ($CD44^{hi}CD62L^{lo}$). Cytokine analysis of *in vitro* restimulated MLN cells indicates that exposure to TCDD reduces IFN-γ and IL-2 production by 50%. These findings suggest that TCDD-mediated suppression of host resistance to influenza virus may be due in part to impaired activation and expansion of T lymphocytes in the regional lymph nodes.

752 SINGLE-DOSE ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN PRODUCES A TIME- AND DOSE-DEPENDENT ALTERATION IN THE MURINE BONE MARROW B LYMPHOCYTE MATURATION PROFILE.

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The halogenated aromatic hydrocarbon, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous, highly toxic environmental contaminant that has been shown to produce immunotoxic effects in mammals. Although its immunotoxicity has been widely reported, little is known regarding its effect upon the development of immune system cells, especially the B lymphocyte. The present study's purpose was to assess the effect that single-dose administration of TCDD has over time upon bone marrow B cell progenitors and pro/pre-B, immature B and mature B cell subpopulations. Results showed that the mature B lymphocyte subpopulation varied in a cyclic manner with a significant increase one day following TCDD treatment (30 μg/kg bw) followed by a significant decrease at day nine, and a return to near vehicle levels by day thirty-one. Pro/pre-B and immature B cell subpopulations were significantly decreased at days six and nine. The earliest B cell progenitor subpopulation increased until day nine and then decreased to vehicle-treated levels. We have shown that TCDD produces a rapid alteration in the B cell maturation profile which affects both less mature and mature subpopulations and is cyclical over time. This change correlates with increased numbers of B cell progenitors at the time at which the mature B subpopulation is at its lowest.

753 INVESTIGATION OF REYES-LIKE SYNDROME AND INFLUENZA MORTALITY IN DIOXIN-EXPOSED MICE.

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Mortality is increased in B6C3F1 mice exposed to 0.1μg TCDD/ kg before infection with influenza A virus. But, viral titers were not elevated and antiviral antibody titers were only suppressed at 10μg TCDD/kg, suggesting that viral overgrowth, secondary to immunosuppression, was not the proximate cause of death. We tested the hypothesis that a Reye's-like syndrome (RLS, mito- chondrial toxicity and dysfunction) was responsible for increased mortality in exposed, infected mice, based on similarities between biochemical and immunological events in RLS and TCDD-exposed animals. B6C3F1

mice were given a single i.p. injection of 0, 0.25, 0.5 or 10μg TCDD/kg 7 d before infection by intranasal instillation of 25 PFU of influenza A Hong Kong/8/68 (H3N2). Sub-groups of noninfected control and high dose group were evaluated for baseline values. Five days after infection, 7 mice were killed from each dose group; serum, lung lavage fluid (BALF) and lung tissue were collected for clinical chemistries, cell counts, cytokine analysis and viral titers. Viral titers were similar in all treatment groups. There was no effect on serum NH_3 or glucose concentrations, two prominent indicators of the altered mitochondrial oxidative metabolism typically observed in RLS. Total cell counts in the BALF were increased by TCDD exposure alone and to a greater extent in combined exposure/infected groups. An increased percentage of neutrophils relative to macrophages was found in the infected/high dose group, possibly due to an observed increase in BALF levels of MIP-2. The remaining 20 animals/group were followed for mortality; increased mortality was only observed at the highest dose. These results suggest that RLS is an unlikely explanation for increased influenza mortality in TCDD-exposed mice. (This abstract does not represent EPA policy.)

754 EXPOSURE TO TCDD CAUSES DELETION OF ACTIVATED ANTIGEN-SPECIFIC CD4+T CELLS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant that suppresses both cell mediated and humoral immune responses. The mechanism by which TCDD exerts its immunosuppressive properties is not completely understood. In addition, the cellular target(s) of TCDD is not known. T helper (CD4+) cell-mediated immune responses are particularly sensitive to the effects of TCDD. We hypothesize that one potential mechanism by which TCDD causes immune suppression is by augmented apoptosis in the antigen- responsive CD4+ population. Apoptosis plays a crucial role in immune function during lymphocyte maturation, activation, effector function, and down regulation of immune responses. In order to test our hypothesis, we used the DO11.10 transgenic T cell receptor (Tg-TCR) adoptive transfer model. This model allows us to track a small population of transgenic DO11.10 CD4+ T cells in syngenic Balb/c mice using a monoclonal antibody (KJ1-26) that recognizes the Tg TCR. Maximal expansion of the splenic antigen-specific CD4+ T cells (CD4+KJ1-26+) occurs 3 days after immunization with 2 mg of ovalbumin in complete Freund's adjuvant (OVA/CFA). Exposure to TCDD (15 μg/kg) did not affect the maximal expansion of the CD4+KJ1-26+ T cells. However, TCDD significantly decreased the percentage and number of CD4+KJ1-26+ T cells on days 5-7 post OVA/CFA administration in a dose responsive manner. This decrease correlated with a significant increase in the level of phosphatidylserine (PS) exposure, an early marker of apoptosis. These results suggest that TCDD is enhancing the deletion of the activated antigen-specific CD4+ T cells, at least in part through an induction of apoptosis of those cells. We also examined the expression of Fas, a molecule important in the induction of apoptosis which is upregulated upon activation. Surprisingly, Fas expression was decreased on the CD4+KJ1-26+ T cells from TCDD-treated animals 3-7 days after antigen administration. Additional studies are necessary to determine the mechanism by which TCDD causes the deletion of the antigen-specific T helper cells. (Supported by NIH grant ES00040.)

755 AHR KNOCKOUT MICE AND 2,3,7,8 TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IMMUNOTOXICITY: IMMUNE SUPPRESSION INDUCED BY TCDD IS DEPENDENT ON EXPRESSION OF AHR IN BONE MARROW-DERIVED CELLS.

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TCDD, a highly toxic environmental contaminant, suppresses both humoral and cell-mediated immune responses in the mouse. Many of TCDD's toxic effects are known to be dependent on the AhR, a cytoplasmic receptor which binds TCDD, translocates to the nucleus, and induces gene transcription. Although the AhR is implicated in the immune dysfunction caused by TCDD, some controversy exists as to its obligatory role. Data generated from *in vivo* studies have collectively supported the hypothesis that TCDD suppresses the immune response in an AhR-dependent manner. However, results from some *in vitro* studies have suggested that the suppression is AhR-independent. In order to resolve this issue, we examined the immune response of AhR knockout mice treated with an immunosuppressive dose of TCDD (15μg/kg). We found that TCDD exposure did not suppress the ability of AhR-/- mice to generate an antibody response to sheep red blood cells (SRBC) or a cytotoxic T cell (CTL) response to allogeneic tumor cells, while these responses were significantly suppressed in AhR+/+ mice. These results are consistent with previous *in vivo* studies and provide compelling evidence that the AhR is required for TCDD-induced immune suppression. Additional experiments using chimeric animals were performed to test the hypothesis that the immune suppression induced by TCDD results from a direct effect on hematopoietic cells. In these experiments, AhR+/+ mice were lethally irradiated and then reconstituted with bone marrow cells from either AhR+/+ or AhR-/- animals. When the chimeric animals were treated with TCDD, only those reconstituted with AhR+/+ bone marrow were sensitive to TCDD-induced suppression of the SRBC and CTL response to allogeneic tumor. Taken together, these results support the hypothesis that TCDD-induced immune suppression is AhR-dependent and results from a direct effect on hematopoietic cells. (Supported by NIH #ES03966 and ES00040.)

756 SENSITIVITY OF THE SRBC PFC ASSAY VS ELISA FOR DETECTION OF IMMUNOSUPPRESSION BY TCDD AND TCDD-LIKE CONGENERS.

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The antibody plaque-forming cell (PFC) assay to sheep red blood cells (SRBC) is considered to be one of the most sensitive assays for detection of immunosuppression, and traditionally has been used in immunotoxicity testing. However, an enzyme-linked immunosorbent assay (ELISA) (Temple et al., 1993), is gaining popularity as a more convenient and less expensive alternative. The PFC assay quantifies the number of anti-SRBC producing plasma cells in the spleen, while the ELISA measures SRBC-specific IgM antibody in the serum. This study was performed to compare the sensitivity of these two assays to detect immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and TCDD-like congeners. Two sets of 6-7 groups of female B6C3F1 mice (6-7 mice/grp, 8-weeks-old) were given a single oral exposure to corn oil or different doses of TCDD or congeners. Seven days later, mice were immunized i.v. with SRBC. The first set of mice was evaluated using the PFC assay and the second using the ELISA, on day 4 or 5 post-immunization, respectively. The four TCDD congeners tested were: 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 2,3,4,7,-pentachlorodibenzofuran (4PCDF), 3,3',4,4',5-pentochlorobiphenyl (PCB126) and 2,3',4,4',5-pentachlorobiphenyl (PCB118). The ED50s for TCDD and each congener were determined from the PFC and ELISA data. For all the chemicals tested the ED50s for the ELISA were lower than the ED50s for the PFC, with percent differences ranging from 33 to 84%. These results indicate that the ELISA is a more sensitive assay for detecting suppression of the SRBC-specific IgM response of dioxin-like chemicals in this rodent model and supports its use as a sensitive alternative to the PFC assay. (This abstract does not reflect EPA policy. This work was supported in part by The Dow Chemical Co. and The DuPont Co.)

757 EFFECTS OF TCDD ON PRIMARY TOXOPLASMOSIS IN C57/BL6 MICE.

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In the current study, the effect of of 2,3,7.8-tetrachlorodibenzo-p-dioxin (TCDD) on infection caused by *Toxoplasma gondii*, a protozoan parasite was studied. C57BL/6 mice were fed 25 tissue cysts of *T. gondii* and treated with sodium sulfadiazine to prevent death from acute disease. Six weeks later, mice were treated with 50μg/kg body weight TCDD and the extent of infection and immune status was determined. TCDD treatment of chronically infected mice did not cause differences in brain lesions or *T. gondii* tissue cyst numbers. It was, however, observed that there was a significant decrease in the thymic cellularity in uninfected or *T. gondii*-infected mice treated with TCDD but not the vehicle. Furthermore, alterations in the percentage and absolute numbers of CD4+, CD8+, CD4+CD8+and CD4-CD8- T cells were observed in *T. gondii*-infected mice following TCDD treatment. However, there were no changes in the splenic cellularity and T cell subsets in all groups of mice. Next, whether prior treatment with TCDD would influence the course of an acute infection with *T. gondii* in C57/BL6 mice was investigated. The results demonstrated that 50% of the vehicle-treated *T. gondii* infected mice and 100% of TCDD-treated *T. gondii* infected mice died from acute toxoplasmosis 11 to 13 days post-infection as indicated by the presence of tachyzoites in liver and lung impression smears. Results of the present study

indicate that prior exposure to TCDD can exacerbate a primary *T. gondii* infection in C57/BL6 mice.

758 MODELING THE CORTICOSTERONE AND STRESS-INDUCED SUPPRESSION OF TH1 AND TH2-RELATED ANTIBODY RESPONSES AND CYTOKINE RESPONSES IN B6C3F1 MICE.

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This work is part of project investigating the role of chemical-induced stress responses in immunosuppression. The goal of this study was to develop mathematical models relating an important stress mediator, corticosterone, and suppression of humoral immune responses and related cytokine responses. Female B6C3F1 mice were treated with corticosterone or restraint stress and the area under the corticosterone vs. time curve (AUC) was determined (*Tox. Sci.* 49:272). This parameter is an important part of these modeling studies, because it reflects cumulative corticosterone exposure over time, and it is a better predictor of immunological outcome than corticosterone concentration at any single time point. In separate experiments, mice were treated with the same regimen of corticosterone or restraint at an optimum time before immunization with keyhole limpet hemocyanin (KLH, 100 μg/mouse, i.p.). Two weeks later IgG1 and IgG2a antibodies specific for KLH were measured by ELISA. The results demonstrate similar patterns of suppression of the anti-KLH responses (both IgG1 and IgG2a) by restraint and by exogenous corticosterone, when the data are plotted as relative antibody response vs. corticosterone AUC. The slopes of the restraint and corticosterone lines were not significantly different, but the elevations of the restraint lines were significantly lower than those obtained with corticosterone, indicating greater suppression of the antibody response (at equivalent cumulative corticosterone exposure) in restrained mice. Similar patterns of suppression were noted for a Th1 cytokine (IL-2) and a Th2 cytokine (IL-4), except that restraint did not significantly decrease IL-2 production. Linear models from these studies should permit the prediction of at least the minimum expected suppression of antibody and IL-4 responses to KLH on the basis of the corticosterone AUC. In addition, the data demonstrate that the widely accepted view that glucocorticoids selectively suppress Th1 responses is not correct under all circumstances. (Supported by NIEHS grant ES09158.)

759 QUANTITATIVE ASSESSMENT OF CHANGES IN CYTOKINE GENE EXPRESSION AND I KAPPA B AND NF-KAPPA B PROTEINS INDUCED BY CORTICOSTERONE IN B6C3F1 MICE.

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Cytokines contribute to the toxicity of a number of drugs and chemicals. A number of these pro-inflammatory cytokines activate the hypothalamic-pituitary-adrenal axis, and the resultant increase in glucocorticoids is important in regulating cytokine production and preventing or diminishing adverse effects. However, the concentration and duration of endogenous glucocorticoids required to prevent or decrease cytokine synthesis has not been systematically investigated. In the present study, the major endogenous glucocorticoid of mice, corticosterone, was administered subcutaneously in a suspension of 2% beta-cyclodextrin in Hanks' Balanced Salt Solution. In a previous study, we determined the area under the corticosterone vs. time curve (AUC) produced by selected dosages of corticosterone (*Tox. Sci.* 49:272). This parameter is an effective measure of cumulative corticosterone exposure, and several corticosterone dosages have been identified that yield corticosterone AUC values similar to those induced by chemical or psychological stressors. In the present study these dosages of corticosterone were evaluated for their effect on the production of cytokines, using an RNAse protection assay with probes for 10 cytokines (Pharmingen, Inc.). Preliminary experiments indicated that the experimental design allowing the greatest sensitivity was as follows: Corticosterone (or dexamethasone as a positive control) was administered subcutaneously, then cytokine production was stimulated three hours later by intravenous administration of anti-CD3 antibody (50 μg). The spleen was removed 1.5 hr after anti-CD3 administration, and RNA was isolated. The RNAse protection assay indicated dose-dependent suppression of Interleukins 2, 4, 6, 13, and 15 as well as Interferon-gamma. In addition, there was a dose responsive increase in IL-10. It has been suggested that glucocorticoids suppress the expression of some cytokine genes by upregulating IkB gene expression and thereby preventing the activation of NF-kB. Initial experiments to determine the effects of corticosterone and dexamethasone *in vivo* on basal levels of these molecules indicate that there was a significant increase in IkB and a decrease in NF-kB, but these changes were transient, and further work is needed to demonstrate that they are responsible for changes in expression of IL-2 or other cytokines. These results quantify the corticosterone exposure required to regulate potentially harmful cytokine responses. (Supported by NIEHS grant ES09158.)

760 CPG DNA MOTIFS DO NOT ACTIVATE THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS OR AFFECT ITS ACTIVATION BY OTHER MEANS.

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Bacterial DNA containing unmethylated CpG motifs (CpG DNA) has adjuvant effects. Clinical trials are underway to evaluate the effectiveness of CpG DNA as an adjuvant in human vaccines. The adjuvant-associated increase in cytokines, however, has not been completely studied. Some cytokines activate the HPA axis and could lead to subsequent immunosuppression. Alternatively, if CpG DNA suppressed activation of the HPA axis, this could increase the probability of cytokine-induced shock syndrome. Therefore, this study was done to examine the effects of CpG DNA on activation of the HPA axis. Administration of CPG DNA (30-300 μg, kindly provided by Dr. Art Krieg) alone did not significantly increase serum corticosterone concentrations 1-4 hr after administration, nor did calf thymus DNA which was used as a control. Administration of CpG DNA (30 μg/mouse) prior to endotoxin (LPS) caused an increase in tumor necrosis factor-alpha (TNF-α) indicative of an adjuvant effect (7590 pg/ml by ELISA) compared to LPS alone (822 pg/ml). LPS, however, induced similar levels of corticosterone with (726 ng/ml by RIA) or without (676 ng/ml) CpG DNA, and these values were roughly half of the maximal stress-induced level. LPS alone over a broad range of dosages caused peak corticosterone levels similar to LPS plus CpG DNA. Exogenous TNF-α administered *in vivo* induced comparable peak levels of corticosterone with (560 ng/ml) or without (527 ng/ml) CpG DNA. An alternative stressor (restraint) yielded similar peak levels of corticosterone with (649 ng/ml) or without (660 ng/ml) CpG DNA. These results suggest that CpG DNA does not activate the HPA axis or alter its normal activation by other stimuli. Although it remains possible that such effects may occur under other circumstances, the present results do not indicate that alterations of the HPA axis will adversely affect the safety of CpG DNA adjuvants.(Supported by NIH grants ES09158 and AA09505.)

761 ROLE OF CORTICOSTERONE IN ETHYL CARBAMATE-INDUCED SUPPRESSION OF ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN FEMALE BALB/C MICE.

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A possible role of corticosterone in ethyl carbamate-induced immunosuppression was investigated in female BALB/c mice using the antibody response to a T-cell dependent antigen, sheep red blood cells, and/or subpopulation changes of thymocytes and splenocytes. When mice were treated with ethyl carbamate ip for 7 consecutive days at 100, 200 and 400 mg/kg, the antibody response was significantly suppressed and the weights of thymus and spleen were reduced. In addition, the level of serum corticosterone was increased. Flow cytometric analyses demonstrated that the numbers of splenic macrophages, B- and T-cells and thymic CD4+CD8- and CD4-CD8+ cells were significantly decreased by ethyl carbamate. Ethyl carbamate-induced suppression of antibody response was partially recovered when adrenalectomized (ADX) mice were treated with ethyl carbamate. In addition, the decrease in splenic numbers of B- and T-cells and macrophages by ethyl carbamate was partially blocked in the ADX mice. Moreover, a single dosing of corticosterone at 25 mg/kg to ADX mice caused a significant suppression of antibody response. These results indicate that ethyl carbamate-induced increase in corticosterone level may closely be related with ethyl carbamate-induced immunosuppression in female BALB/c mice. (Supported by a grant from the Ministry of Science and Technology, Korea.)

762 RESTRAINT STRESS MODIFIES DNCB-INDUCED LYMPH NODE CYTOKINE PRODUCTION, BUT NOT T CELL PROLIFERATION.

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The timing of a stressful event with respect to antigen exposure affects the development of the immune response. We have demonstrated that restraint applied prior to sensitization alters the ear swelling response and the pattern of cytokine production differently than restraint applied prior to challenge. We hypothesized that restraint would also modify the immune response to chemical in the draining lymph nodes. Male BALB/c mice were exposed on the dorsum of both ears on days 1, 2 and 3 with 1% or 0.5% di-nitrochlorobenzene (DNCB; n = 5) or vehicle only (n = 10), and restrained for 2 hours prior to chemical application on day 1 or day 3. We assessed T lymphocyte proliferation on day 5. To evaluate lymph node cytokine production, lymph nodes were removed on day 5 and cultured for 24, 48 and 72 hours. To assess these parameters following chemical challenge, we sensitized mice on the flank on days 1 and 2, challenged on day 6 immediately following restraint, and assessed T lymphocyte proliferation on day 8. We determined that, for all treatment paradigms, DNCB stimulated significant T cell proliferation which was not altered by restraint. Furthermore, we determined that DNCB-activated lymph node cells (LNCs) produced IL-6 and IFN-γ and that restraint enhanced both cytokines at all timepoints. For example, at 48 hours, the concentration of IFN-γ in non-restrained mice measured 83.5 pg/ml, whereas restrained mice produced 232 pg/ml. IL-6 production at 48 hours measured 87.5 pg/ml in non-restrained mice and 289 pg/ml in restrained mice. These data suggest that restraint stress modulates the lymph node immune response to chemical through changes in cytokine production that do not alter significantly T cell proliferation.

763 MECHANISMS OF METALLOTHIONEIN-MEDIATED IMMUNE SUPPRESSION: EFFECTS ON ANTIGEN PRESENTATION.

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Metallothionein (MT) is a stress-induced protein that can modulate the consequences of exposure to heavy metals and to reactive oxygen intermediates. MT can be induced by toxicants such as metal cations and acute phase cytokines. Recent work from a number of laboratories has suggested that metallothionein also plays an integral part in cellular mechanisms that are part of the signal transduction cascade. Metallothionein can serve as an essential reservoir of trace metals, regulate the activity of Zn-dependent transcription factors, and can act to maintain a reduced cytoplasmic environment. These roles are essential to normal cellular processes, including those that are part of an effective immune response. Previous work from our laboratory has demonstrated that exogenous MT can downregulate the developing immune response, reducing levels of antigen-specific humoral and cellular immune responses. In contrast, MT-null mice display increases in mitogen-responses and in antigen-specific humoral responses. This enhanced immune function in MT-null animals correlates with an increased NF-kB activity that is associated with the metallothionein-null phenotype. The experiments described here show that metallothionein can interfere with antigen processing and presentation, a hallmark of an effective immune response. By utilizing OVA-specific TH cell clones, we have shown that metallothionein interferes with IL-2 secretion from T cells after activation with antigen. For instance, Do11.10 T cells have a 20-30% decrease in IL-2 secretion when MT is added to cultures utilized in antigen presentation assays. This decrease in T cell function may be due to changes in lymphocyte apoptosis that occur in the presence of extracellular metallothionein. The observation that antigen-specific T cells isolated from MT-null mouse spleens have an approximate 30% increase in IL-2 secretion in antigen presentation assays further extends the idea that metallothionein synthesis is important in determining the effectiveness of an immune response. These results may imply that toxicants which upregulate metallothionein levels can modulate immune capacity through alterations in T cell survival, antigen processing and presentation. This would suggest a novel therapeutic avenue that might lead to clinical manipulations of toxicant-induced immune.

764 CHARACTERIZATION OF THREE ANTIBODY RESPONSE MODELS IN THE MOUSE: KINETICS AND SENSITIVITY TO CLASSICAL IMMUNOSUPPRESSANTS.

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The primary component of the Environmental Protection Agency (EPA) immunotoxicity guidelines (OPPTS 870.7800), is the antibody response to a T-dependent antigen; sheep red blood cells (SRBC). The selection of this functional endpoint was based in part on the results of the National Toxicology Program (NTP) validation studies that included an intralaboratory comparison and the characterization of over 50 different compounds. The EPA guidelines also indicate that the antibody response can be measured by either a plaque-forming cell (PFC) assay or an enzyme-linked immunosorbent assay (ELISA). The selection of the PFC assay was based on the substantial database generated by the NTP studies. The ELISA is not nearly so well characterized, but it is an attractive alternative because of numerous technical and methodological advantages. The overall goal of this investigation was to compare the sensitivity of the antibody response models to the classical immunosuppressants cyclophosphamide (CY) and benzo(a)pyrene (B(a)P). In addition to comparing the anti-SRBC response in the PFC assay, we included the antibody response to a second T-dependent antigen, Keyhole limpet hemocyanin (KLH), which was also measured by an ELISA. The selection of KLH was based on the observation that it appears to be the second most prevalent antigen in previous immunotoxicity studies and on the fact that it has been used in humans to assess immunocompetence. The three antibody response models were initially optimized as to the antigen concentration and the kinetics of the response. The results indicated a comparable sensitivity with the anti-SRBC PFC assay and the anti-SRBC ELISA in agreement with a previous study (Temple et al., 1993). In contrast, the anti-KLH ELISA was less sensitive to treatment with either cyclophosphamide or B(a)P. It is premature to conclude that KLH is an inappropriate choice as a second T-dependent antigen in immunotoxicology studies. Future studies will compare the sensitivity of these three antibody response models to additional chemicals.

765 EFFECTS OF EXERCISE STRESS OR PYRIDOSTIGMINE BROMIDE (PSB) ON IMMUNE FUNCTION PARAMETERS IN B6C3F1 MICE.

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Pyridostigmine bromide (PSB) and physiological stress have been identified as potential agents that contributed to symptoms reported by Gulf War veterans. These exposures in combination with other compounds may result in toxicity to the neuro-endocrine-immune axis thereby leading to the reported manifestations of the Gulf War veterans. To assess the immune effects of these exposures independently, adult female B6C3F1 mice were either gavaged daily for 14 days with PSB (0, 1, 5, 10, or 20 mg/kg) or were challenged daily for 14 days to physiological stress via forced exercise on a treadmill (0, 20, 40, or 60 minutes). Immune parameters assessed were lymphoproliferation, natural killer cell activity, the plaque-forming cell (PFC) response, thymus and spleen weight and cellularity, and thymic and splenic CD4/CD8 lymphocyte subpopulations. No effect was observed in lymphoproliferation or natural killer cell activity following either treatment. Exposure to PSB had no effect on thymic or splenic weights but exercise stress resulted in significant decreases in both spleen and thymus weight at the 40 and 60 minute time points. Both agents significantly decreased the PFC-response (at 1, 5, and 20 mg PSB/kg and at 40 and 60 minutes of exercise, respectively) and altered splenic and thymic CD4/CD8 subpopulations. These studies are a result of our intial efforts to determine the immunological effects due to combined exposure to exercise stress and PSB. Additionally, exposure to these agents will be assessed concurrently with other Gulf War relevant environmental agents such as JP-8 jet fuel and n,n,-diethyl-m-toluamide.

766 α-TOCOPHERYL SUCCINATE (TS) PREVENTS MITOCHONDRIAL COMPLEX I INHIBITOR-INDUCED HEPATOCYTE TOXICITY.

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Our laboratory has demonstrated that the exposure of rat hepatocytes to the glutathione (GSH) depleting agents, diethyl maleate (DEM) and ethyl methane sulfonate (EMS) results in mitochondria-mediated oxidative damage and cell death, that are prevented by TS (25 μM) treatment. Here we exam-

ined the role of GSH depletion (using a non-toxic concentration of DEM) and TS treatment in the toxicity induced by four specific mitochondrial complex inhibitors using freshly isolated rat hepatocyte suspensions. The inhibitors of mitochondrial complex I, rotenone (ROT, 100 μM) and complex II, thenoyltrifluoroacetone (TTFA, 100 μM), caused oxidative damage, as evidenced by the increase in lipid peroxidation and LDH leakage (an indicator of cell death) as well as the decrease of mitochondrial membrane potential (MMP). The depletion of GSH with 1 mM DEM dramatically potentiated both ROT and TTFA-induced oxidative damage to hepatocytes. TS (25 μM) totally prevented oxidative damage induced by either ROT or ROT + DEM but did not prevent TTFA or TTFA + DEM-mediated oxidative damage. Blockade of complex III by antimycin A (AA, 50 μM) and IV by cyanide (CN, 1 mM) collapsed MMP and caused cell death but did not increase lipid peroxidation. DEM or TS, however, had little effect on both AA and CN-induced toxicity. Our findings demonstrate that complex III and IV-induced cell death is not mediated by oxidative stress. In contrast, cell death caused by the inhibition of mitochondrial complexes I and II is related to oxidative stress and modulated by the depletion of GSH. TS treatment protects hepatocytes from oxidative damage resulting from the inhibition of mitochondrial complex I. Interestingly, the oxidative damage induced by mitochondrial complex II inhibitor was not prevented by TS, suggesting that TS cytoprotection may be associated with complex II activity. (Supported by NIH grant #R01ES05452.)

767 PHOSPHATIDYLCHOLINE HYDROPEROXIDE DECREASES MITOCHONDRIAL MEMBRANE POTENTIAL AND OXIDIZES CARDIOLIPIN.

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Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is a member of the family of selenium dependent enzymes that catalyze the reduction of hydroperoxides, using glutathione or other thiols as reducing substrates. Unlike the three other members of the family, PHGPx can directly reduce membrane phospholipid hydroperoxides. A mitochondrial-targeted form resides in the contact sites between the inner and outer mitochondrial membrane. A previous study using guinea pig cells showed that treatment with phosphatidylcholine hydroperoxide (PCOOH) induces loss of mitochondrial membrane potential ($\Delta\Psi m$) and that overexpression of PHGPx protects against this loss. The present study investigates whether the PCOOH-induced loss of $\Delta\Psi m$ is associated with oxidation of cardiolipin, a mitochondrial membrane phospholipid that is required for function of adenine nucleotide translocator (ANT), a component of the permeability transition pore. Mouse embyronic fibroblasts treated with PCOOH (0, 5, and 50 μM) showed a loss of $\Delta\Psi m$ ($p<0.01$), as indicated by a decrease in CMX-rosamine fluorescence analyzed by confocal imaging. In addition, the loss in $\Delta\Psi m$ was correlated with cardiolipin oxidation, indicated by a decrease in 10-nonyl acridine orange (NAO) fluorescence ($p<0.05$). Cytotoxicity also increased in a dose-dependent manner with PCOOH treatment ($p<0.01$). These results support the hypothesis that PHGPx protects against the loss of $\Delta\Psi m$, at least in part, by protecting mitochondrial membrane cardiolipin. Further studies will examine the interaction between PHGPx, ANT, and formation of the permeability transition pore, with which PHGPx is co-located. (Supported by NIH Grants ES07033 and ES07032.)

768 DOXORUBICIN-INDUCED CUMULATIVE AND IRREVERSIBLE MITOCHONDRIAL DYSFUNCTION.

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Interference with mitochondrial calcium regulation has been proposed to be a primary causative event to explain doxorubicin-induced cardiotoxicity. We previously reported disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration. The present study was designed to characterize the dose-dependent and cumulative mitochondrial calcium regulation and to assess the reversibility of this functional lesion. Sprague-Dawley rats were treated with 2 mg/kg/wk doxorubicin s.c. for 4-8 weeks. With succinate as substrate cardiac mitochondria isolated from rats after 4 weeks of treatment with doxorubicin expressed a lower calcium loading capacity compared to control. This suppression of calcium loading capacity increased with successive doses to 8 weeks of treatment ($p<0.05$), and persisted for 5 weeks after discontinuation of doxorubicin treatment. The decrease in cardiac mitochondrial calcium loading capacity was not due to bioenergetic changes in the electron transport chain, as the mitochondrial coupling efficiency was not altered by doxorubicin treatment. Preincubation of mitochondria from treated rats with tamoxifen, dithiothreitol, amifostine, or monobromobimane did not reverse the diminished calcium loading capacity. In contrast, incubation with cyclosporin A abolished any discernible difference in mitochondrial calcium loading capacity between doxorubicin-treated and saline-treated rats. Furthermore, it was found that the ADP/ATP translocase content was significantly diminished in mitochondria from rats receiving 8 weeks of doxorubicin treatment. These data indicate that doxorubicin treatment *in vivo* causes an irreversible and dose-dependent decrease in mitochondrial calcium loading capacity. The suppression of adenine nucleotide translocase content is possibly a critical factor responsible for the decreased mitochondrial calcium loading capacity, which in turn may account for the clinically observed cumulative and irreversible loss of myocardial contractility in patients receiving doxorubicin chemotherapy (Supported by NIH grant HL-58016.)

769 EFFECTS OF HYPEROXIA ON LUNG COASH AND COASSG CONCENTRATIONS IN THE RAT.

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CoASH is compartmentalized preferentially in the mitochondria and CoASH and its mixed disulfide with GSH (CoASSG) undergo thiol/disulfide exchange reactions with GSH and GSSG *in vitro*. We measured CoASH and CoASSG in freeze-clamped lung tissues from Fischer-344 (F344) and Sprague-Dawley (SD) rats exposed to >95% O2 or maintained in room air for 48 h to test the hypothesis that oxidant stresses on lung thiol status would be expressed predominantly in the mitochondria. Lung tissue concentrations of CoASH and CoASSG were not different in F-344 or SD rats, but CoASH levels were decreased following hyperoxia (example: F-344 declined from 6.4+/-0.8 to 3.0+/-0.6 nmol/g). The decline in CoASH levels was not accompanied by increases in CoASSG contents, which also declined from 0.9+/-0.15 to 0.5+/-0.13 nmol/g of lung; AcCoA levels were also lower in the lungs of rats exposed to hyperoxia. Lung mitochondrial SDH activities were not diminished in hyperoxia, indicating that the decreases in CoASH are not attributable to general mitochondrial destruction. Pulmonary edema was observed in hyperoxic rats and accounted for some of the declines in CoASH, per g of tissue or per mg of protein, but CoASH contents per total lung also declined. Total lung CoASSG contents were not lower in the rats exposed to hyperoxia, and CoASH/CoASSG ratios were lower in animals exposed to hyperoxia, consistent with an oxidant stress on this thiol/disulfide redox couple. In contrast, lung GSH/GSSG ratios did not decrease, which suggests a mitochondrially compartmentalized oxidant stress response to hyperoxia in the lungs of the rats is consistent with some form of compartmentalization or selectivity in the oxidant stress responses. The mechanisms and pathophysiological consequences of the decreases in lung CoASH levels are not evident from these studies, but the loss of more than half of the tissue contents of this critical biochemical intermediate is likely to generate additional effects.

770 INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION *IN VITRO* BY CARBOXYLIC ACIDS.

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Induction of the mitochondrial permeability transition (MPT) has been implicated in the mechanism of tissue injury for a variety of foreign chemicals, including oxidants and weak acids. We recently reported that acrylic acid induces the MPT *in vitro*, which we suggested might be a critical event in the acute inflammatory and hyperplastic response of the olfactory epithelium. The purpose of the present investigation was to determine if induction of the MPT is a general response to short chain carboxylic acids or if there are critical physical chemical parameters for this response. Freshly isolated rat liver mitochondria were incubated in the presence of varying concentrations of selected carboxylic acids. Induction and progression of the MPT was monitored spectrophotometrically. All of the acids that we tested caused a concentration-dependent induction of the MPT, which was blocked by cyclosporine A. Although the C5 carboxylic acids were slightly more potent than the C4 acids, there was no correlation with the degree of saturation, the octanol/water coefficient (logP) or the dissociation constant (pKa) of the acids that we tested. We conclude that induction of the MPT *in vitro* is a general response to short chain carboxylic acids having a pKa of 4-5 and that induction of the MPT may be an important causative factor in the acute inflammatory and/or hyperplastic response to this important class of compounds.

771 DIBROMOACETONITRILE (DBAN), A DRINKING WATER CONTAMINANT, INDUCES OXIDATIVE DAMAGE AND BASE EXCISION REPAIR IN MOUSE FIBROBLASTS.

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DBAN, a water chlorination byproduct detected in drinking water is a direct acting genotoxic agent. The mechanisms of DBAN adverse effects are not known. This study examines the mechanism of DBAN effects in mouse fibroblasts (Mβ16tsA) as these cells are widely distributed and participate in several repair processes *in vivo*. Fibroblasts were exposed to various concentrations (5 μM- 40 μM) of DBAN for 10- 240 min. Phase contrast microscopic and morphologic evaluation of DBAN-treated cells indicated features of apoptosis at low exposure levels and severe membrane damages at higher concentrations of DBAN. The data indicate a concentration and time dependent depletion (up to 80%) of total GSH, until 40 min. At later times, however, GSH concentrations were rebound. Malondialdehyde (MDA), was significantly increased (up to 5 fold). Increase in membrane damage was indicated by leakage of lactate dehydrogenase (up to 250% of control) and decreased viability (18% of control). The ability of fibroblasts to undergo DNA repair was studied using base excision repair assay (BER). BER was up regulated in cells exposed to 5μm DBAN for 10 min. and was inhibited at higher concentrations and longer time periods. These studies indicate that DBAN causes oxidative stress leading to membrane and nuclear damage in fibroblasts.

772 THE COMPARABILITY OF THE AMOUNTS OF 8-OXODG ON ISOLATED DNA DETECTED BY THE METHODS USING ELECTROPHORESIS AND HPLC-ECD.

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Quantitative detection of oxidative DNA damage in tissues is an important factor for risk assessment, especially of tumor development. First, 8-oxodG was produced on lambda DNA by methylene blue and visible light. Second, 8-oxodG was cut off by fpg protein enzymatically. And then we measured the number of fpg protein sensitive sites as oxidative DNA damage maker on isolated DNA using electrophoresis developed by Sutherland et al. and compared with the number of damaged DNA, especially 8-oxodG, measured by HPLC-ECD. When we define X as the number of 8-oxodG / 10 to the fifth dG measured by HPLC-ECD method and Y as the number of 8-oxodG / 10 to the fifth dG measured by electrophoresis method, Y and X were fitted to Y=1.19X+0.91 by simple regression analysis. Correlation coefficient was 0.93. Even though the specificity of the electrophoresis method is lower than that of the HPLC-ECD method, the values by two methods were similar. Furthermore, it is considered that this electrophoresis method is more useful than HPLC-ECD method when we measure several oxidative DNA damages at the same time, because we could measure other oxidative DNA damages by changing only DNA glycosylase. We are now planing to establish the measurement of oxidative DNA damages in living cells using electrophoresis method.

773 LIPID PEROXIDATION FORMS N^2,3-ETHENOGUANINE BY DIRECT ALKYLATION: STUDIES USING [$^{13}C_{18}$]-ETHYL LINOLEATE.

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Recently, a number of endogenous DNA adducts have been attributed to the reaction of DNA with products of lipid peroxidation. Here we demonstrate that N^2,3-ethenodeoxyguanosine (εG) is formed from the reaction of either deoxyguanosine (dGuo) or calf thymus DNA (ctDNA) with [$^{13}C_{18}$]-ethyl linoleate ([$^{13}C_{18}$]-EtLA) under peroxidizing conditions. [$^{13}C_{18}$]-EtLA was reacted with 200 nmol dGuo in the presence of *tert*-butyl hydroperoxide (t-BuOOH) at 50°C for 24 hr. εG was analyzed by immunoaffinity chromatography-gas chromatography-high resolution mass spectrometry (IA/GC/HRMS) of the pentafluorobenzyl derivative of its base. This reaction, using a 10-fold molar excess of [$^{13}C_{18}$]-EtLA, resulted in a 5700- and 400-fold increase in [$^{13}C_2$]-εG and unlabeled εG, respectively, compared to controls. This same reaction with [$^{13}C_{18}$]-EtLA in equimolar concentration resulted in a 1000- and 130-fold increase in [$^{13}C_2$]-εG and unlabeled εG, respectively, compared to controls. dGuo incubated in the presence of t-BuOOH, but no lipid, resulted in a 60-fold increase in εG, compared to controls. Increases in unlabeled εG suggested an alternative mechanism to direct alkylation by EtLA. To test the hypothesis that deoxyribose oxidation products form εG, dGuo was reacted with equimolar concentrations of [$^{13}C_{18}$]-EtLA and a 10-fold molar excess of thymidine, a source of deoxyribose. This reaction resulted in a 840- and 110-fold increase in [$^{13}C_2$]-εG and unlabeled εG, respectively, compared to controls. The amount of εG formed in the presence or absence of thymidine was comparable suggesting that dGuo was the source of unlabeled εG. In addition, [$^{13}C_{18}$]-EtLA was reacted with 300 μg ctDNA in the presence of t-BuOOH at 37°C for 24 hr. This reaction, using a 10-fold molar excess of [$^{13}C_{18}$]-EtLA, resulted in a 27- and 4-fold increase in [$^{13}C_2$]-εG and unlabeled εG, respectively, compared to controls. These data show εG is formed primarily from direct alkylation by ethyl linoleate under peroxidizing conditions. These data also show εG is formed from an alternative mechanism that may result from dGuo oxidation. (Supported by NIH grants ES07017, ES05779 and ES05948.)

774 REDUCTION OF WHITE BLOOD CELL DNA DAMAGE (COMET ASSAY) BY BLACK TEA CONSUMPTION IN SMOKERS AND NON-SMOKERS.

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DNA damage can occur in humans following exposure to endogenous and exogenous agents. DNA damage may lead to gene mutation, conformational changes in chromosomes and modulation of gene expression. Since DNA damage has been implicated in the process of tumorigenesis, aging, and other diseases, a reduction of DNA damage is an important strategy for chemoprevention agents. Black tea has been shown to possess antioxidant and anticarcinogenic properties. Using the Comet assay (single cell gel electrophoresis) we examined whether black tea consumption prevented DNA damage in smokers and non-smokers. Volunteers (males and females, 25-35 years of age; cigarette smokers (20 + /day) and nonsmokers) were provided a black tea beverage (2.4~4.8g tea solids) for 2 weeks and a placebo for 2 weeks. After treatment, blood was collected, white blood cells were separated, and assayed for DNA damage using the alkaline comet assay. While variation was seen between individuals, in general, smokers exhibited an increase in the Comet tail length and moment compared to nonsmokers. Consumption of black tea resulted in a significant decrease in Comet tail length and moment in smokers and in nonsmokers. In addition, oxidized purines in the WBC nuclei were also determined by using Fpg (formamidopyrimidine-DNA glycolase) enzyme pretreatment. Smokers showed a significant increase Comet tail length and moment in Fpg treated cells which was reduced following black tea consumption. Similar trends were seen in nonsmokers. These results suggest that black tea consumption had a protective effect against DNA damage (as measured by the Comet Assay) in smokers and non-smokers.

775 THE ROLE OF RAT PLASMA IN MANIFESTING TOXICITY OF ERYTHROCYTES BY MENADIONE: INVOLVEMENT OF REACTIVE OXYGEN SPECIES GENERATION.

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Our previous studies have demonstrated that nonenzymatic reaction of menadione with thiols in plasma generated reactive oxygen species, resulting in potentiation of the menadione-induced platelet toxicity. Since menadione, one of the representative quinone compounds, has been reported to cause hemolytic anemia *in vivo*, we hypothesized that erythrocytes could be one of the potential target tissues to menadione in the presence of plasma. To investigate the role of plasma in the erythrocyte toxicity by menadione and to identify reactive oxygen species derived from the reaction of menadione with plasma, rat plasma was treated with menadione sodium bisulfite (MSB), water soluble menadione. Treatment with MSB increased oxygen consumption rate as well as luminol- and lucigenin-amplified chemiluminescence in a dose-dependent manner. The chemiluminescence enhanced by luminol or lucigenin was inhibited by superoxide dismutase (SOD) addition, suggesting that superoxide anions were generated. When erythrocytes were suspended in plasma or buffer, MSB-induced chemiluminescence in plasma was larger than that in buffer, indicating that erythrocytes suspended in plasma were exposed to greater extent of free radicals than those in buffer. Consistent with these findings, we observed MSB-induced hemolysis only in erythrocytes suspended in plasma while not in those suspended in buffer. In order to identify the reactive oxygen species associated with cytotoxicity, various radical scavengers were tested to inhibit MSB-induced hemolysis. Addition with catalase or mannitol resulted in significant inhibition of hemolysis, while SOD had no effect. These results suggest that hydrogen peroxide and hydroxyl radical

rather than superoxide appeared to be involved in erythrocyte cytotoxicity although the reaction of plasma thiols with MSB was accompanied by superoxide generation.

776 PEROXIDATION AND EXTERNALIZATION OF PHOSPHATIDYLSERINE IN PLASMA MEMBRANE OF HL-60 CELLS DURING *TERT*-BUTYL HYDROPEROXIDE-INDUCED APOPTOSIS: ROLE OF CYTOCHROME *C*.

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We have previously identified selective phosphatidylserine (PS) oxidation as a potential signaling pathway preceding PS externalization in apoptotic cells following oxidant exposure. Here we address the subcellular localization and potential mechanism of PS oxidation during oxidant-induced apoptosis. We found that 20 min exposure of HL-60 cells to 150 μM *tert*-butyl hydroperoxide (*t*-BuOOH) was sufficient to induce apoptosis as shown by DNA fragmentation and changes in nuclear morphology. Apoptosis was accompanied by the translocation of cytochrome *c* from mitochondria to cytosol, as well as, activation of caspase-3. Lipid peroxidation in individual phospholipid classes was monitored using the oxidation-sensitive fluorescent fatty acid, *cis*-parinaric acid, metabolically incorporated in cellular phospholipids. Oxidation of major phospholipids, including phosphatidylcholine (PC) and phosphatidylethanolamine, was also observed 40 min after the 20-min *t*-BuOOH treatment. PS oxidation, however, was significantly greater than in other phospholipids and was accompanied by PS externalization determined by fluorescamine labeling of externalized aminophospholipids. Analysis of phospholipid oxidation in various subcellular fractions obtained after *t*-BuOOH treatment revealed that PS in plasma membrane was oxidized to a greater extent than PS contained in other cell organelles. HL-60 cells were "loaded" with exogenous cytochrome c in the presence of mild sonication. Selective oxidation of PS was observed after cytochrome *c* loading alone and was especially marked when "loaded" cells were challenged with *t*-BuOOH. Using a cell-free model system we observed that cytochrome c/H_2O_2 could effectively oxidize arachidonyl residues in purified PS but not in PC. Thus, selective PS oxidation during oxidant-induced apoptosis occurs in plasma membrane and may, in part, be mediated through the redox activity of cytochrome *c* released into the cytosol. PS oxidation may be a component of apoptotic signaling pathways serving to modulate PS externalization within the plasma membrane.

777 LIPID PEROXIDATION CAUSED BY SELENOCYSTINE AND ITS ENHANCEMENT BY INHIBITOR OF SELENIUM METHYLATION IN MICE.

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Selenocystine can be decomposed to selenide, and then selenide can be methylated to dimethyl selenide or trimethylselenonium ion *in vivo*. The methylation is regarded as detoxification process, because these methylated selenium compounds are excreted into exhalation and urine, respectively. On the other hand, selenide has the potency to generate active oxygen species if selenide reacts with oxygen molecule, and the lipid peroxidation is one of possible mechanisms of selenium toxicity. The effects of a methylation inhibitior, periodate-oxidized adenosine (PAD), on the lipid peroxidation and toxicity by selenocystine were examined in the present investigation in mice. Serum glutamate oxaloacetate transaminase (GOT), hepatic lipid peroxide (thiobarbituric acid reactive substances; TBA-RS), and content of selenium in the liver increased in a dose dependent manner six hours after the administration of selenocystine at a dose of 0, 10, 20, or 40 micromole/kg orally. Co-administration PAD with selenocystine increased GOT, TBA-RS, and the content of selenium in the liver significantly as compared with those of mice treated with selenocystine alone. Selenocystine also increased blood urea nitrogen (BUN), but not TBA-RS in the kidney; co-administration of PAD did not increase BUN nor TBA-RS. These results suggest that lipid peroxidation, possibly caused by selenide, is involved in the manifestation of toxicity of selenocystine in the liver.

778 METABOLISM OF 4-HNE BY RAT KUPFFER CELLS.

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The importance of Kupffer cells in CCl_4 and ethanol-induced liver damage has recently been demonstrated. Common to both of these animal models of hepatotoxicity is the cytotoxic process of lipid peroxidation. Lipid peroxidation is a cellular process that releases aldehydic products such as *trans*-4-hydroxy-2-nonenal (4-HNE), and is believed to play a major role in liver damage. 4-HNE is believed to directly activate Kupffer cells, resulting in production of biologically active products that modulate inflammatory responses, tissue and matrix remodeling, and regulate hepatocyte function. In order to determine whether freshly isolated rat Kupffer cells have the capacity to metabolize 4-HNE, we analyzed this cell type for alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and glutathione-*S*-transferase (GST) enzyme systems. Spectrophotometric analysis of ADH and ALDH enzyme activity determined that activity in Kupffer cells is difficult to quantitate through this particular method. Total GST enzyme activity was also determined spectrophotometrically using 1-chloro-2, 4-dinitrobenzene as a substrate. GST activity observed was 103±5.4 μmol/min/mg protein. Immunoblots demonstrate that Kupffer cells contain GST classes Ya, Yb, and P1-1, as well as ADH Class I and Class II ALDH. HPLC measurements of 4-HNE metabolism studies were conducted in freshly isolated Kupffer cells in which the cells were treated with 25μM 4-HNE. These studies indicate that 4-HNE is metabolized in a biphasic manner with a production of 4-hydroxy-2-nonenoic acid suggesting involvement of the ALDH enzyme system. These data suggest that Kupffer cells may have the capacity to metabolize and detoxify 4-HNE. (Supported by NIAAA F31 AA05536-02.)

779 EFFECT OF THE OXIDATIVE STRESS PRODUCED BY ETHANOL, ACETALDEHYDE AND ENDOTOXIN ON INTERLEUKIN-8 SECRETION BY HEPG2 CELLS.

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Chronic inflammatory responses, associated with large neutrophil influxes, occur following acute or chronic liver injury in response to a variety of hepatotoxic chemicals and infectious agents. Oxidative stress play an important role in these events. Cytokines, particularly tumor necrosis factor-α (TNF-α), and chemokines, such as interleukin-8 (IL-8), are important regulators of hepatic injury and repair following the insult. The objetive of the present work was to study the effect of oxidative stress produced by ethanol (EtOH), acetaldehyde (Ac) and LPS on IL-8 secretion by HepG2 cells. Cells were treated during 24 h with 50 mM EtOH, 175 μM Ac or 1μg/ml of LPS. Neutral red probe and MTT assay were performed to study the physiological damage produced by the toxics. Lipid peroxidation damage was determined by MDA production in the presence of thiobarbituric acid. GSH content was determined by Ellman's reaction and SOD activity by Winterbourn method. IL-8 secretion was determined by ELISA. Lisosomal damage, determined by neutral red probe, showed that EtOH treatment produced a 17% and Ac 41% decrease of OD comparing with control values. MTT assay resulted in an increase of 15% in EtOH and 43% in Ac of OD in control values. Lipid peroxidation damage increased 30% in EtOH, 43% in Ac and 33% in LPS comparing with control cells. GSH content decreased in 31% with EtOH, 42% with Ac and 21% with LPS. SOD activity diminished in all treatments, presenting the lower value the Ac treated cells. IL-8 secretion was determined in presence of the toxics, and the effect 10 mM of antioxidant N-acetyl-L-cysteine (NAC) and 1,1,3,3-tetramethyl-2-thiourea (TMTU) and policlonal anti-human TNF-α were also studied. IL-8 secretion increased 38% in EtOH, 41% in Ac and 38% in LPS treated cells. Both antioxidant treatments showed a decrease under control values in IL-8 secretion. Anti TNF-α treatment produced also a decrease in IL-8 secretion. GSH protects against oxidative damage, and oxidative stress has been associated with a decrease in these levels. In our study GSH content decreased while lipid peroxidative damage increased. SOD activity was also diminished as a result of hepatotoxic damage. HepG2 cells readily responded to antioxidant agents and anti TNF-α to control IL-8 secretion, an evidence that oxidative stress is a mediator in IL-8 production. An indirect participation of TNF-α in this process was also evaluated showing that is an important mediator of damage in this process.

780 EFFECTS OF BLEOMYCIN (BLM) ON LIVER ANTIOXIDANT ENZYMES AND THE ELECTRON TRANSPORT SYSTEM FROM *AD LIBITUM* AND DIETARY RESTRICTED FEMALE AND MALE FISCHER 344 RATS.

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Dietary Restriction (DR) is the only known intervention that delays aging and age-related diseases. Among the proposed mechanisms are: lowering free radical production, increased free radical detoxification and/or removal of oxidatively damaged macromolecules. In the present study, the effect of BLM on antioxidant enzymes and the electron transport system (ETS) complexes has been evaluated in different cellular fractions of liver in female and male Fischer 344 rats. Animals were fed *ad libitum* (AL, n=5) or 60% of the AL intake (DR, n=5) for 6 weeks. A single intra peritoneal injection of either 2.5, 5 or 10 mg BLM/kg body weight was given to rats at 20 weeks of age and they were sacrificed 4 weeks after a drug treatment. BLM significantly increased glutathione peroxidase and lactate dehydrogenase activities in liver cytosol of male AL rats more than in females. Similar changes were also noted for glutathione reductase and glucose 6-phosphate dehydrogenase activities in BLM-treated AL rats. Drug treatment had no significant effect on these enzyme activities in DR animals. In liver mitochondria, glutathione peroxidase was increased in female and male AL rats, but more so in females. Profound effects of BLM were noted in activities of complexes I, III and IV of ETS, in both AL and DR female and male rats; however, complex II demonstrated no significant diet or treatment effect. Induced antioxidant enzyme activities in BLM-treated AL rats may be a response to excessive free radical generation due to BLM metabolism in AL animals that is mitigated by DR. Further, dysfunction of ETS due to altered complexes I, III and IV (but not complex II) might suggest its role in a secondary mechanism for generation of free radicals during BLM metabolism contributing to its toxicity in AL animals which was modulated by DR.

781 *IN SITU* DETECTION OF QUINONE-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION IN INTACT CULTURED HEPATOCYTES USING LUMINOL CHEMILUMINESCENCE.

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Luminol-enhanced chemiluminescence (LCL) has been widely used to measure the production of reactive oxygen species (ROS) in cell-free systems or in subcellular fractions. Quantitative determination of drug-induced increased ROS production in intact cells has, however, remained difficult. We adapted the LCL method for measurement of ROS production in intact cultured hepatocytes because increased ROS production has been implicated in cell signaling and hepatic toxicity of numerous drugs. Short-term cultured murine hepatocytes were incubated with the redox-cycling agent menadione (2-methyl-1,4-naphthoquinone, MNQ). In the presence of luminol (10 μM) and horseradish peroxidase (13 U/ml) LCL was quantitated on-line with a thermostated chemiluminescence plate reader. MNQ-induced LCL increased non-linearly in a concentration- and temperature-dependent manner. Both catalase and dimethylthiourea strongly attenuated the signal, indicating that the signal is largely dependent on the presence of hydrogen peroxide. To further validate the method, other quinones, including 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), and 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), were used. As expected, ROS production was most pronounced in the presence of MNQ and DMNQ followed by NQ, whereas exposure of hepatocytes to BQ exhibited no detectable ROS production. These findings are in accordance with the known redox-cycling activities of DMNQ and MNQ and the increasingly prevailing arylating capacities of NQ and BQ. The data demonstrate that different quinones can be differentiated with respect to their ROS-producing capacities and that LCL measurements allow for a reliable detection and quantitation of drug-induced ROS production in intact cultured hepatocytes.

782 OXIDANT STRESS IN RAT LIVER FOLLOWING LIPOPOLYSACCHARIDE ADMINISTRATION: ROLE OF NITRIC OXIDE.

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The role of inducible nitric oxide synthase (iNOS) in LPS-induced hepatic injury was evaluated using the iNOS inhibitor L-iminoethyl-lysine (L-NIL). Male rats (250 g) were divided into three groups. One group received LPS (S. minnesota) 2 mg/kg i.v. A second group received LPS plus L-NIL (3 mg/kg i.p.) at the time of LPS administration followed by a second dose 3 hr later. A third group received saline i.v. At 6 hr, the rats were killed by an overdose of pentobarbital. Blood and liver were collected. Serum nitrate/nitrite (metabolic products of NO) levels (n=5) were increased from 5.4±1.5 nmole/ml in the saline group to 360±48 nmole/ml in the LPS group. Values for the LPS+L-NIL group was significantly reduced to 35±7 nmole/ml. Tissue malondialdehyde levels were increased from 0.20±0.02 nmole/mg (n=4) in the saline group to 0.41±0.03 (n=4) in the LPS group. L-NIL significantly reduced the values to 0.29±0.02 nmole/mg (n=4). 4-Hydroxylnonenal protein adducts levels were increased 3.6 fold by LPS treatment as compared to saline. L-NIL significantly reversed the levels to 1.6 fold (n=4). Intracellular glutathione levels were decreased from 8.49±0.64 nmole/mg (n=4) in the saline group to 5.63±0.51 nmole/mg in the LPS group (n=7). L-NIL significantly increased the levels to 7.04±0.46 nmole/mg (n=7). These data indicate that LPS-induced NO generation can result in oxidant stress in the liver and that inhibitors of iNOS may offer some protection in LPS-induced hepatic toxicity.

783 EFFECT OF N-ACETYLCYSTEINE AND α-TOCOPHEROL IN OXIDATIVE STRESS INDUCED BY LIPOPOLYSACCHARIDE AND ACETAMINOPHEN IN MICE.

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Lipopolysaccharide, an endotoxin produced by gram negative bacteria, has been known to induce endotoxemia in human and animals. Hyperthermia, anorexia, fatigue and tachycardia, etc. are well known symptoms observed in the case of endotoxemia.Acetaminophen is widely used as an antipyretic drug but its side effects of hepatotoxicity and oxidative damage are concerned.In this study, we investigated the profile of oxdative stress induced by lipopolysaccharide (LPS) and acetaminophen (AC) in mice with the indices of glutathione (GSH), glutathione disulfide (GSSG), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in serum or in liver of mice. And then, the anti-oxidative effects of N-acetylcysteine (NAC) or α-tocopherol (TC) on the oxidative stress induced by LPS or AC single or combined treatment.GSH and GSSG in serum and liver were decreased but MDA in liver was increased at 48 hours after the second treatment of LPS(5, 10 mg/kg B.W., ip). GSH in serum was decreased but GSSG in serum and GSH and GSSG in liver were decreased at 4hours after the 8th treatment of clinical dosage of AC(35 or 105mg/kg B.W., 2times per day for 4days orally). NAC(2 or 5 mmol/kg, ip) inhibited the GSH decrement and MDA increment induced by LPS and the decrement of GSH induced by AC. TC inhibited the increment of GSSG and MDA induced by LPS but showed none effect on the oxidative stress induced by AC. Finally, the decrease of GSH induced by LPS was inhibited more effectively by the combined treatment of AC and NAC than single treatment of AC and the increase of MDA induced by LPS was inhibited more effectively by the combined treatment of AC and TC than single treatment of AC.These results show oxidative stress induced by LPS can be alleviated by combined treatment of NAC or TC with the common antipyretic agent, AC.

784 PEROXYNITRITE CONTRIBUTES TO OZONE-INDUCED LUNG INJURY.

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Inhalation of ozone (O_3) causes epithelial cell damage and Type II cell hyperplasia. This is associated with the accumulation of macrophages in the lower lungs which have been shown to contribute to toxicity. Alveolar macrophages (AM) release a number of mediators with cytotoxic potential including nitric oxide and superoxide anion. These can react leading to the formation of peroxynitrite, an even more potent oxidant. To analyze the role of these mediators in O_3 toxicity we used transgenic animals lacking the gene for inducible nitric oxide synthase (NOSII) or overexpressing the gene for superoxide dismutase (SOD). Treatment of wild type control animals with O_3 (0.8-1 ppm) for 3 hr resulted in an increase in bronchoalveolar lavage (BAL) fluid protein reaching a maximum 24-48 hr after exposure. This was correlated with increased expression of NOSII protein and mRNA by alveolar macrophages and increased production of nitric oxide as well as peroxynitrite. O_3 inhalation also resulted in the appearance of nitrotyrosine staining in the lungs, a marker of peroxynitrite damage. In contrast, BAL protein was not increased in NOSII knockout mice or SOD overexpressors demonstrating that these mice were protected from O_3 induced epithelial injury. Moreover, AM from the transgenic mice did not produce increased amounts of nitric oxide or perox-

ynitrite even after O_3 inhalation. There was also no evidence of nitrotyrosine staining in the lungs. These data suggest that O_3 induced lung injury is mediated by peroxynitrite. To test this possibility directly we used a peroxynitrite scavenger, aminoethylselenourea (AESeU). AM from wild type control mice pretreated with AESeU (10-30 mg/kg) twice daily for three days produced significantly less nitric oxide than cells from ozone treated mice, and produced negligible quantities of peroxynitrite. Mice pretreated with AESeU were also protected from O_3 induced tissue injury as evidenced by reduced BAL fluid protein and the absence of nitrotyrosine staining. Taken together these data demonstrate that peroxynitrite contributes to O_3 toxicity. (Supported by ES04738, ES06897& Burroughs Wellcome Fund.)

785 NITRIC OXIDE-INDUCED COPPER DELIVERY BY METALLOTHIONEINS TO APO-SUPEROXIDE DISMUTASE.

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In mammalian metabolic pathways, toxicity of excess copper is prevented by its sequestration in: (i) the metal-binding metallothioneins (MT), or (ii) export via the copper-translocating ATPases. How effective are metallothioneins in these copper sequestration mechanisms and how are they linked to other mechanisms of copper regulation is unknown. We studied Cu transfer from Cu-MT to apo/Zn-SOD in cell-free model system and found that Cu_5-MT and Cu_{10}-MT were able to reconstitute SOD activity only in the presence of a nitric oxide donor, (Z)-[N-(3-ammoniopropyl)-amino]-diazen-1-ium-1,2-diolate (NOC-15). The percentage of reconstitution by Cu_5-MT and Cu_{10}-MT was 34% and 83% respectively, compared with that reconstituted by free Cu. Non-denaturing PAGE staining for SOD activity and protein showed that reconstitution of enzymatically active protein and shift from non-Cu containing band to 2-Cu containing bands only occurred in the presence of NOC-15 for both Cu_5-MT and Cu_{10}-MT. 2, 2'-Dithiodipyridine titration of MT SH-groups showed a 70% decrease after incubation with NOC-15. The transfer of Cu from Cu-MT to apo/Zn-SOD was not accompanied by enhanced Cu-dependent generation of ascorbate radicals or hydroxyl radicals as measured by EPR spectroscopy. Cu chelation assay using bathocuproine sulfonate (BCS) showed that NOC-15 induced reconstitution of apo/Zn-SOD did not require release of free Cu (from Cu_5-MT). Overall, our results suggest Cu-MT may function in a nitric oxide-dependent physiologic pathway for delivery of Cu to apo-SOD.

786 FREE RADICAL MEDIATED MECHANISMS OF METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT)-INDUCED DOPAMINERGIC TOXICITY IN PC12 CELLS.

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Despite the known neurotoxic effects of manganese on the dopaminergic system, methylcyclopentadienyl manganese tricarbonyl (MMT) has recently been legalized for use as a fuel additive in the United States. Data regarding the mechanism of action of this organic manganese compound on the CNS is greatly needed for appropriate risk assessment. It has been shown that MMT is a mitochondrial toxin, and inhibits aerobic respiration at Complex I in isolated mitochondria. Previously, we have shown that MMT dose-dependently increases cytotoxicity, extracellular dopamine (DA), and reactive oxygen species (ROS) in PC12 cells, an *in vitro* model of dopaminergic neurotoxicity. In order to further understand the causal factor in this toxicity, these endpoints were examined after 15 min pretreatment with antioxidants followed by MMT challenge. After exposure to MMT for 1 hr, cytotoxicity was measured by either the Trypan Blue Dye Exclusion Assay or by the LDH release method, DA was measured by HPLC-EC, and ROS were measured by the 2,7-dichlorofluorescin method. It was determined that Trolox, a Vitamin E analogue, prevents ROS formation, and also protects the cells against MMT-induced cytotoxicity. Further mechanistic studies in our lab have shown that MMT treatment causes NF-κB translocation, caspase activation, PKC translocation/cleavage and cytochrome C release within 2 hours of exposure, and these changes are consistent with mitochondrial damage and ROS mediated cell death. These data indicate that free radical-mediated mechanisms may be operative in MMT-induced dopaminergic toxicity, and further studies are being undertaken to determine if exposure could initiate or accelerate neurodegenerative processes that lead to dopaminergic disorders such as Parkinson's disease.

787 EXPOSURE OF H441 CELLS, A PULMONARY-DERIVED CELL LINE, TO 7-KETOCHOLESTEROL LEADS TO CYTOTOXICITY, WHICH IS PRECEDED BY INCREASES IN INTRACELLULAR GLUTATHIONE DISULFIDE (GSSG) CONTENTS.

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Premature infants commonly develop severe chronic lung disease, which is called bronchopulmonary dysplasia (BPD). The histological characteristics of BPD are arrested lung growth, lung cell damage and fibrosis. There is abundant evidence that BPD develops, in part, as a consequence of oxidant stress to the lung. However, the mechanisms by which oxidant stresses mediate cell injury and altered cell growth are unknown. Oxysterols, which are products of oxidation of cholesterol, have been studied in the context of atherogenesis and have been found to lead to cytotoxicity that was associated with alterations in cellular glutathione (GSH) contents and increased formation of reactive oxygen species. In preliminary studies, we detected oxysterols in the tracheal aspirate samples of intubated newborn infants. The purpose of the present study was to test the hypotheses that a specific oxysterol, 7-ketochosterol, mediates cytotoxicity in a pulmonary-derived cell line, and that the cytotoxicity is preceded by alterations in cellular GSH and/or GSSG contents. We exposed H441 cells, a transformed human lung cell line with characteristics of Clara cells, to 0, 5, 10 or 50 nmol/ml of 7-ketocholesterol for up to 48 h. At each time point, cell and media LDH activities were determined, as well as cellular contents of GSH and GSSG. We found that media LDH activities doubled in the plates of cells exposed to 50 nmol/ml for 24 h and in the plates of cells exposed to 10 nmol/ml for 48 h, suggesting that these two doses of 7-ketocholesterol lead to significant cytotoxicity. Cellular LDH activities were also diminished in plates of cells exposed to the two highest doses of 7-ketocholesterol. Surprisingly, there was no effect of 7-ketocholesterol on cellular GSH contents in any of the plates of cells. However, cellular GSSG contents showed a marked dose response to 7-ketocholesterol as early as 12 h of exposure (1.91±0.03 vs. 2.46±0.12, vs. 3.26±0.58, vs. 4.40±0.73 in cells exposed to 0, 5, 10 and 50 nmol/ml respectively, $P<0.05$). These surprising early increases in cellular GSSG contents in cells exposed to 7-ketocholesterol suggest that the compound's cytotoxicity is mediated through a rapid increase in cellular peroxide production, or by attenuating the reduction or export of GSSG from the cells. Studies to determine mechanisms for the increases in cellular GSSG levels mediated by 7-ketocholesterol are warranted and may lead to important mechanistic insights into the cellular basis for cytotoxicity mediated by 7-ketocholesterol.

788 A PRELIMINARY STUDY ON THE EFFECTS OF DIETARY VITAMIN E AND C SUPPLEMENTATION ON ANTIOXIDATIVE ENZYMES IN RATS.

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Sponsor: R. R. Dalvi.

The aim of this study was to evaluate the effect of high levels of dietary supplementation of vitamin E and C on the antioxidant status of rat tissues [pectoralis major (PM), sartorius (S) and Liver (L)]. Male rats were fed either Control (rodent diet), vitamin C (VC) supplemented, vitamin E (VE) supplemented or combination of vitamin E and C (CVEC) supplemented diets for 28 days. At the end of experiment, all rats were sacrificed and PM, S and L tissues were analyzed for selected antioxidative enzymes, catalase (Cat) and gluthathione peroxidase (GPx) levels were assayed. GPx activity was lower in PM with VC, VE, and CVEC supplementation when compared to control. GPx activity was lower in S with both VC and VE and was higher with the CVEC. GPx activity was lower in L with VE and showed no changes when compared to other diets or with control. Cat activity in PM was higher with all vitamin supplementations when compared to control. Cat activity in S was higher in both VC and CVEC. However, VE increased Cat activity in S compared to control. This preliminary data indicates that dietary supplementation of VC and CVEC increased Cat activity. Whereas the supplementation of VC, VE and the CVEC reduced the activity of GPx in both Pm and S.

789 ANTIOXIDANT LOADING PROTECTS FROM BLAST OVERPRESSURE-INDUCED OXIDATIVE STRESS.

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Detonation of explosives or firing of large caliber weapons during military operations or training and occupational high energy impulse noise, produce blast overpressure (BOP) waves characterized by rapid rise in atmospheric pressure above ambient. Exposure to BOP waves can cause injury, predominantly to the hollow organs accompanied by hemorrhage, edema, and hypoxia. We observed in rats that BOP induces free radical-mediated oxidative stress characterized by antioxidant depletion, increased lipid peroxidation, and decreased hemoglobin (Hb) oxygenation. We examined whether loading the rats with pharmacological doses of antioxidants can protect from BOP. Sprague-Dawley rats weighing 300-350 g were loaded with vitamin E (vE), vitamin C (vC) or lipoic acid (LA) for 3 consecutive days before a BOP exposure. Antioxidants were administered by gavage using 2-ml vehicle (corn oil or distilled water) alone or containing 800 IU vE, 25 mg LA or 1000 mg vC. Each regimen was subdivided into 4 groups (6 rats/group): vehicle control and exposed, and antioxidant control and exposed. After the 3-day loading, all rats were deeply anesthetized with sodium pentobarbital, then the exposed groups were subjected to a low-level BOP at 62±2 kPa peak pressure of 5 msec duration. One hour after exposure, the rats were euthanized then blood and lungs were analyzed for biochemical alterations. We found that antioxidant loading resulted in elevated Hb oxygenation, decreased lipid peroxidation, and smaller lung weight increases. These observations suggest that preloading with pharmacological doses of antioxidants for only three days can alleviate BOP-induced oxidative stress. The order of antioxidant efficacy was vE>LA>vC. These observations have clinical and occupational implications.

790 FREE RADICAL SCAVENGING *VS* BCL-2 INDUCTION IN ANTIAPOPTOTIC EFFECT OF ESTROGENS IN MCF-7 BREAST CANCER CELLS.

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Since estrogens are known to act as radical scavengers their antiapoptotic activity may be related the antioxidant effects. Alternatively, this activity may be due to estrogen receptor- and estradiol-induced overexpression of the antiapoptotic gene, *bcl-2*. To experimentally resolve these alternative pathways we studied long-term and acute effects of estrogens on phospholipid peroxidation induced in MCF-7 human breast cancer cells by a lipid-soluble azo initiator of peroxyl radicals 2,2'-azobis(2,2-dimethylvaleronitrile), AMVN (addition of 10^{-8}-$5x10^{-7}$M estradiol to the medium immediately or for 14 days preceding AMVN treatment). Incubation of control MCF-7 cells with AMVN resulted in oxidation of major phospholipid classes: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Acute exposure of MCF-7 cells to estradiol resulted in protection against AMVN-induced oxidation of PI and PS, and a trend towards such protection of PE and PC. Long-term exposure to estradiol caused selective protection of PS, whose oxidation is a critical component of the final common pathway for apoptosis. Not surprisingly, protection of PS was accompanied by a 2-fold decrease in the percentage of cells demonstrating apoptotic morphology after a 24 h exposure to AMVN. Additionally, long-term exposure to estradiol yielded an increase in GSH level in MCF-7 cells while short-term incubation had no effect of GSH content. Our results indicate that estrogens may act as both free radical scavengers and antiapoptotic agents in breast cancer cells.

791 MYELOPEROXIDASE-CATALYZED ONE-ELECTRON GENERATION OF ETOPOSIDE PHENOXYL RADICALS IN VIABLE HL60 CELLS.

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Etoposide (VP-16), a widely used phenolic antitumor drug, is also known to cause secondary acute myeloid leukemias. We hypothesized that VP-16 genotoxicity is associated with its one-electron oxidative metabolism to its phenoxyl radical catalyzed by myeloperoxidase (MPO) in bone marrow progenitor cells. Our previous work demonstrated that both purified MPO and MPO activity in homogenates of human leukemia HL60 cells were able to catalyze formation of VP-16 phenoxyl radicals in the presence of H_2O_2. In the present study, we attempted to identify conditions compatible with the detection of VP-16 phenoxyl radicals in viable HL60 cells. We found that VP-16 phenoxyl radical could be directly observed in HL60 cells by EPR spectroscopy if: (i) MPO activity was sufficiently high (≥ 10 nmol quaiacol/min/10^6 cells), (ii) endogenous GSH was substantially depleted (> 75% by 5 min preincubation with a maleimide SH reagent, ThioGlo™-1), and (iii) endogenous catalase was inhibited (by pretreatment with 3-amino-1,2,4-triazole). In HL60 cells grown in the presence of an inhibitor of heme synthesis, succinyl acetone (SA, 500 μM), both decreased MPO activity and VP-16 phenoxyl radical production was observed. Importantly, a significantly enhanced VP-16-induced formation of DNA/topoisomerase II complexes was found in HL60 cells under conditions where GSH concentration was depleted by 54% as a result of ThiolGlo™-1 treatment (1.0 μM, 5 min) and VP-16 phenoxyl radical was immediately detectable by EPR. We conclude that VP-16 phenoxyl radical formation may be essential for VP-16-induced geno- and cytotoxicity through either their direct effects on topoisomerase II and/or indirectly on intracellular GSH.

792 REDOX-CYCLING OF PHENOLS CAUSES DEPLETION OF GSH, OXIDATIVE STRESS AND CYTOTOXICITY IN NORMAL HUMAN EPIDERMAL KERATINOCYTES (NHEKS).

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Exposure to phenolic compounds is associated with neuro-, myelo-, immuno-, geno-, and dermotoxicity. In skin, phenols cause rash and inflammation, contact and irritant dermatitis, leucoderma and cancer promotion. The biochemical mechanisms responsible for cytotoxicity of phenolic compounds are not well understood. We hypothesized that the cytotoxic effects of phenolic compounds are due, at least in part, to the generation of phenoxyl radicals via their enzymatic one-electron oxidation which alters the intracellular pool of GSH and protein sulfhydryls. To test this hypothesis, we measured cell viability, intracellular levels of GSH and protein SH-groups, as well as lipid peroxidation in NHEKs exposed to twelve different phenolic compounds. We found that an 18 h incubation of cultured NHEKs in the presence of 100-500 μM 1,4-benzenediol (hydroquinone), *o*-hydroxybiphenyl (2-phenylphenol), bis-(4-glycidyloxyphenyl)-methane, bis (4-hydroxyphenyl)-dimethylmethane (bisphenol A), 4-*tert*-butylcatechol, 1,2-benzenediol (catechol), *iso*-eugenol (2-methoxy-4-propenylphenol), eugenol (4-allyl-2-methoxyphenol) or phenol caused pronounced cytotoxicity and significant depletion of GSH. In addition, concentration-dependent cell detachment, loss of cell-cell contacts, and disruption of the cellular membrane were observed. Importantly, the phenolic compounds that did not decrease thiol levels, such as 3-n-pentadecylphenol, 4-*tert*-butylphenol, or bis (4-hydroxyphenyl) methane, did not induce cytotoxicity after a 18 h treatment. Incubation of ascorbate-preloaded keratinocytes with phenols produced an EPR-detectable signal of ascorbate radicals indicating that redox-cycling of the one-electron oxidation product of phenol, its phenoxyl radical, is likely involved in these oxidative effects. While phenolics are known to act as radical scavengers, their enzymatically formed metabolites, phenoxyl radicals, can react with vital thiol reductants and cause cytotoxic effects.

793 OXIDATIVE STRESS FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: ASCORBATE RADICAL AS A TOOL TO DETECT FREE RADICAL ACTIVITY.

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Several studies failed to detect generation of oxygen radicals following traumatic brain injury (TBI) using ESR of spin traps. We attempted to detect any spin adducts of oxygen radicals in the ESR spectra of brain samples taken at different times after TBI (1.0, 2.5, and 6 hr) from animals injected with high doses of DMPO (ip, 80 mmol/kg). We found that the concentration of DMPO in the brain 1 h after injection was ≈10.0 mmol/kg and declined by 6h to ≈2.0 mmol/kg. We demonstrated that endogenous ascorbate was able to outcompete DMPO as the radical scavenger, hence prevent any detection of DMPO adducts with oxygen radicals. We used a catalytic system to generate hydroxyl radicals (H_2O_2/Fe^{2+}) in brain homogenates in which we manipulated concentrations of DMPO and endogenous ascorbate (using ascorbate oxidase).

The formation of DMPO-OH adducts was detectable after elimination of ascorbate by ascorbate oxidase. In all other conditions, only ESR signal of ascorbate radical was readily discernable in the spectra. We further utilized ascorbate as an endogenous spin trap in the brain. We found that 1.5, 4 and 6 h after trauma brain homogenates produced higher steady-state concentrations of ascorbate radicals than controls. Accordingly, the content of ascorbate was significantly lower in the samples of traumatized brain than in the controls (53.9 in control vs 34.8 nmol ascorbate/mg protein vs 6 hr after trauma, respectively). We conclude that monitoring ascorbate radical formation in oxidatively challenged brain is instrumental for detection of free radical activity.

794 PHOSPHINE-INDUCED OXIDATIVE DAMAGE IN RATS.

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Phosphine (PH_3), from hydrolysis of aluminum, magnesium and zinc phosphide, is an insecticide and rodenticide. Earlier observations on PH_3-poisoned insects, mammalian cell lines and humans led to the proposed involvement of oxidative damage in the toxic mechanism. This investigation focused on PH_3-induced oxidative damage in rats and antioxidants as candidate protective agents. Male Wistar rats were treated ip with PH_3 at 2 mg/kg. Thirty min later the brain, liver, and lung were analyzed for glutathione (GSH) levels and lipid peroxidation (as malondialdehyde and 4-hydroxyalkenals) and brain and lung for 8-hydroxydeoxyguanosine (8-OH-dG). PH_3 caused a significant decrease in GSH concentration and elevation in lipid peroxidation in brain (36-42%), lung (32-38%) and liver (19-25%) and significant increase in 8-OH-dG in brain (70%) and liver (39%). Antioxidants administered ip 30 min before PH_3 were melatonin, vitamin C and β-carotene at 10, 30 and 6 mg/kg, respectively. The PH_3-induced changes were significantly or completely blocked by melatonin while vitamin C and β-carotene were less effective or inactive. These findings establish that PH_3 induces and melatonin protects against oxidative damage in the brain, lung and liver of rats and suggest the involvement of reactive oxygen species in the genotoxicity of PH_3.

795 6-HYDROXYDOPAMINE RELEASES IRON FROM SERUM TRANSFERRIN: OXYGEN RADICAL PRODUCTION AND TOXICITY.

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The dopamine analogue, 6-hydroxydopamine (6-OHDA), is selectively toxic to catecholaminergic neurons. Because of its selectivity for neuroblastic cells in the sympathetic nervous system, 6-OHDA has been suggested as a chemotherapeutic agent for targeted treatment of patients with neuroblastoma. We tested the hypothesis that toxicity of 6-OHDA is caused by its interaction with serum ferric-transferrin (Fe-TF) resulting in release of iron, that through its redox-cycling by 6-OHDA triggers generation of reactive oxygen species. 6-OHDA-induced release of iron from Fe-TF was demonstrated by: 1) Decay of the characteristic low-temperature EPR signal of Fe-TF (g=4.3) and appearance of the high-spin signal from iron chelated by 6-OHDA oxidation products. 2) Spectrophoto-metrically-detectable complex of iron with Fe^{2+} chelator, ferrozine. 3) Redox-cycling of ascorbate yielding EPR-detectable ascorbate radical formation. 4) Generation of hydroxyl radicals as evidenced by EPR of its adduct with a spin trap, DMPO. In human plasma, 6-OHDA caused iron release only under nitrogen but not under air or oxygen. The absence of 6-OHDA effect in plasma under aerobic conditions was due to its ferroxidase activity (hence, reuptake of Fe(III) by apo-TF) and catalytic oxidation of 6-OHDA by ceruloplasmin. Modeling of these plasma activities by a stable nitroxide radicals, TEMPOL, resulted in protection of plasma Fe-TF against iron release under nitrogen. Parenteral administration of 6-OHDA to mice resulted in iron release from Fe-TF as evidenced by transformation of the Fe-TF EPR signal that was indistinguishable from that seen *in vitro*. Administration of the iron chelator, deferoxamine, to mice prior to administration of toxic doses of 6-OHDA resulted in a decrease in activity impairment of mice as compared to that seen with 6-OHDA alone. These findings suggest that iron-chelators may be used for prevention of 6-OHDA toxicity.

796 SUPEROXIDE RADICALS ARE INVOLVED DURING REDUCTION OF ALAMAR BLUE.

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AlamarBlue (AB; Alamar Biosciences, Inc.), a fluorogenic compound, is used to assess cell proliferation and cell-mediated cytotoxicity in various mammalian and bacterial cells. Because the AB assay offers the advantages of technical simplicity, freedom from radioisotopes and reproducibility and sensitivity, it is now the method of choice for assessing cellular phenomena. However, the mechanism(s) of such reduction by cellular activity has not been elucidated. We found that superoxide radicals (O_2-), generated by xanthine oxidase (X.O.) acting on xanthine, were able to reduce AB and this reduction was found to be susceptible to inhibition by superoxide dismutase (SOD) at pH 6.0, as well as at pH 7.0. The rate of reduction of AB by xanthine + X.O., and sensitivity of this reduction to inhibition by SOD were both pH-dependent. The iron-chelator desferoxamine (10^{-4}M), hydroxyl radical scavenger mannitol (6 mM), and catalase (1,000 units), all had trivial effects on the rate of AB reduction. EPR spectroscopy in combination with spin trapping techniques, using the X.O. system in the presence of DMSO to generate O_2- and DMPO as a spin trapping agent, was used to monitor the O_2- scavenging ability of AB. In this assay, AB was found to inhibit DMPO-O_2- adduct formation in a dose-dependent manner. These data clearly demonstrate that AB is a potent scavenger of O_2- and assays utilizing AB, to assess the metabolic activity of cells, may actually be monitoring the intracellular production of O_2-.

797 TOCOPHERYL SUCCINATE CYTOPROTECTION IS ASSOCIATED WITH A UNIQUE UPTAKE ADVANTAGE IN ISOLATED RAT HEPATOCYTES.

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Previous studies from our laboratory have demonstrated that administration of 25 μM tocopheryl succinate (TS) completely protects hepatocyte suspensions from the toxic effects of ethyl methanesulfonate (EMS). EMS is an alkylating agent that depletes cellular glutathione resulting in lipid peroxidation and cell death and has an onset of action approximately 90 minutes after exposure. In contrast to TS, protection toward EMS was not observed with administration of equimolar tocopherol (T) or tocopheryl acetate (TA). We propose that the selective cytoprotection afforded by TS is associated with the rapid uptake and subsequent hydrolysis of TS to T in mitochondria, the subcellular source of reactive oxygen species in EMS toxicity. To test this hypothesis, we measured cellular and mitochondrial T levels by HPLC in hepatocytes exposed to TS, T and TA. Unfortunately, the lipophilic nature of vitamin E prevented an accurate assessment of these compounds in cell homogenate and subcellular fractions. We therefore determined the cellular and mitochondrial antioxidant capacity as a measure of active T incorporation using a novel fluorimetric lipid peroxidation assay which examines the susceptibility of membranes to ferrous/ADP:ascorbate (FeAs) prooxidant stress. Results from these studies showed that hepatocytes incubated with TS were completely protected against FeAs-induced lipid peroxidation within 1 h, as were the mitochondria isolated from these cells. In contrast, hepatocytes required over 2 h incubations with T and TA to provide significant resistance to FeAs-induced lipid peroxidation. Thus the antioxidant capacity of hepatocytes and mitochondria from incubations with T, TS or TA correlated with the relative protective abilities of these compounds toward EMS toxicity. We conclude that the rapid uptake and hydrolysis of TS in mitochondria results in enhanced antioxidant protection of mitochondria and hepatocytes against the acute toxic effects of EMS. (This work was supported by NIEHS/NIH grant # R01ES 05452.)

798 FREE RADICAL INVOLVEMENT IN THE POTENTIATION OF NOISE INDUCED HEARING LOSS (NIHL) BY CARBON MONOXIDE (CO).

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Potential hypotheses for cochlear toxicity following simultaneous exposure to noise and carbon monoxide (CO) includes free radical involvement. Simultaneous exposure to noise and CO results in potentiation of noise induced hearing loss (NIHL) by CO. In this study, protective effects of phenyl-*N-tert*-butylnitrone (PBN - a known free radical scavenger) against potentiation of NIHL by CO has been demonstrated. In addition, electroparamagnetic spectroscopy (EPR) was used to demonstrate the presence of free

radicals in the cochlea. Auditory sensitivity was evaluated in Long Evans hooded rats exposed to conditions that show optimum potentiation. Treatment groups included rats administered PBN(ip) pre- *and* post-combined exposure to noise and CO. In addition, protective effects seen with this pre- *and* post-combined exposure treatment were compared to protective effects of PBN when administered only post combined exposure versus repeated administration post combined exposure. End-points evaluating auditory sensitivity included compound action potential (CAP) and cochlear microphonic (CM). Results demonstrate effective protection of PBN when administered pre and post combined exposure. Partial protection was obtained with post administration of PBN and with repeated post administration of PBN. EPR spectra revealed PBN adducts with trapped free radicals in animals exposed to noise and CO. These results show that toxic pathways involved in the potentiation of noise induced hearing loss (NIHL) by CO includes free radicals which are generated early in the process of combined exposure. Occupational exposure to noise and CO is common in work environments of fire-fighters and automobile/aircraft workers Elucidation of these mechanisms is relevant not only to set exposure limits in work environments, but also in order to develop effective preventive and protective regimens. (This study was supported by NIOSH grant #03481 and NIEHS grant ES 08082 to L D Fechter. Additional support was obtained through a cooperative agreement with NSF EPSCoR program [EPS9550478].)

799 EFFECTS OF *PLUCHEA SYMPHYTIFOLIA* IN CHOLESTEROL AND TRIGLYCERIDES BLOOD LEVELS.

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Pluchea symphytifolia is a common plant around the world. It has been used as stimulant, diuretic, tonic and digestion helper. This investigation studied the effect of Pluchea symphytifolia in cholesterol and triglycerides levels in blood samples taken from CF-1 mice. Thirty five (35) male mice from the species CF-1 were divided in seven groups with 5 mice in each group. Group Control I had a diet based on rabbit food with 2% of cholesterol and was treated with 12.5% plant extract. Control II-A had a normal diet and water. Control II-B had food elevated in lipids and drink only water. Experimental groups were divided in two groups of 10 mice (I and II). One group received a normal diet (I) and the other group a diet elevated in lipids (II). Each group was then subdivided in two groups (IA, IB, IIA and IIB). Experimental Groups IA and IIA were treated with 12.5% extract and Experimental Groups IB and IIB with 7.5% extract. Food and treatment was ad libitum. Blood samples from the eyes were taken initially(before starting the treatment), two weeks and four weeks after the treatment. The experiment duration was six weeks. Results indicate a significant decrease on triglycerides levels after the treatment. Also, they suggest some decrease an cholesterol levels. Further studies in relation to cholesterol levels will be performed in order to established blood levels decrease.

800 COMPARISON OF CYTOTOXIC EFFECTS OF OCHRATOXIN A AND B ON HUMAN, RAT AND PORCINE RENAL CELLS.

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Chronic dietary intake of ochratoxin A (OTA) via contaminated food (bread, beer, coffee, wine, meat) has been associated with increased incidences of urothelial tumors and nephropathy in humans (e.g., Balkan Endemic Nephropathy, BEN). It has also been demonstrated to be carcinogenic in rodents, with distinct species- and sex-differences in susceptibility. The mechanism(s) leading to these sensitivity differences have yet to be elucidated. This study investigated the cytotoxic effects of OTA and its structural analogue OTB on primary renal cortex cells from humans (biopsy material), both sexes of F344 rats, and from improved German hybrid pigs. Furthermore, a porcine renal cell line, LLCPK-1, was used for comparison. Cells were exposed to OTA and OTB at concentrations ranging from 1 nM to 100 μM for 24 to 96 hours. Following incubation several cytotoxicity endpoints were assessed: Neutral red uptake, MTT reduction and cell number analyses (Coulter counter) to show differences in proliferation. Supravital fluorescent staining techniques as well as electron microscopy were employed to detect toxin induced morphological changes. A range of fluorescent labeled antibodies to cytoskeletal proteins (e.g., vimentin, cytokeratins, actin, talin, tubulin) were used to demonstrate OTA- and OTB-induced morphological changes. Although all cells showed similar toxin induced morphological changes, distinct species-differences were observed in respective sensitivities: porcine kidney cells > human kidney cells > rat kidney cells/ LLCPK-1.

801 INTER-SPECIES VARIATIONS IN OCHRATOXIN A PROTEIN BINDING AND UPTAKE.

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Chronic dietary intake of ochratoxin A (OTA) via contaminated food (bread, beer, coffee, wine, meat) has been associated with increased incidences of urothelial tumors and nephropathy in humans (e.g., Balkan Endemic Nephropathy, BEN). It has also been demonstrated to be carcinogenic in rodents, with distinct species- and sex-differences in susceptibility. The mechanism(s) leading to these sensitivity differences have yet to be elucidated. The aims of this study were to investigate the possibility that such differences could arise due to variations in OTA protein binding and/or uptake. Protein binding studies were carried out with a classical competitive assay using [3H]OTA and homogenates from human, porcine, rat and mouse renal cortex tissue. Species-specific binding capacities were observed: human >> rat > pig > mouse. OTA bound human and porcine homogenates with higher affinity than rat and mouse with regard to competition with bromosulftophthalein (BSP). Concentration-dependent inhibition of [3H]OTA binding was also seen with piroxicam, indomethacin, nalidixic acid, ethacrynic acid, furosemide and probenecid, but not with fumonisin B1, para-amminohippurate (PAH) and several bile acids. [3H]-OTA uptake was measured over 60 min in human (HKC) and porcine (PKC) primary renal epithelial cells and in NRK52E and LLCPK-1 cell lines. Uptake over time remained relatively constant, however, distinct differences were observed in the amount of [3H]-OTA taken up by the different cell types. Both HKC and PKC displayed a 10-15 fold higher uptake than that observed in LLCPK-1 and NRK-52E cells.

802 ANTIPROLIFERATIVE AND CELL CYCLE SPECIFIC EFFECTS OF OCHRATOXIN A IN LLCPK-1, NRK-52E AND PORCINE PRIMARY PROXIMAL KIDNEY CELLS.

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Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by certain Aspergillus and Penicillium species. It is commonly found as a contaminant of both human and animal foodstuffs. All human blood samples tested to date have proved positive for OTA. Average daily intake in humans is estimated to be approximately 5–10ng/kg. OTA has been demonstrated to induce nephropathy as well as to be immunotoxic in pigs (antiproliferative effect in T-cells) and teratogenic in several species including rat, mouse, hamster and chick. Although little is known about its potential for human teratogenesis, OTA displays similarities in animal models to other well-characterized human teratogens e.g., valproate and thalidomide, especially with respect to dose-dependency and gestational sensitivity periods. A disruption in the normal cellular proliferation control could contribute to the observed induction of nephropathy, immunotoxicity and teratogenicity of OTA. Therefore, the effect of OTA on proliferation rates of LLCPK-1, NRK-52E and porcine primary proximal kidney cells (PKC) following acute exposure was investigated. Cells were exposed to increasing concentrations of OTA over 24, 48 and 72hrs. Antiproliferative effects were determined using a Coulter counter. The concentration producing a 50% proliferative arrest (GI50) in LLCPK-1 and NRK-53E cells lines was 21mM, with PKCs displaying a GI50 of 15mM. Standard flow cytometric analysis of propidium iodide stained DNA was used to investigate the nature of this growth inhibitory effect. OTA was found to increase the percentage of cells residing in the G2/M phases of the cell cycle. More detailed analysis, (fluorescence-microscopic mitotic index of Hoechst-stained DNA) demonstrated no increased numbers of cells in M-phase thus suggesting an OTA mediated specific blockade of the cell cycle located to the G2 phase in all three cell types.

803 A COMPARISON OF RECONSTITUTION TIMES FOR CROTAB AND ANTIVENIN (*CROTALIDAE*) POLYVALENT [WYETH].

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Background: Both antivenom products have been shown to be effective in patients with crotalid snake envenomation. It is generally believed that the less purified Wyeth antivenom requires more time to dissolve in its diluent than the CroTAb antivenom, but there is no literature available to support or dispute this perception. **Objective:** To compare the reconstitution times for CroTAb and Wyeth antivenom by randomized analytical and blinded observational methodology. Methods: In the first phase, three vials of each antivenom were injected with 10 ml of recommended diluent, and then 10 ul aliquots of antivenom were taken from all vials at 5 minute intervals from 0 to 90 minutes. The samples were diluted, randomized and spectrophotometrically analyzed according to the method of Bradford (1976) as modified by Pierce using the Coomassie Plus Protein Assay Reagent. In the second phase, time required for complete dissolution of five vials of each antivenom was empirically judged by three medical professionals at 5-minute intervals for a 90-minute duration. **Results:** Two Factor ANOVA indicated there was a statistically significant difference over time ($p<0.001$) and between each group ($p<0.002$) based on the reconstitution/time curves for each antivenom in the analytical phase. In the observational phase the average time to reconstitution for CroTAb was 38 minutes (SD+12 min.), but none of the Wyeth vials were determined to be reconstituted at the end of the 90-minute trial. The higher proportion of CroTAb vials reconstituted within 90 minutes was statistically significant (two-tailed Fisher?s Exact test, $p=0.008$). **Conclusions:** CroTAb had a significantly shorter reconstitution time by both analytical determination and clinical observation. Since the time required for each product to dissolve into solution delays treatment, a significant difference in reconstitution time would be clinically useful in determining antivenom selection.

804 EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND INFLAMMATORY CYTOKINES BY α-HEDERIN IN MACROPHAGES.

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α-Hederin, a triterpenoid saponin which exists in some oriental herbs, was known to decrease the hepatotoxicity of cadmium by inducing expression of metallothionein. However, the mechanism(s) by which α-Hederin induces metallothionein is not well investigated. Inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, and nitric oxide were suggested as mediators of up-regulation of metallothionein. In the present study, we investigate the role of inflammatory cytokines and iNOS in α-Hederin-induced up-regulation of the metallothionein in murine peritoneal macrophages. The induced expression of metallothionein in α-Hederin-treated cells was accompanied by increase of transcripts for IL-1β, IL-6, TNF-α, and iNOS in a dose-dependent manner by RT-PCR analysis. The increase in NO, as demonstrated by measurement of nitrite was found to correlate well with an increase in transcripts of iNOS. Since the promoter in IL-1β, TNF-α, and iNOS gene contains binding motifs for NF-κB/Rel, the effect of α-Hederin on the activation of this transcription factor where determined. Using DNA fragments containing the NF-κB/Rel binding sequence, α-Hederin was shown to activate the protein/DNA binding of NF-κB/Rel to its cognate site as measured by electrophoretic mobility shift assay. Collectively, the results of these experiments indicate that expression of IL-1β, IL-6, TNF-α, and iNOS were induced by α-Hederin and these results show that the inflammatory cytokines and iNOS that are induced by α-Hederin may play an important role in the α-Hederin-induced up-regulation of metallothionein. (Supported by KOSEF Grant 981-0505-028-2 and 1999-2-214-002-5.)

805 EFFECTS OF HEPATIC CYTOCHROME P450 BY GLYCYRRHIZIN AND GLYCYRRHETINIC ACID.

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There have been numerous reports of the antihepatotoxic activity of glycyrrhizin, a triterpenoid saponin of Glycyrrhiza glabra L. as well as its genuine aglycon, 18beta-glycyrrhetinic acid, but little attention has been paid to regarding to its effects of cytochrome P450 (P450). Therefore, in this study, we investigate the effects of glycyrrhizin and 18beta-glycyrrhetinic acid on the constitutive and inducible microsomal activities and expression of P450 in rat. The administration of glycyrrhizin and 18beta-glycyrrhetinic acid to rats significantly decreased the activities of microsomal pentoxyresorufin O-dealkylase and aniline hydroxylase, representative activities of P4502B1/2 and P4502E1, respectively, in a dose- and time-dependent manner. However, ethoxyresorufin O-deethylase and methoxyresorufin O-demethylase, a representative activity of P4501A1 and P4501A2, respectively, was decreased to a lesser extent. Glycyrrhizin and 18 beta-glycyrrhetinic acid also decreased inducible monooxygenase activities in the same manner. Suppressions of P450 isozyme expression occurred in glycyrrhizin and 18beta-glycyrrhetinic acid treated hepatic microsomes, as determined by immunoblot analysis in a manner consistent with that of the enzyme activity levels. These results suggest that glycyrrhizin and 18beta-glycyrrhetinic acid may act as a more specific suppressor for P4502B1/2 and P4502E1 than P4501A/2. (upported by KOSEF Grant 981-0505-028-2 and 1999-2-214-002-5.)

806 COUMARIN DOES NOT INDUCE UNSCHEDULED DNA SYNTHESIS (UDS) IN THE *IN VIVO* RAT HEPATOCYTE DNA REPAIR ASSAY.

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In this study we have examined the ability of coumarin to induce UDS in hepatocytes after *in vivo* administration to male Sprague-Dawley CD rats. From a preliminary toxicity study, the oral maximum tolerated dose (MTD) of coumarin was determined to be 320 mg/kg body weight. In the UDS study rats (n=3 per group) were treated with single oral 32, 107 and 320 mg/kg (i.e., $^1/_{10}$, $^1/_3$ and $^1/_1$ MTD) doses of coumarin. Negative controls were treated with corn oil and positive controls with either 20 mg/kg dimethylnitrosamine (DMN, for the 2-4hr expression of UDS) or 50 mg/kg 2-acetylaminofluorene (2-AAF, for the 12-16 hr expression of UDS). Hepatocytes were isolated by liver perfusion either 2-4 hr or 12-16 hr after treatment. The cells (viability 85-91%) were cultured in medium containing [^{3}H]thymidine for 4 hr and then in medium containing unlabelled thymidine for 12-24 hr. UDS was quantified by grain counting of autoradiographs. Coumarin at doses of 32, 107 and 320 mg/kg had no significant effect on UDS in rat hepatocytes either 2-4 hr or 12-16 hr after dosing. In contrast, significant increases in UDS were observed in hepatocytes from rats treated with either DMN for 2-4 hr or 2-AAF for 12-16 hr. Net grain counts were significantly increased from -0.33 to 5.69 and from -0.03 to 11.44 by treatment with DMN and 2-AAF, respectively. Both genotoxins also increased the percentage of hepatocyte nuclei with >5 and >10 net grains. These results demonstrate that coumarin does not induce UDS in hepatocytes of male Sprague-Dawley CD rats after oral administration at doses up to the MTD of 320 mg/kg. (Conducted as part of a Coumarin Research Program and supported by RIFM, Inc., IFRA (Geneva, Switzerland), IOFI (Geneva, Switzerland) and Rhodia Inc.)

807 SAFETY RISK ASSESSMENT OF THE MYCOTOXIN PATULIN IN APPLE JUICE AND APPLE JUICE PRODUCTS.

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Patulin is a highly toxic heterocyclic lactone, produced by a variety of molds including *Penicillium* and *Aspergillus*. Patulin can be found in various apple products, and is associated with moldy apples used for juice production. Patulin inhibits protein synthesis and impairs several enzymes, including alcohol dehydrogenases, and mixed function oxidases. Rodent carcinogenicity studies have yielded inconclusive results. No documented cases of human illness associated with patulin have been observed. However, the FDA has estimated a Tolerable Daily Intake (TDI) of 0.43 μg/kg bw based upon application of a 100-fold safety factor to the No Observed Adverse Effect Level observed in a 109 week gastric intubation patulin study in the rat. Because of patulin's toxicity and the large consumption of apple juice and apple juice products by U.S. children particularly in relation to their body weight, the FDA has proposed setting a limit of 50 ppb for patulin. To estimate this limit, apple juice consumption data from the 1987-88 USDA National Food Consumption Survey (NFCS), and patulin levels from 1660 samples of apple juice were used to estimate exposure. At the 50 ppb limit, all age categories, including infants and young children, are adequately protected, *i.e.*, are exposed to levels of patulin equal to or below the TDI, even a two year-old 90th percentile consumer.

808 SAFETY/RISK ASSESSMENT OF THE CONSUMPTION OF PEAR BRANDIES CONTAINING METHANOL.

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Some pear brandies can contain methanol (MeOH), a compound associated with pronounced visual and central nervous system toxicity. This assessment addresses the potential health hazards of acute and chronic consumption of pear brandy due to MeOH exposure. Various pear brandy products (n=134) were analyzed for MeOH content by gas chromatography with a flame ionization detector. The MeOH content of these products ranged from 0.03 to 0.87 (mean = 0.41) % volume. The estimate of exposure to MeOH from brandy consumption was based on brandy MeOH content and point estimates of brandy intake (USDA, CFSII, 1989-1992). Mean and 90-99th percentiles of the average MeOH exposures were estimated for one-day and chronic consumption of brandy. The tolerable daily intake (TDI, mg/kg/day) for MeOH for one-day exposure is 7.1 (human NOAEL, FDA, 1984) and for chronic exposure is 0.5 (RfD, IRIS, EPA, 1993). The average single-day exposure to MeOH from ingestion of pear brandy was at or below the TDI for the mean, 90th and 95th percentile brandy intake levels, but exceeded it at the 99th percentile level. One-day MeOH exposure for the 134 individual pear brandies exceeds the one-day TDI 7.5, 46.3, 51.5, 85.1% of the time at the mean, 90th, 95th and 99th percentile intake levels, respectively. The average chronic exposure to MeOH from pear brandies exceeds the chronic TDI at several intake percentiles (mean, 90-99th). Thus, the possibility exists that some pear brandy products at some exposure levels of varying duration may represent a hazard to human health.

809 DETERMINATION OF AD50 AND LD50 OF *CLERODENDRON SIPHONANTHUS* LEAF EXTRACT FOR ANTIFERTILITY AND MORTALITY OF *CALLOSOBRUCHUS CHINENSIS* (L.) BY TOPICAL, DIPPING AND INJECTION METHODS.

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AD50 with the effect of *Clerodendron siphonanthus* leaf extract on hatchability has been noted. These median antifertility doses are 0.7828ug./weevil, 0.4682% per weevil and 0.5346% per weevil in topical, dipping and injection (12ug/weevil) methods respectively. Probit analysis for dose response data for *Clerodendron siphonanthus* showed that linear relation ship existed between the concentration (Log X) and percentage antifertitlity induced (Probit Y). LD50 was observed with effect of antifertility agent *Clerodendron siphonanthus* leaf extract during hatchability i.e. 0.8217 µg per weevil, 0.5164 percent per weevil 0.4813 percent per weevil in topical, dipping and injection (12ul per weevil method respectively). Probit analysis showed that a linear relation ship existed in between the concentration (Log X) and percentage mortality induced (Probit Y), AD50-Antifertility dose, LD50-Lethal dose.

810 DISTURBANCES IN CA^{2+} SIGNALING FOLLOWING AROCLOR® 1254 INVOLVE INTRA- AND EXTRACELLULAR CA^{2+} POOLS IN A TEMPORALLY DISTINCT SEQUENCE.

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Polychlorinated biphenyls (PCBs) are ubiquitous, persistent bio-accumulative toxicants (PBTs) that affect many types of cells and for which alterations of intracellular Ca^{2+} homeostasis is a recurring theme. Using single cell, fura-2 ratio fluorescence imaging we recently reported that addition of Aroclor 1254 (A1254; UltraScientific Inc.) to developing neocortical cells (maintained in culture < 8 days) resulted in an immediate, transient increase of intracellular Ca^{2+} (latency < 0.5 min, duration 3.9 ± 0.3 min) followed 3-16 min later by the onset of recurring Ca^{2+} oscillations (duration, 10-30 sec each). These later disturbances were dependent on extracellular Ca^{2+} and blocked by the L type voltage-gated Ca^{2+} channel (VGCC) antagonist, nifedipine (1 µM) (Soc. Neurosci. Abstr. 25, 500.17). Here, the initial Ca^{2+} transient was studied and found to be concentration-dependent (1- 25 µM), reaching 1 -1.5 µM intracellular [Ca^{2+}] with 20 µM A1254 from a basal level of 0.1 µM. The source of Ca^{2+} during the initial transient (intra- vs. extracellular) was localized to intracellular pools since neither Ca^{2+}-depleted buffer (in presence of 1 mM EGTA) nor pre-treatment with nifedipine (1 µM) prevented the initial increase of intracellular [Ca^{2+}] by A1254 (20 µM). Following administration of thapsigargin (10 µM for 10 min) to deplete intracellular Ca^{2+} stores, both carbachol-stimulated intracellular Ca^{2+} release and the A1254 -induced Ca^{2+} transient were blocked. Current studies are focused on determining the involvement of specific intracellular stores (e.g. ryanodine- or IP_3- sensitive). Effects of PCB congeners were structure-dependent wherein addition of ortho-substituted PCB 4 and PCB 138, but not the non-ortho substituted PCB 15, PCB 77, or PCB 126 (10 µM each), also caused this initial Ca^{2+} transient. These observations are consistent with A1254 causing an initial transient which is dependent on intracellular Ca^{2+} stores, followed by Ca^{2+} oscillations which are dependent on extracellular Ca^{2+} and mediated by L-type VGCC. (This abstract does not necessarily reflect US EPA policy.)

811 ACUTE EXPOSURE TO PCB153 AND PCB28 SUPPRESSES LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES.

M. E. Gilbert. *USEPA, Research Triangle Park, NC.*

Long-term potentiation (LTP) is a model of synaptic plasticity that encompasses the physiological changes associated with memory. We have demonstrated a reduction in hippocampal LTP in adult rodents whose exposure to PCBs was restricted to the perinatal period (Gilbert and Crofton, 1999). LTP has also been shown to be involved in activity-dependent synapse formation at very early stages of hippocampal development (Durand et al., 1996). The present study examined the effects of acute exposure to two ortho-substituted congeners (PCB28, PCB153) on synaptic plasticity *in vitro*. Hippocampal slices were prepared from adult male rats and maintained in physiological buffer for 2 hrs prior to recording. Stimulating electrodes were placed in stratum radiatum of area CA1. Evoked field potentials were recorded from the CA1 pyramidal cell layer and the stratum radiatum. No change in population spike (PS) amplitude recorded from pyramidal cells was evident in slices maintained in 0.2% DMSO vehicle, PCB 153 (3 and 10µM) or PCB28 (10µM) over a 60 min recording period. LTP (15 100 Hz 4-pulse bursts) was induced 1-2 hrs after exposure to PCB-containing medium. Potentiation of the PS was reduced by 10µM PCB28. PCB153 also demonstrated a trend for reduced PS LTP at concentrations of 3 and 10µM. To the extent that functional synapse induction and LTP are mechanistically similar processes (Durand et al., 1996), the acute action of PCBs to suppress LTP may disturb synaptogenesis when PCBs are present during hippocampal development following *in vivo* dosing. Altered synaptic networks may contribute to a reduced capacity to support LTP in the adult offspring of PCB-exposed dams. (This abstract does not necessarily reflect US EPA policy.)

812 DEVELOPMENTAL EXPOSURE TO A1254 ALTERS SYNAPTIC TRANSMISSION IN THE DENTATE GYRUS *IN VIVO*.

J. L. Blanton, M. M. Taylor, K. M. Crofton and M. E. Gilbert. *USEPA, Research Triangle Park, NC.*

Previous work has demonstrated a reduced capacity to support long-term potentiation (LTP) in animals exposed to a PCB mixture, Aroclor 1254 (A1254) (Gilbert and Crofton, 1999). Assessment of "normalized" input/output (I/O) functions collected prior to LTP induction failed to reveal differences in baseline synaptic transmission between control and PCB-exposed groups. The present study was designed to systematically evaluate excitatory and inhibitory synaptic transmission using a more extensive I/O analysis and tests of paired pulse depression. Pregnant dams were exposed to 6 mg/kg/day of A1254 from GD6 to PND21. This dosage of A1254 produces perinatal hypothyroxenemia, hearing loss, and impaired LTP in offspring. Adult male offspring were anesthetized with urethane and stimulating and recording electrodes placed in the perforant path and dentate gyrus. An ascending series of 15 stimulus intensities was delivered (100-1500µA) to the perforant path. Population spike (PS) amplitudes recorded in the dentate gyrus were slightly but significantly enhanced in PCB-exposed animals relative to controls only in the midrange intensities. No group differences were observed in EPSP slope amplitudes. Inhibitory synaptic transmission was assessed by delivering pairs of stimulus pulses at 5 intervals (IPI=10-70 msec) and 3 intensities (50, 80,100% maximum). Paired pulse depression was decreased at the intermediate IPIs (20 and 30ms), a change restricted to the lowest stimulus intensity. These data demonstrate subtle alterations in synaptic function in hippocampus as a result of early PCB exposure. These data support previous conclusions that PCB-induced LTP deficits are not readily attributable to reductions in synaptic excitability. (This abstract does not necessarily reflect US EPA policy.)

813 DEVELOPMENTAL EXPOSURE TO THE 2,2',4,4',5,5'-HEXACHLOROBIPHENYL (PCB153): THYROID HORMONES AND NEUROBEHAVIORAL EFFECTS.

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Polychlorinated biphenyls (PCBs) are pervasive environmental contaminants that have been shown to detrimentally affect somatic and behavioral endpoints. Primiparous Long-Evans rats were exposed to 0, 1, 5, 20 or 60 mg/kg/day PCB153 via oral gavage from Gestation Day 6 through Postnatal Day 21. Offspring were evaluated at various ages for circulating thyroid hormone concentrations [total serum triiodothyronine (T3) and thyroxin (T4)], survival, body weight, eye opening, motor activity development, auditory startle response and auditory thresholds. A dose-dependent moderate reduction in T4 concentrations was apparent from PND4 to PND21, with a maximal effect at 60 mg/kg (52% decrease at PND21). T4 in dams on PND22 was deceased up to 32% at 60 mg/kg. Concentrations of T3 did not differ from controls for pups or dams. There was no body weight gain reduction in either the dams or the offspring. There was no alteration in eye opening or survival. There was an age- but not dose-dependent increase in motor activity from PND7 to 14. Further, auditory startle amplitudes were not affected on PND24. These data suggest that PCB153 is much less potent as a thyroid hormone disruptor during development compared to Aroclor 1254. Furthermore, PCB153 at the doses tested does not seem to alter many of the endpoints previously shown to be sensitive to Aroclor 1254 exposure. (This abstract does not necessarily reflect US EPA policy.)

814 SEX-DEPENDENT BEHAVIORS AND STEROID HORMONE CONCENTRATIONS IN RATS AFTER MATERNAL EXPOSURE TO A RECONSTITUTED MIXTURE OF PCBS.

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Previous work from our group indicated reduced serum testosterone concentrations and feminized saccharin preference in adult male rats and decreased aromatase (CYP19) activity in the hypothalamus of newborn male rat pups maternally exposed to a PCB-mixture which was reconstituted according to the congener pattern found in human breast milk. Effects of the reconstituted mixture were more pronounced than effects of Aroclor 1254. In the present study the dose-response relationship for the reconstituted PCB-mixture was examined. Female rats were fed PCB-loaded diets resulting in an average daily intake of about 0, 0.5, 2 or 4 mg/kg b.wt. Exposure started 50 days prior to mating and was continued throughout mating and gestation until parturition. Adult male, but not female offspring exhibited dose-dependent elevations of saccharin preference indicating a feminization of this sexually dimorphic behavior. Evaluation of conditioned place preference revealed a preference for the testosterone-paired side in adult male rats of the highest exposure group (4 mg/kg b.wt.). In addition, there were dose-dependent reductions in serum testosterone levels in female pups on postnatal day (PND) 21 and in adult male rats. Serum levels of the secosteroid 1,25-$(OH)_2$-vitamin D_3 were decreased in dams on 0 and PND 21 and on PND 21 in offspring in a dose-related manner. Taken together, maternal exposure to a reconstituted PCB-mixture resulted in dose-dependent and long-lasting alterations of sex-specific behaviors and sex hormone levels in male rats. (Grant PUG 97004, State of Baden-Wuerttemberg, Germany, to H.L.).

815 GENDER-SPECIFIC CORTICAL ASYMMETRY OF RAT BRAIN FOLLOWING PRENATAL EXPOSURE TO TCDD.

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Sexually dimorphic patterns of cortical asymmetry in rats show that males exhibit pronounced right hemisphere dominance while females exhibit more diffuse lateralization patterns but tend to greater left hemisphere dominance. Prenatal TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) exposure produces demasculation of offspring sexual behavior. This study aimed to determine gender-specific differences in cortical lateralization in Holtzman rats exposed prenatally to TCDD. Pregnant females were given a single p.o. dose of 180 ng/kg on gestational day 18 (GD 18). Cortical depth measurements were taken in selected brain regions in offspring 300 days of age. Areas 2, 3, 17, and 18a, at bregmas -1.8, -3.8, and -5.8, were chosen for analysis. Morphometric indices were collected using digitized, enhanced images produced by a photomicroscope fitted with a special color camera. In several brain areas of male offspring, a marked reversal from right hemispheric dominance or symmetry to left hemispheric dominance was observed. Female offspring brains tended to show a contrary but less pronounced tendency to deviate from symmetry to right hemisphere dominance. In general, prenatal exposure of males to TCDD produced a change in the pattern of cortical asymmetry consistent with the behavioral demasculation reported in the literature. (Supported by grants ES 08958 and ES 01247 from NIEHS.)

816 MOTOR ACTIVITY OF RAT OFFSPRING AFTER A SINGLE PRENATAL EXPOSURE TO 2,3,7,8-TCDD.

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Altered neurobehavioral development is becoming recognized as one of the most sensitive consequences of prenatal TCDD exposure. Still to be elucidated, however, are such issues as dose-response relationships, critical periods of exposure, and the types of neurobehavioral functions susceptible to exposure. In one phase of a program to evaluate the offspring of Holtzman rats born to TCDD-exposed females, we measured motor activity at various times during postnatal development. On gestational day 18 (GD18), pregnant rats were given single p.o. doses of 0, 20, 60, or 180 ng/kg TCDD. Motor activity (one male and one female per litter) was assessed during 15-min periods in a small chamber equipped with a Doppler effect-detection device on postnatal days (PND) 30, 38, 90, and 150. Onset and termination of movements were recorded and analyzed for each session to capture the episodic nature of activity. Results obtained on PND30 were analyzed separately because a different size activity chamber was used at that time. All the groups followed a similar developmental pattern of activity. At PND90, the number and duration of episodes were smallest and the intervals (IRTs) between episodes the longest of any of the test ages. TCDD exerted little influence on this developmental pattern, except that TCDD-exposed males at PND90 tended to exhibit fewer movements and longer IRTs than controls ($p=0.051$, and 0.052, respectively, based on Kruskal-Wallis tests). These results suggest that maternal exposure to TCDD on GD18 may influence one aspect of motor activity in the offspring. (Supported by grants ES 08958 and ES 01247 from NIEHS.)

817 LOW LEVEL PRENATAL TCDD AFFECTS OPERANT RESPONDING FOR MOTOR REINFORCEMENT IN FEMALE RATS.

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Wheel-running behavior, and other measures of activity in sexually mature female rats are linked to the 4-5 day estrus cycle. Such behaviors are sensitive to acute exposure to estrogenic substances or to developmental exposure to substances that disturb the organization of the brain regions that mediate incentive motivation or motor output. Operant responding for food is also linked to the estrus cycle. During behavioral estrus, females resemble males in that they respond at higher rates on certain reinforcement schedules. In the current procedure, both wheel running and operant responding for food were examined in rats that were exposed to a single, low prenatal dose of 2,3,7,8, tetrachlorodibenzo-p-dioxin (TCDD). Pregnant Holtzman rats were administered 20, 60, or 180 ng/kg TCDD p.o. on gestational day 18. Beginning on postnatal day 80, male-female pairs of littermates were trained to lever press for food on a random ratio schedule. A separate group of female offspring were trained to lever press on a fixed-ratio schedule for a brief opportunity to run in specially-designed wheels. Both random ratio and fixed-ratio requirements were increased across sessions. Vaginal lavage data were also collected to monitor cyclicity and were later compared to the behavioral data. In the wheel procedure, TCDD reduced the number of completed fixed-ratio schedules, reduced the lever response rate, and reduced the number of wheel revolutions per session in correspondence with dose. These data indicate that TCDD can induce subtle motor or motivational decrements in adult female rats responding for other primary reinforcers and extend previous findings on the effects of TCDD on male sexual behavior. (Supported by grants ES 08958 and ES 01247 from NIEHS.)

817A EFFICACY OF RECOMBINANT C FRAGMENT OF HEAVY CHAIN BOTULINUM NEUROTOXIN SEROTYPE B AGAINST LETHAL AEROSOL CHALLENGE OF BOTULINUM B TOXIN IN RHESUS MONKEYS.

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Botulinum neurotoxin B (BoNTB) is a distinct protein subtype of neurotoxins normally considered to be foodborne pathogens. These neurotoxins cause flaccid paralysis at the motor endplate by inhibiting the function of proteins associated with the release of vesicles containing acetylcholine. Similarly, aerosolized botulinum B causes flaccid muscle paralysis with loss of funtion of the muscles of respiration ultimately causing death in both routes. Because adjunctive and therapeutic methods are limited by their need of logistical support and recognition of exposure respectively, prophylaxis is the current method to combat deliberate, aerosol exposure of BoNT. The current study is part of a series of studies evaluating the toxicity of the different serotypes of BoNT in rhesus monkeys and the efficacy of their corresponding recombinantly derived C fragment of the H_c chain (carboxy terminus of the heavy chain, receptor binding domain) to protect against effects of aerosolized toxin. Specifically this study was to determine the efficacy of rBoNTB H_c to protect against aerosolized BoNTB in the rhesus monkey and whether a difference exists between two candidate dosages. Animals were given three inoculations of either 1 or 5 µg rBoNTB Hc (4 weeks apart, IM, Alhydrogel adsorbed). Serial serum neutralization assays (SNA) and enzyme-linked immunosorbant assays (ELISA) were performed at various time points before aerosol challenge. Monkeys were then challenged with an aerosol concentration of BoNTB 50 times the BoNTB LCT_{50} at 6 weeks after the last inoculation. All vaccinated animals produced a detectable SNA and ELISA titer and survived the aerosol challenge while the vaccine controls did not indicating that rBoNTB is both immunogenic and protective in this model.

818 TESTOSTERONE REVERSES ETHANOL-INDUCED DEFICIT IN SPATIAL REFERENCE MEMORY IN CASTRATED RATS.

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The present study was designed to evaluate the effects of ethanol, testosterone and combination of ethanol and testosterone, on spatial reference memory in castrated rats. Male Sprague-Dawley rats (120-150g), were adapted to 12-hour light: 12-hour dark illumination cycle for 7 days. After the adaptation period, animals were castrated bilaterally under pentobarbital anesthesia. Animals were monitored for a recovery period of 10-14 days. Ethanol (3g/kg as 22.5% solution in saline, i.p.), testosterone (2.5 mg/ rat in 0.2ml olive oil S.C. in the dorsal region) and combination of the drugs were administered to rats at 9:00 h. Control animals were injected i.p. with 2 ml saline. After a training period of 4 days, spatial reference memory was evaluated in treated and control rats for 7 days using the Morris water maize. The time to find the platform (latency) was significantly increased in ethanol treated rats as compared to control, indicating that ethanol induces deficit in spatial reference memory. On the other hand, testosterone administration improved spatial reference memory by significantly decreasing the latency period. In addition, there was a significant decrease in latency period in the animals treated with combination of ethanol and testosterone. These findings clearly indicate that administration of testosterone not only improved the reference memory but also reversed the spatial memory deficit induced by ethanol in castrated rats. (Supported by NIH grant RR03020.)

819 NEUROPROTECTION OF ALCOHOL AGAINST HIV-1 GP120 NEUROTOXICITY.

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Human Immunodeficiency Virus type 1 (HIV-1) induces brain damage leading to dementia. A large number of individuals with AIDS consume alcohol. However, the effects of alcohol on neurodegeneration associated with HIV are still unstudied. Many reports indicate that the HIV glycoprotein gp120, is a potential mediator of HIV neurotoxicity. Its toxicity is mediated, at least, by excitotoxicity mechanisms including stimulation of glia to release factors (particularly arachidonic acid, AA) which increase extracellular glutamate and activation of NMDA receptors which involve excessive Ca++ influx. Likewise NMDA receptors and glia are alcohol targets. Our recent study shows a neuroprotection effect of alcohol, which mediated by astrocytes dependent-function. In this study, we examine the effects of alcohol pretreatment on gp120 induced neurotoxicity, in rat brain organotypic slice cultures (HEC), in term of AA release and LDH leakage. Our results show that gp120 (100 pM) induces an increase of AA release (150%) after 15 min, accompanied by increase of NO liberation and IL6 production. In parallel, LDH release increased by 209% after 24 h of treatment as compared to control. After 6 days of pretreatment with alcohol (30 mM), the increase of AA and LDH significantly prevented. However, the increase of IL6 and NO remained strongly correlated. Our finding suggests that the neuroprotective effect of alcohol, is in part, mediated by the following: 1- Modulation of AA cascade, 2- activation and/or proliferation of astrocytes, which occurred in presence of gp120, leading to increase the glutamate uptake.

820 NICOTINE-ETHANOL INTERACTIONS ARE ALTERED IN ADOLESCENT *VS.* ADULT RATS.

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Nicotine and ethanol are two of the most widely used drugs in the world. They are often used together, but their interactive effects are not well understood. Both nicotine and ethanol use often begins during adolescence. It is not known whether the interaction of these drugs differs in adolescence vs. adulthood. The current study was conducted to determine the interactive effects of nicotine and ethanol in adolescent and adult rats. Forty-two female Sprague-Dawley rats (18 adolescents 49 days old and 24 adults 69 days old) were injected with vehicle, nicotine (0.2 mg/kg SC), ethanol (2.5 g/kg, IP) or ethanol + nicotine (N=4-7/age/treatment). Effects on body temperature (15 minutes after injection) and locomotor activity (20-80 minutes after injection) were measured. Locomotor activity was measured in the figure-8 apparatus. Unique interactions of ethanol and nicotine were seen in adolescents vs. adults with both measures. Body temperature was lowered by both ethanol and nicotine to a similar extent when given alone to adolescent and adult rats. However, the combination of nicotine and ethanol caused a significantly greater decrease in body temperature of adolescent rats than adults ($p<0.005$). In the locomotor activity test, ethanol caused a similar degree of sedation in adolescents and adults. Nicotine did not cause a discernable effect in either age group. When nicotine was given in combination with ethanol, there was a substantial reversal of the ethanol-induced hypoactivity in adults, but not in adolescents ($p<0.05$). Adolescence appears to be a unique period for vulnerability to the effects of nicotine and ethanol. (This research was supported by NIDA grant DA11943.)

821 ADOLESCENT NICOTINE EXPOSURE CAUSES PERSISTENT CHANGES IN RAT BRAIN.

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Whereas numerous studies have examined fetal or adult nicotine exposure, few studies have concentrated on exposure during adolescence, the period in which tobacco use typically begins. We gave nicotine to adolescent rats on postnatal days 30-47 via minipump infusions that achieve plasma nicotine levels found in smokers, and compared the results with similar infusions in adult rats. There were major differences in the regional specificity and persistence of nicotinic cholinergic receptor upregulation. In adolescents, upregulation was uniform across all regions during the infusion period, whereas in adults, there was a distinct regional hierarchy, hippocampus > cerebral cortex > midbrain; accordingly, receptors in the adolescent midbrain were upregulated far more than in adults. With adolescent nicotine treatment, significant increases were still apparent in male rats one month after the termination of exposure. We also obtained evidence for cell damage in nicotine-exposed adolescents. There were statistically significant changes in cell number (DNA content) and size (protein/DNA) in cortex and midbrain. In female hippocampus, nicotine evoked increases in expression of p53 mRNA, accompanied by increases in the cell membrane protein concentration, again suggestive of cell damage. Adolescent nicotine exposure thus elicits persistent changes in the brain, effects that may contribute to increased addictive properties and behavioral deficits. (Supported by a STAR fellowship from the US Environmental Protection Agency and by a grant from the Smokeless Tobacco Research Council.)

822 ADAPTATION OF THE 2-DEOXYGLUCOSE METHOD TO FISH FOR CHARACTERIZING KNOWN AND UNKNOWN AQUATIC NEUROTOXINS.

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We have developed a unique and much needed system to aid in the characterization of neurotoxins, and contribute to human and environmental risk assessment studies. This method is based on the pioneering work of L. Sokoloff (1977), which shows a direct relationship between glucose metabolism and regional brain activity. The product of the breakdown of 2-deoxyglucose (2-DG) is deoxyglucose-6-phosphate, which lacks the necessary oxygen for further enzyme recognition and metabolism, and thus remains trapped in the tissue, indicative of regional neural activity. Living, healthy fish (10-20g body weight) are injected intramuscularly with 1uCi of ^{14}C 2-DG and immediately allowed to swim freely in water with dissolved neurotoxins, mimicking real-life environmental exposure. At completion of the experimental time-course (20 minutes), the fish are sacrificed with a lethal dose (0.5g/L) of MS222, and brains are removed and fixed in 10% NBF overnight. Brains are then cryopreserved in 20% sucrose overnight and stored frozen at -20°C. Brains are cryosectioned (10-20μm) and the sections are analyzed by LM autoradiography and H & E staining. A microscope fitted with both dark and bright field optics is used to facilitate visualization of both cytoarchitecture and 2-DG localization within the same field. Preliminary data using goldfish and known optic lobe stimuli (strobe-light and dark exposure) shows a 10-30% regional redistribution of glucose in the brain in response to the stimuli. Our system is intended to aid in defining the mechanisms of action of neurotoxins and supply vital information regarding neural targets. More importantly, assessment of toxins can be done prior to identification and/or purification. It is a novel approach to the characterization of environmental toxins, which is an increasingly important part of today's research. (Supported by NIEHS grants P01ES09563-01 and 5-T32ES07263.)

823 A RAT MODEL OF THE COGNITIVE IMPAIRMENT CAUSED BY *PFIESTERIA PISCICIDA*.

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Pfiesteria piscicida, a dinoflagellate found in estuaries along the east coast of the United States has been associated with cognitive deficits in humans. We have developed a rat model of this cognitive deficit using the radial-arm maze to demonstrate the causal relationship between exposure to Pfiesteria-containing waters and cognitive impairment. Sprague-Dawley rats injected once (sc) with Pfiesteria-containing water (35,600 or 106,800 cells/kg of rat body weight) showed a significant deficit in radial-arm maze choice accuracy during the learning phase of training. Learning deficits have been seen ten weeks after a single exposure to Pfiesteria. Three different types of Pfiesteria culture samples have been found to cause radial-arm maze choice accuracy deficits. Juvenile male and female as well as adult female rats showed significant Pfiesteria-induced deficits. Locomotor activity was not as sensitive as radial-arm maze choice accuracy to the Pfiesteria-induced effects. Measures of the functional observational battery were largely unaffected by Pfiesteria exposure. Radial-arm maze choice accuracy deficits caused by exposure to Pfiesteria-containing water may be useful in helping to identify the critical toxin(s) of Pfiesteria piscicida. (This research was supported by the US-EPA, NOAA, NIEHS and the State of North Carolina.)

824 BREVETOXIN-INDUCED AUTOCRINE EXCITOTOXICITY IS ASSOCIATED WITH MANIFOLD ROUTES OF CA^{2+} INFLUX.

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A fluorescence imaging plate reader (FLIPR&Oχιρχ;, Molecular Devices, Sunnyvale, CA) was used to monitor real-time alterations in intracellular Ca^{2+} ($[Ca^{2+}]_i$) in fluo-3-loaded cerebellar granule neurons (CGNs) exposed to the brevetoxin, PbTx-1. PbTx-1 produced rapid and concentration-dependent increases in neuronal $[Ca^{2+}]_i$ with a potency nearly identical to that determined previously for PbTx-1-induced neurotoxicity. The N-methyl-D-aspartate (NMDA) receptor antagonists MK-801, dextrorphan and D-AP5, and tetanus toxin (TT), an inhibitor of Ca^{2+}-dependent exocytotic neurotransmitter release, effected significant reductions in both the integrated fluo-3 fluorescence response and excitatory amino acid (EAS) release, and protected CGNs against PbTx-1 neurotoxicity. The L-type Ca^{2+} channel antagonist nifedipine produced a modest reduction in the fluo-3 response, but reduced substantially the plateau phase of the PbTx-1 increment in $[Ca^{2+}]_i$ when combined with MK-801. When nifedipine and MK-801 were combined with the Na^+/Ca^{2+} exchange inhibitor, KB-R7943, the PbTx-1 increment in $[Ca^{2+}]_i$ was nearly completely attenuated. These data show that Ca^{2+} entry into PbTx-1-exposed CGNs occurs through three primary routes: NMDA receptor ion channels, L-type Ca^{2+} channels, and reversal of the Na^+/Ca^{2+} exchanger. There was a close correlation between reduction of the integrated fluo-3 fluorescence response and level of neuroprotection against PbTx-1 challenge afforded by blockers of each Ca^{2+} entry pathway; however, simultaneous blockage of L-type Ca^{2+} channels and the Na^+/Ca^{2+} exchanger, while reducing the integrated $[Ca^{2+}]_i$ response to a level below that provided by NMDA receptor blockage alone, failed to completely attenuate PbTx-1 neurotoxicity. This finding suggests that in addition to total $[Ca^{2+}]_i$ load, neuronal vulnerability is governed selectively by the NMDA receptor Ca^{2+} influx pathway.

825 A RECEPTOR SCREEN TO IDENTIFY TOXICANTS AND TOXINS OF BRAIN NEUROTRANSMITTER RECEPTORS.

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A protocol was developed to identify neurotransmitter receptor targets of neurotoxic agent(s) that mediate cognitive function. Current radioactive ligand binding assays for acetylcholine (nicotinic and muscarinic), glutamate (AMPA and NMDA), dopamine (D1 and D2) and GABAA receptors vary greatly in tissue preparation, temperature, incubation time, volume and buffer. This protocol uses a single membrane preparation, isolated from a rat brain homogenate in 20 mM Tris-HCl buffered modified Krebs Ringer, by centrifugation at 40,000xg for 20 min. The pellet is washed by recentrifugation four times and then suspended in 50 mM Tris-HCl, pH 7.4 to yield 1 mg protein/ml. This tissue preparation is divided into 25 mL samples, each of which is incubated for 60 min at 23°C with a specific radioactive ligand for each receptor, in a final volume of 250 mL; at a concentration that produces <5% receptor occupancy. Bound radioactive ligand is separated from unbound by vacuum filtration on GF/B filter and quantitated by liquid scintillation counting. Nonspecific binding is determined in presence of a saturating concentration of the selective receptor antagonist. One rat brain is sufficient for >300 assays. This assay is applicable for high throughput screening (Supported by NIEHS grant No. PO1 ES09563.)

826 CONVULSANT ACTIVITY OF NOVEL 6-HYDRO-8-METHOXYQUINOLONE ANTIBIOTICS.

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Most quinolone antibiotics are known to have convulsant activity. This activity has been related to inhibition of γ-aminobutyric acid, subtype A ($GABA_A$) receptors and is enhanced in the presence of nonsteroidal antiinflammatory agents such as 4-biphenylacetic acid (4BPA). Novel 6-hydro, 8-methoxy-quinolone antibiotics (PGE-9262932, PGE-4175997, PGE-9509924 and PGE-510629) were compared to benchmark quinolone antibiotics (gatifloxacin, trovafloxacin, ciprofloxacin) using the following assays: $GABA_A$ receptor binding in the absence or presence of 4BPA and proconvulsant activity in mice using pentylenetetrazole (PTZ) as the convulsant. For the $GABA_A$ receptor assay, bovine cerebellum suspension (4 mg/ml), [^{3}H]-GABA (5 nM), quinolone (1 μM to 6 mM) with or without 4BPA (1 mM) were incubated at 0°C for 60 min, cold vacuum filtered and assayed by liquid scintillation. An IC_{50} was determined from percent specific binding vs. the logarithm of the concentration. For the proconvulsant assay, CD-1 mice (21 per group) were dosed with 200 mg/kg IP of a quinolone or vehicle and 1 hr later given PTZ, 50 mg/kg IP, and observed for convulsions for 15 min. All of the compounds had IC_{50}s at the $GABA_A$ receptor of ≥ 800 μM, except for PGE-9509924 where it was 120 μM. The poor binding of ciprofloxacin and trovafloxacin to the $GABA_A$ receptor in the absence of 4BPA suggests this assay does not provide a reliable estimate of quinolone convulsant potential. $GABA_A$ receptor binding in the presence of 4BPA was only slightly increased (< 5-fold) with all compounds except trovafloxacin and ciprofloxacin (>200-fold). None of the 6-hydro-8-methoxy-quinolones enhanced the convulsant activity of PTZ, but activity was enhanced by gatifloxacin, trovafloxacin and ciprofloxacin. The 6-hydro-8-methoxy-quinolones appear to have lower convulsant activity than gatifloxacin, trovafloxacin and ciprofloxacin.

827 THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONIST, CP-101,606, DOES NOT PRODUCE NEURONAL VACUOLATION OR NECROSIS IN THE POSTERIOR CINGULATE/RETROSPLENIAL (PC/RS) CORTEX OF RATS: A MAJOR POINT OF DIFFERENTIATION FROM COMPETITOR NMDA RECEPTOR ANTAGONISTS.

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CP-101,606, the dextrorotatory enantiomer of (1S, 2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, is a NR2B subunit antagonist of the NMDA receptor under clinical development as a neuroprotectant in head trauma and stroke. The potential of CP-101,606 to produce morphological changes in cerebrocortical neurons of rats was evaluated since other NMDA receptor antagonists (e.g., MK-801, CGS 19755) produce neuronal vacuolation and necrosis in the PC/RS cortex of rodents in a dose-, time- and age-dependent manner. Sprague-Dawley rats (5/sex/dose/time point) received a single i.v. bolus dose of 0, 2, 10 or 50 mg/kg CP-101,606, followed 20 minutes later by a 3.67 hour i.v. infusion dose of 0, 1, 6, or 30 mg/kg/hr CP-101,606, respectively, or 4.4 mg/kg MK-801 (s.c., positive control, females only) prior to necropsy/perfusion fixation at 4, 12 and 24 hours after the start of dosing. The age of animals and time points selected for histologic examination in this study are most appropriate for assessing pathomorphologic changes with other NMDA receptor antagonists such as MK-801. Neuronal vacuolation and necrosis were observed in the PC/RS cortex of rats treated with the positive control agent, MK-801 within 4 and 24 hours after dosing, respectively. In contrast, CP-101,606 did not affect neuronal morphology. Steady-state plasma CP-101,606 levels at the low dose were equivalent to the efficacious level in rat head trauma models (~0.2 μg/ml) and at the high dose were within the maximum tolerated range (~8 μg/ml) based on previous rat studies. These data suggest that blockade of the NMDA receptor is not necessarily associated with pathomorphologic changes in the PC/RS cortex and that a critical factor may be the site of interaction of the antagonist within the receptor complex.

828 FLUOROCARBONS CFC-12 (DICHLORODIFLUOROMETHANE) AND HFC-134A(1,1,1,2-TETRAFLUOROETHANE) INDUCE DEPOLARIZATION OF BRAINSTEM AND HIPPOCAMPAL NEURONS.

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Replacement of the chlorofluorocarbon refrigerant CFC-12 by HFC-134a has led to concerns about the comparative toxicity that is especially related to cardiorespiratory control. The actions of CFC-12 and HFC-134a were tested on neurons in cardiorespiratory areas of the brainstem (dorsal motor nucleus, DMN; nucleus tractus solitarius, NTS; locus coeruleus, LC) and a non-cardiorespiratory area (hippocampus CA1). Perforated patch recordings were made in slices (rat, 200-350 μm, P1-14) visualized using infrared video microscopy. Both chemicals were saturated at the slice after 15 min (72.5 mg/l and 438.6 mg/l for CFC-12 and HFC-134a, respectively). Bath application of HFC-134a or CFC-12 (20 min) induced depolarization, increased firing rate, and decreased input resistance (R_{in}) in all brainstem neurons tested (8.6± 2.5 mV; R_{in} -32%). 30% of brainstem neurons tested depolarized suddenly (>Δ20 mV) and became swollen after 2-15 min of exposure to CFC-12 (12.1-72.5 mg/l) or HFC-134a (35.7-438.6 mg/l). The majority of brainstem neurons survived, 40% of which recovered from 20 min of exposure. There were no significant differences among the neurons from LC, DMN and NTS in response to either HFC-134a or CFC-12 exposure. When concentrations for both HFC-134a and CFC-12 were reduced to 0.38-1.6 mg/l at the slice, both chemicals induced slight depolarization associated with decreased R_{in} in 40% of neurons tested. No neuronal damage or death was observed. All CA1 neurons tested survived 20 min exposure of either chemicals. HFC-134a induced depolarization associated with decreased R_{in} in CA1 neurons. CFC-12, however, induced a 5% decrease in R_{in} but no significant membrane potential change. Thus, HFC-134a depolarized neurons in both brainstem and hippocampus, but only neurons in the brainstem were irreversibly depolarized. CFC-12 affected neurons in the brainstem only (ONR, NIH HL56683).

829 NEUROLOGIC, MORPHOMETRIC AND PATHOLOGIC EVALUATION OF NERVE FUNCTION AND ULTRASTRUCTURE IN BEAGLE DOGS ADMINISTERED THALOMID (THALIDOMIDE) OVER 53 WEEKS.

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Thalidomide has been approved in the US. A potential adverse event in humans is the development of peripheral neuropathy after prolonged use. Evaluations of nerve function and ultrastructure were performed as part of a 53-week toxicity study in Beagle dogs. 28 male and 28 female dogs were orally dosed with thalidomide capsules at 0, 43, 200 or 1000 mg/kg/day for 53 weeks with subsequent recovery for 5 weeks. All dogs were neurologically examined at weeks -2, 13, 26, 38, 51 and 58. Nerve function was assessed by measuring sensory nerve action potential (SNAP), action potential amplitude, duration and latency from the tibial nerve of the gastrocnemius muscle. Sensory nerve conduction velocity (SNCV) was also determined. Dogs were sacrificed at interim (26 weeks), end of dosing (53) and recovery (58) time points. Mean myelin thickness and mean axonal diameter of sural nerve fibers were determined using light microscopy at each sacrifice. Morphometric and pathologic evaluations were performed using electron microscopy. The distal spinal cord was also evaluated using light and electron microscopy at weeks 53 and 58. Distal spinal cord measurements included average thickness of myelin sheath, area of entire nerve fiber (myelin sheath plus axon), area of each axon cylinder and area of each myelin sheath. Thalidomide did not induce any neurological deficits. It had no effect on nerve function. There were no thalidomide-related ultrastructural morphometric and pathologic changes in sural nerve and distal spinal cord at all 3 sacrifice time points. The Beagle dog is therefore not predisposed to develop thalidomide-induced peripheral neuropathy.

830 MYELINOPATHY FOLLOWING SUBCHRONIC ADMINISTRATION OF ETHYL METHACRYLATE IN THE RAT.

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To identify potential morphological alterations in the nervous system resulting from exposure to ethyl methacrylate (EMA), sections were examined from the forebrain, brainstem, spinal cord and sciatic nerve of rats exposed to 0.1, 0.2, 0.5% EMA in drinking water for 60 days. While a decrease in the density of neurons was apparent in regions of the forebrain, brainstem and spinal cord, more striking were spongiform-like alterations in major fiber tracts. In the spinal cord, clusters of enlarged axons were equally distributed throughout the dorsal, lateral and ventral columns. These clusters of enlargements involved internodal portions of adjacent axons, and there was no evidence of more than one enlargement along the trajectory a single axon. In the sciatic nerve, cytoskeletal aberrations were apparent in sparsely distributed axons and some large caliber axons exhibited relatively thin myelin sheaths indicative of remyelination. This pattern of alterations is consistent with a central-peripheral myelinopathy, rather than the primary axonopathy typically associated with the structurally related neurotoxicants, acrylamide and glycidamide.

831 GAMMA-DIKETONE NEUROPATHY: ULTRASTRUCTURAL CHARACTERIZATION OF PERIPHERAL NERVE FIBERS.

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We have shown that exposure to neurotoxic doses of the gamma-diketone, 2,5-hexanedione (HD), produces axonal swellings and atrophy in rat peripheral nerves (Lehning et al. Toxicol. Appl. Pharmacol. 135:58, 1995). In the present study, we used transmission electron microscopy to characterize the morphologic and ultrastructural appearance of swollen and atrophic fibers in proximal sciatic and posterior tibial nerves. Rats (250-275 gm) were given daily gavage doses of 250 mg/kg HD and were sacrificed at slight, moderate or severe levels of hindlimb weakness. Tibial and sciatic nerves exhibited occasional swollen myelinated axons which were distended by excess, abnormally arrayed neurofilaments. Atrophic myelinated axons, however, were

abundant in both nerve regions but were more prominent in distal tibial when compared to proximal sciatic nerve. In addition, the magnitude and frequency of atrophy progressed as a function of HD exposure duration. Atrophied fibers displayed shrunken linear or irregular profiles which were correlated to reductions in ultrastructurally evident neurofilaments and increases in microtubule density. These morphologic observations were corroborated by immunoblot analyses demonstrating a decrement in neurofilament protein subunits with no change in tubulin content (See companion abstract by Opanashuk et al.). This study provides ultrastructural morphologic evidence that axon atrophy associated with gamma-diketone peripheral neuropathy results in cytoskeletal simplification where a selective decrease in neurofilament content occurs in conjunction with microtubule conservation. (Supported by ES07912.)

832 HEXANEDIONE NEUROPATHY: RELATIONSHIP OF AXONAL ATROPHY WITH ALTERATIONS IN NEUROFILAMENT GENE EXPRESSION.

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We have previously used quantitative morphometric analysis to demonstrate that axonal atrophy is the primary neuropathic feature associated with 2,5 hexanedione (HD) peripheral distal axonopathy. In this study, we tested the hypothesis that alterations in neurofilament content is the mechanism of HD-induced axonal atrophy. Rats were exposed by gavage to 3 dosing rates (100, 250, or 400 mg/kg/d) of HD until moderate changes in gait were observed (99,69, and 22 days, respectively). Dorsal root ganglia were removed and processed for isolation of total RNA. Alterations in neurofilament (NF) subunit (NF-H, -M, and -L) gene expression were assessed by northern blot. Proximal sciatic and distal tibial nerve proteins were subjected to immunoblot analysis for NF-H, -M, -L and tubulin content. HD exposure resulted in decreased levels of NF-H, -M, and -L mRNA at all dosing rates. Changes in RNA expression were paralleled by dose-rate dependent reductions in NF protein content, which were more prominent in distal tibial nerves. The greatest decrement in NF protein levels were detected in the long-exposure/low-dose (175 mg/kg/d) treatment groups. Slower-migrating NF-H immunoreactive bands were detected in nerves following 400mg/kg/d HD exposure. However, the presence of these higher molecular weight species do not account for the decline in neurofilament content because they were inconsistently detected on a spatiotemporal basis. Alterations in tubulin expression were not found in response to HD exposure. These findings indicate that reduced NF gene expression is a selective cytoskeletal effect, which could be related to the mechanism of HD-induced axonal atrophy. (Supported by NIH grant ES07912.)

833 FORMATION OF ACRYLAMIDE (ACR) AND GLYCIDAMIDE (GLY) HEMOGLOBIN ADDUCTS IN ACR-EXPOSED RATS.

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We have shown previously that oral ACR exposure produced abundant peripheral nerve axon degeneration whereas ip injection was not associated with fiber loss (Lehning et al., Toxicol. Appl. Pharmacol. 151:211-221, 1998). The basis for this differential expression of axon degeneration is uncertain but could involve exposure-dependent differences in metabolite production. GLY is an epoxide metabolite of ACR which may contribute to neurotoxicity (J. Toxicol. Env. Health 39:447-464, 1993). Both ACR and GLY readily adduct hemoglobin. Evidence suggests the extent of hemoglobin adduction in blood correlates positively with development of neurotoxicity and reflects concentrations of ACR and GLY protein adducts in tissue. Therefore, in the present study, ACR and GLY hemoglobin adducts were quantified as methyl ester derivatives by GC-MS. Adducts were determined in rats at several times during exposure to ACR by ip injection (50 mg ACR/kg/day) or in drinking water (2.8mM ACR). At times of equivalent neurobehavioral toxicity, ACR adduct contents were approximately equal for both exposure routes. However, GLY adduct levels were 4-7 times greater in rats exposed to ACR by drinking water than by ip injection ($p < 0.05$). These results indicate more GLY is formed in rats receiving ACR in drinking water than by ip injection and suggest GLY might be involved in peripheral nerve axon degeneration. (This research is supported by NIH grant ES03830.)

834 THE XENOESTROGEN 4-OCTYLPHENOL (4-OP) INCREASES THE EXPRESSION OF AMYLOID PRECURSOR-LIKE PROTEIN-2 (APLP-2) MRNA IN THE HYPOTHALAMUS OF THE SNAPPING TURTLE.

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The gonadal steroid estradiol (E2) is important for the development and regulation of hypothalamic function and many aspects of reproduction in vertebrates. Pollutants, such as 4-OP, that mimic the actions of estrogens are therefore candidate endocrine disrupting chemicals. We employed a differential display strategy (RNA-arbitrarily primed PCR) to isolate partial cDNA sequences of neurotransmitter, developmental and disease-related genes that may be regulated by 4-OP or E2 in the turtle hypothalamus. Hatchling and year-old male snapping turtles (Chelydra serpentina) were exposed to 10 ppb waterborne 4-OP or E2 for 17 days. One transcript (421 bp) regulated by 4-OP and E2 was 83% identical to human APLP-2. APLP-2 and the amyloid precursor protein (APP) regulate neuronal differentiation and are also implicated in the genesis of Alzheimer's disease in humans. Northern blot analysis determined that the turtle hyothalamus contains a single APLP-2 transcript of 3.75 kb in length. Exposure to 4-OP upregulated hypothalamic APLP-2 ($p<0.05$) mRNA levels 2-3 fold in month old and yearling turtles. E2 did not affect APLP-2 in hatchlings but in contrast stimulated a 3-fold increase ($p<0.05$) in APLP-2 mRNA levels in yearling males. The protein beta-amyloid, a selectively processed peptide derived from APP is also involved in neuronal differentiation, and accumulation of this neurotoxic molecule results in neuronal degeneration in the Alzheimer brain. Therefore, we wished also to determine the effects of estrogens on its expression. Using homology cloning based on known sequences, we isolated a cDNA fragment (474 bp) from turtle brain with 88% identity to human APP. Northern blot analysis determined that a single 3.5 kb transcript was expressed in the turtle hypothalamus. In contrast to APLP-2, waterborne 4-OP and E2 did not affect the expression of APP. Our results indicate that low levels of 4-OP are bioactive and can specifically alter the expression of APLP-2. Given that APLP-2 is involved in neuronal development, we hypothesize that 4-OP exposure may disrupt hypothalamic development in young turtles. (Supported by CNTC.)

835 ABILITY OF AG AND NAC TO ACT AS SCAVENGERS OF 4-HYDROXY-2(E)-NONENAL.

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Oxidative degradation of polyunsaturated lipids leads to the formation of α, β-unsaturated aldehydes, such as 4-hydroxy-nonenal (HNE), which have been linked to the progression of several age-related diseases including atherosclerosis and Alzheimer's Disease. Recent efforts in our laboratory have focused on the abilities of *N*-acetylcysteine (NAC) and aminoguanidine (AG) to prevent the reaction between HNE and proteins and mitigate subsequent tissue damaging effects from HNE. Using ^{13}C NMR spectroscopy and ^{13}C-labeled HNE, we found both NAC and AG can effectively compete with BSA for scavenging HNE. Although NAC had little effect on Michael adducts already formed on BSA, AG was able to substantially reverse the adduction and effectively remove HNE from the protein. Neither scavenger had any effect on pyrrole adducts once formed on the protein. Gel electrophoresis techniques showed that AG can effectively compete with β-lactoglobin (LG) for HNE to reduce and partially reverse protein cross-linking over time when pretreated with AG. AG, known to interfere with advanced glycation chemistry, may also act *in vivo* to effectively scavenge α, β-unsaturated aldehydes that arise from lipid peroxidation. (Supported by NIEHS Grants ES06387 and ES07028.)

836 DISULFIRAM IS A SELECTIVE SCHWANN CELL TOXICANT THAT PRODUCES S-(N,N-DIETHYLCARBAMOYL)-CYSTEINE PROTEIN ADDUCTS.

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Disulfiram (tetraethylthiuram disulfide or Antabuse®) is a dithiocarbamate drug used clinically for alcohol aversion therapy. In certain individuals, disulfiram administration produces a sensorimotor peripheral neuropathy. Disulfiram metabolism produces carbon disulfide (CS_2), but previous investigations have demonstrated minimal CS_2-mediated protein cross-linking to result from disulfiram administration, arguing against CS_2 as the proximate

neurotoxic metabolite of disulfiram. Identification of an S-(N,N-diethylcarbamoyl) glutathione metabolite of disulfiram suggested that covalent protein modification resulting from the electrophilic metabolites methyl N,N-diethylthiocarbamoyl sulfoxide or sulfone may contribute to disulfiram neurotoxicity. In the current study, male Sprague-Dawley rats were administered 1% w/w disulfiram in their diet. Hemoglobin and spinal cord axonal proteins were isolated at 2-, 4- and 7-week intervals, and separate animals were used to obtain spinal cord and peripheral nerve sections for light and electron microscopy. Protein samples were either hydrolyzed and analyzed by LC-MS/MS or examined intact by MALDI-MS for the presence of S-(N,N-diethylcarbamoyl) cysteine. Modified cysteine was detected at all time points and reached maximum levels at 2 and 4 weeks for hemoglobin and axonal proteins, respectively. A unique peak was identified in globin preparations analyzed by RP-HPLC that exhibited a mass consistent with the formation of an S-diethylthiocarbamoyl adduct on the beta3-globin chain. Elimination of modified beta3-globin obeyed first order kinetics with a half-life on the order of 11 days. Morphological changes detected in the peripheral nerves of disulfiram-exposed animals initiated with vacuole formation in the Schwann cell cytoplasm and progressed to segmental demyelination with little change observed within the axon. The formation of S-(N,N-diethylcarbamoyl) cysteine proteins adducts presents a potential mechanism through which disulfiram may target Schwann cells. (Supported by NIEH grants ES06387, ES07028, ES05764, & ES00267.)

837 FUMONISIN B1-INDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN HUMAN NEUROBLASTOMA CELLS UNDER GLUTATHIONE DEPLETION.

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Fungal growth in moisture problem houses often leads to exposure to large number of microbes many of which produce microbial toxins. They may cause deleterious health effects in animals and man. The typical symptoms of exposure to indoor air bioaerosols are fatigue and respiratory tract disorders such as infections, irritation of respiratory airways and the lungs, and febrile reactions, if the concentrations of the bioaerosols are sufficiently high. Recent findings also suggest that microbial toxins, e.g., fumonisin B1 (FB1), produced by Fusarium moniliforme, frequently present in mold problem houses, may cause serious effects in the CNS of animals and man. We have studied the effects of FB1 on the production of reactive oxygen species (ROS), levels of intracellular glutathione (GSH), and cell viability in human SH-SY5Y neuroblastoma cells. The cells were exposed to FB1 at concentrations of 0.01, 0.1, 1, 10, and 100 μM up to 36 h in 48-well plates. The production of ROS, the levels of GSH, and cell viability were measured at 0, 0.5, 1, 2, 3, 4, 5, 12, 24, and 36 h by using fluorescent probes, dichlorofluorescein, monochlorobimane, and propidium iodide, respectively. The exposures were carried out in the presence and absence of diethyl maleate (DEM) that effectively depletes intracellular GSH and thereby renders the cells more susceptible to oxidative stress. Exposure of the cells to FB1 induced a transient dose-dependent increase of ROS production from 0 to 5 hours, both in the presence and absence of DEM, that was not apparent at 12, 24, or 36 h. FB1 did not affect the GSH levels or cell viability at any time point. Number of studies have shown that these cell are resistant to oxidative stress. It is also tempting to speculate that the initial and transient ROS production may have lead to activation of transcription factors and subsequent alterations in gene expression that may have rendered the cells resistant to deleterious effects of FB1. (Supported by the Academy of Finland.)

838 NEURODEGENERATIVE DISEASE IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MICE.

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During pregnancy, the antioxidative enzyme G6PD protects the embryo from endogenous and xenobiotic-initiated DNA oxidation and teratogenicity mediated by reactive oxygen species (ROS) (Nicol et al., FASEB J. [in press] 1999). Here we determined if G6PD also might protect against endogenous ROS-mediated neurodegeneration in aged G6PD-deficient mice. Six normal (+/+) and 5 heterozygous G6PD-deficient (+/-) male and female mice, about 2 years of age, were killed by cervical dislocation and the brains were isolated for subsequent histological sectioning and H&E staining. Morphological observations were examined in a blinded fashion by a certified veterinary pathologist. In addition to the typical changes relating to aging in all animals, significant degenerative changes were observed in the brains of +/- G6PD-deficient mice compared to age-matched G6PD-normal controls. In the deficient mice, there was prominent cerebellar vacuolation of the white matter and increased pathological loss of Purkinje cells with concurrent astrocyte proliferation. In the frontal cortex, an increase in the glia-to-neuron ratio in the mid- to outer regions and an increased number of chromatolytic or pyknotic (dead or dying) neurons were observed, along with numerous condensed and pyknotic centrally chromatolytic neurons at the lateral border of the hippocampus. There were also scattered examples of large vacuolated mononuclear cells adjacent to blood vessels in the hippocampus. Lipofuscin accumulation was more pronounced in +/- G6PD-deficient mice. These results are consistent with an endogenous ROS-mediated mechanism of neurodegeneration, and suggest that G6PD-deficient mice may be a useful model for these diseases. Furthermore, G6PD appears to be an importanat neuroprotective enzyme, and hereditary G6PD deficiencies, which are the most common human enzymopathies, may constitute an important risk factor for some neurodegenerative diseases. (Support: Medical Research Council of Canada.)

839 EFFETS OF SDZ PSC 833 ON GLUCOSE METABOLISM IN PRIMARY CULTURES OF RAT NEURONAL AND RAT GLIAL CELLS.

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Impaired glucose metabolism plays a fundamental role in many heriditary and drug-induced neurological disorders. It was the purpose of the present study to evaluate, whether disturbances of the glucose metabolism in neural cells might also contribute to the reversible neurological side effects of PSC833 in rats. PSC833 and cyclosporine A (CsA) were investigated in primary cultures of rat neuronal and glial cells at the concentration of 0, 0.1, 1, 10 and 20 μM for 24 and 48 hours. Lactate dehydrogenase (LDH) was determined as a marker of cytotoxicity. Proliferation was determined in astrocytes. Glucose metabolism was investigated in cells by 13C-NMR using (1-13C)-glucose as a substrate. Glucose and lactate concentrations were determined spectrophotometrically in the cell culture supernatants. PSC833 at the concentrations of 10 μM PSC833 was neither cytotoxic in neuronal and glial cells, nor did it inhibite proliferation in astrocytes 24 hours after incubation. Under the same conditions, the determination of the (1-13C)-glucose metabolism revealed in both cell types, significantly increased glucose consumption by the formation of (3-13C)-lactate, as well as decreased levels of Krebs cycle intermediates in comparison to controls. In the cell culture medium 10 μM PSC833 treatment resulted from both cell types in increased glucose consumption and lactate production, same as CsA at 20 μM. The non-neurotoxic PSC833 metabolite M9 did not cause significant changes in glucose consumption and lactate formation not even at concentrations of 20 μM. The present results suggest, that PSC833-mediated impairment of the neuronal and glial glucose metabolism and the impaired TCA (tricarboxylic acid) cycle flux, resulting in decreased Krebs cycle metabolite formation, can cause energy depletion and acidosis, which might contribute to the reversible neurological symptoms of PSC833. The relevance of the present findings is strongly supported by the similar concentrations applied *in vitro* and those found *in vivo* in the brain in rats and by the finding, that the non-neurotoxic PSC833 metbolite M9 did not cause significant changes in all glucose metabolism parameters.

840 THE MITOCHONDRIAL PERMEABILITY TRANSITION: ROLE IN DIFFERENTIAL SUSCEPTIBILITY OF SY5Y NEUROBLASTOMA AND C6 GLIOMA CELLS AS A MODEL FOR 1,3-DINITROBENZENE-INDUCED ENCEPHALOPATHY.

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1,3-Dinitrobenzene (DNB)-induced encephalopathy is mediated by oxidative stress and mitochondrial dysfunction. The ability of DNB to induce mitochondrial permeability transition (MPT) was investigated in SY5Y neuroblastoma and C6 glioma cells. The present studies evaluate the differential susceptibility to induction of MPT following exposure to DNB. Neuroblastoma cells were more sensitive to the cytotoxic effects of DNB than glioma cells, as measured by the capacity of mitochondria to reduce the tetrazolium compound, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). The IC_{50} values for inhibition of MTT reduction by DNB were 107±25 μM in SY5Y cells and 1047±101 μM in C6 cells. DNB induced abrupt depolarization of mitochondria in both neuroblastoma and glioma cells that was inhibited by trifluoperazine. Onset of MPT occurred at ten-fold lower concentration of DNB in SY5Y cells than in C6 cells, consistent with the observed differential sensitivity to DNB-induced mitochondrial dysfunction.

Antioxidants, deferoxamine and α-toxopherol acid-succinate, prevented DNB-induced MPT in C6 and SY5Y cells, indicating that oxidative stress was involved in the induction of MPT by DNB. Exposure to DNB also caused a decrease in mitochondrial calcium in SY5Y and C6 cells, concurrent with onset of MPT. Mitochondrial calcium release was blocked by trifluoperazine, deferoxamine, and α-toxopherol, suggesting that DNB-induced mitochondrial calcium efflux requires induction of oxidative stress and opening of the permeability transition pore. These data demonstrate that SY5Y neuroblastoma and C6 glioma cells are differentially susceptible to DNB-induced MPT and suggest that differences in regulation of MPT may contribute to cell-specific oxidative injury in the central nervous system. (Supported by PHS-NIH grants ES08846 and ES06103.)

841 BCL-2-FAMILY PROTEIN EXPRESSION IN DIFFERENTIAL SENSITIVITY OF SY5Y NEUROBLASTOMA AND C6 GLIOMA CELLS TO 1,3-DINITROBENZENE-INDUCED MITOCHONDRIAL PERMEABILITY TRANSITION.

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In brain, regional and cellular differences in regulation of the mitochondrial permeability transition (MPT) may mediate sensitivity to endogenous and exogenous oxidants. The correlation between expression of Bcl-2-family proteins and sensitivity to 1,3-dinitrobenzene (DNB)-induced MPT was examined in C6 glioma and SY5Y neuroblastoma cells. C6 glioma and SY5Y neuroblastoma cells were exposed to DNB and evaluated by laser scanning confocal microscopy for loss of mitochondrial membrane potential, measured using the fluorescent cationic dye, tetramethylrhodamine (TMRM). DNB caused a rapid decrease in mitochondrial membrane potential in both C6 and SY5Y cells. By 40 min, membrane potential in SY5Y cells exposed to 50 μM DNB was reduced similarly to that in C6 cells exposed to 500 μM DNB. The first order rate constants for mitochondrial depolarization were: C6, $k = 0.31 \pm 0.02$ min^{-1}; SY5Y, $k = 0.14 \pm 0.01$ min^{-1}. Loss of mitochondrial membrane potential was inhibited in both cell lines by pretreatment with trifluoperazine, indicating onset of MPT. The antioxidants desferoxamine and α-tocopherol prevented DNB-induced loss of mitochondrial membrane potential, suggesting that oxidative stress from DNB exposure induced opening of the PTP. Following 60 min exposure to DNB, cellular ATP levels were reduced to 63% and 22% of control in C6 and SY5Y cells, respectively. The expression of Bcl-2, Bcl-X_L, and Bax was evaluated in C6 and SY5Y cells by western immunoblotting. C6 cells strongly expressed Bcl-X_L and only weakly expressed Bcl-2 and Bax, whereas SY5Y cells expressed lower levels of Bcl-X_L and higher levels of both Bcl-2 and Bax. These results suggest that higher constitutive expression of Bcl-X_L, rather than Bcl-2, correlates with resistance to DNB-induced MPT. (Supported by PHS-NIH grants ES08846 and ES06103.)

842 THE EFFECT OF PHENYLALANINE AND ITS METABOLITES ON ASTROCYTE PROLIFERATION: A POTENTIAL MECHANISM FOR PHENYLKETONURIA?

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Phenylketonuria (PKU) is a genetic defect that, without strict dietary control, results in the accumulation of phenylalanine (Phe) in body fluids. If the proper diet is not maintained during pregnancy, the offspring of phenylketonuric women are born with mental retardation and microcephaly (i.e., maternal PKU (mPKU)). Primary cultures of rat astrocytes and human astrocytoma (132 1N1) cells were used to test the hypothesis that the smaller brain size of individuals with mPKU is a result of reduced astrocyte proliferation. A 24 hr exposure to Phe or to six Phe metabolites (phenylacetic acid (PAA), phenyllactic acid, hydroxyphenylacetic acid, phenylpyruvic acid, methyl phenylalanine, and phenylethylamine (PEA), mandelic acid) did not result in cytotoxicity measured by the release of lactate dehydrogenase. Exposure to Phe, however, decreased cell proliferation measured by the incorporation of [3H]thymidine into cellular DNA. This effect was concentration dependent, and flow cytometry revealed that Phe exposure results in the accumulation of cells in the Go/G1 phase of the cell cycle. In addition, in 132 1N1 cells, exposure to PAA, and in rat astrocytes, exposure to PEA inhibited cell proliferation. Membrane localization is critical for the activation of Ras and RhoA, two small G-proteins that are involved in cell cycle progression. Immunoblot analysis revealed that treatment of both rat astrocytes and 132 1N1 cells with Phe analogs that inhibit proliferation results in an accumulation of Ras and RhoA in the cytoplasm. These results suggest that the central nervous system dysfunction associated with PKU, at least in part, may be due to reduced astrocyte proliferation, and that this effect may involve diminished translocation of Ras and RhoA to the cell membrane. (Supported by T32 ES07032 and P30 ES07030.)

843 COMPARISON OF A DYNAMIC, THREE-DIMENSIONAL, *IN VITRO* BLOOD BARRIER MODEL TO THE TWO-DIMENSIONAL CULTURE INSERT SYSTEM TO STUDY DRUG PASSAGE ACROSS THE BRAIN ENDOTHELIAL MONOLAYER.

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Promising therapeutics aimed at the CNS often fail in clinical trials, since many drugs are excluded by the blood-brain barrier (BBB). An *in vitro* model of the BBB is therefore an invaluable tool for researchers studying drug passage across the brain endothelial monolayer. The purpose of this study was to compare two configurations of an *in vitro* barrier model for use in assaying the ability of compounds to cross the BBB. The first model consisted of a two-dimensional system using bovine endothelial cells seeded onto the apical side of culture insert filters, which were placed into the wells of a 6-well plate. The permeability of the endothelial cell monolayers were measured by adding permeate (14C-Diazepam) and impermeate (3H-Sucrose) compounds. Aliquots were removed from the insert chambers and wells at several time intervals, and permeability values were calculated from the measured 14C and 3H levels. The results show that while the cells did establish a barrier to the compounds relative to blank inserts, the permeability values obtained for diazepam versus sucrose were not found to be significantly different. We then tested a model of the BBB consisting of a three dimensional co-culture system, using several artificial capillaries supported inside a sealed chamber. Bovine endothelial cells were seeded intraluminally, while rat glial cells were seeded in the extraluminal space. 3H-Sucrose and 14C-Diazepam were applied to the *in vitro* BBB intraluminally. Aliquots of media were taken from the extraluminal space and the intraluminal space at several time intervals and assayed for the presence of each compound. The permeability values obtained for diazepam were found to be on the order of 100-fold greater than those obtained for sucrose. This data demonstrates that the dynamic, three-dimensional model is much more sensitive in assessing the ability of compounds to cross the blood brain barrier, and more closely resembles the *in vivo* BBB.

844 POSITIVE MODULATION OF AMPA RECEPTORS PROMOTES CELLULAR REPAIR FOLLOWING EXCITOTOXIC INJURY TO BRAIN TISSUE *IN VITRO* AND *IN VIVO*.

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Ampakines are compounds developed for the treatment of neurological disorders. They enhance the ionotropic activity of AMPA receptors, glutamate-gated channels also known to function as cell-surface signal transducers by interacting with and/or regulating G-proteins, Src-family kinases, and MAP kinase pathways involving CREB and the neurotrophic factor BDNF (Nature 389:502, 1997; ibid. 397:72, 1999; J. Neuroscience 19:2954&5861, 1999). This study tested whether Ampakines can promote cellular viability by augmenting such metabotropic actions. Accordingly, CX516 (1-(quinoxalin-6-ylcarbonyl)piperidine) and other Ampakines were screened for protective effects in hippocampal slice cultures and adult rats treated with excitotoxic levels of AMPA (15-min infusion vs. intrastriatal injection). In the slices, the selective loss of CA1 neurons evident 24 h later was reduced 75% by 100 μM CX516; all subfields exhibited normal cell density and morphology 10 days post-insult when the Ampakine was administered. In the rats, CX516 co-injected with AMPA attenuated the MRI-measured lesion extent by 65% in a dose-dependent manner. Effects on changes in neuronal markers (CAT and GAD) also indicated protection. Persistent injury in the slice model, measured as calpain-induced cytoskeletal breakdown, exhibited Ampakine-mediated recovery in pyramidal and dendritic zones. Such cellular recovery was evident when Ampakines were administered before or after the excitotoxic episode. These data indicate that Ampakines promote signaling cascade(s) underlying neuroprotection. Close correspondence between protective action and electrophysiological influences among micromolar- and nanomolar-potent analogs will be discussed.

845 POTENTIATION OF OCTAVE-BAND NOISE INDUCED AUDITORY IMPAIRMENT BY HYDROGEN CYANIDE INHALATION.

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It is known that noise, hydrogen cyanide (HCN) and carbon monoxide (CO) are present in various environmental situations and can cause serious hazards with neurotoxicological effects. In previous studies from our lab, broadband and octave-band noise induced hearing loss has been found to be potentiated by simultaneous CO exposure. In the present study, octave-band noise induced auditory impairment was studied with the presence of HCN gas at levels of 10, 30 and 50 ppm. The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) is 10 ppm or 11 mg/m3 as a ceiling value, also with a "skin" notation. The octave-band noise had a center frequency of 13.6 kHz and the intensity of noise used was 100 dB (Ln). Experimental animals (rats) were exposed to noise, HCN and their combinations in the chamber for 2 hours. The compound action potential (CAP) and cochlear microphonics (CM) were recorded 4 weeks after the exposure to assess permanent auditory impairment. Noise induced elevation of the CAP threshold and the CM iso-amplitude curve were potentiated by the simultaneous HCN exposure of 10 ppm or higher. HCN potentiation seems dosage-dependent. HCN exposure alone had no effect on CAP and CM. The size of the potentiation shown by CAP and CM was similar, indicating a possible origin of HCN potentiation from damage to the outer hair cells. (This project was funded by NIOSH grant OH03481 to L.D. Fechter and by NSF EPSCoR Cooperative Agreement OSR-9550478.)

846 INTERMITTENT NOISE INDUCED HEARING LOSS AND THE INFLUENCE OF CARBON MONOXIDE.

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Intermittent noise causes less hearing loss than continuous noise of equal intensity. The reduction in damage observed with intermittent noise may be explained by the fact that the auditory system has time to recover between the noise phases. Simultaneous carbon monoxide (CO) exposure produces greater noise-induced hearing loss than does noise alone. In the present study, intermittent noise (octave-band with center frequency of 13.6 kHz, 100 dBLin) of 2-hours total duration, but with different noise duty cycle (% of noise during exposure) was used. The intermittent exposure that had shorter noise duty cycle (more rest periods in noise) induced less permanent threshold shift (PTS) than those that had longer noise duty cycle (or fewer rest periods). This relation between the loss in compound action potential (CAP) sensitivity and the noise duty cycle (or rest period) was abolished by the presence of CO. That is, all noise exposures produced equivalent threshold shifts if CO was present. Cochlear microphonic (CM) amplitude revealed similar results to those seen using the CAP. While intermittent noise did not cause hair cell loss by itself, the combined exposure to noise and CO (1200 ppm) caused remarkable OHC loss in the basal turn. (This work was supported by NIOSH grant OH03481 to L.D. Fechter and by NSF EPSCoR Cooperative Agreement OSR-9550478. The authors thank Dr. D.L. Ding for the helpful technical advice.)

847 COCHLEAR HISTOCHEMICAL CHANGES AFTER LOW MODERATE LEVEL TOLUENE EXPOSURE.

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Toluene is known as an ototoxic agent. However, permanent hearing loss has been reported in rats only after toluene exposure of 1000 ppm for 16 h/d for 5 days. Current permissible exposure limit (PEL) of toluene for human is 100 ppm. The current study evaluated cochlear function succinate dehydrogenase activity and apoptosis acutely following toluene exposure. Changes in succinate dehydrogenase (SDH) activity in auditory hair cells serve as an early marker of cellular stress in cochlear hair cells. In this study guinea pigs were exposed to low level toluene (1000 ppm, 500 ppm and 250 ppm) 8h/d, 5d/w for 4 weeks and distortion product testing was undertaken to assess outer hair cell function. Cochleae were removed immediately after the last exposure and incubated with SDH incubation solution. Reduced SDH activity in hair cells was observed in the basal turn in the animals exposed to 500-ppm and 1000-ppm toluene. However, this change was not seen in the animals with 3-day recovery period after exposure. Interestingly, the changes are comparable to the measurements of distortion product otoacoustic emmission (DPOAE). A few outer hair cells in the very base turn (corresponding to frequencies higher than 24 kHz) also showed signs of programmed cell death (apoptosis) suggesting the development of limited cellular damage undetectable using functional testing. The data show a clear low-level toluene-induced impairment of auditory hair cells. Some impairment may develop into a permanent injury. (Supported by NIEHS grant ES08082 and NIOSH grant OH03481 to L.D. Fechter and by NSF EPSCoR Cooperative Agreement OSR-9550478.)

848 LOW-MODERATE LEVELS OF TOLUENE INFLUENCE ON AUDITORY FUNCTION AND RELATED BLOOD CONCENTRATIONS IN THE GUINEA PIG.

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Toluene is a widely used organic solvent has adverse effects on the human auditory system, the mechanism of which is unclear. The current permissible exposure limit (PEL) for inhalation by humans is 100 ppm, which is also the lowest-observed-adverse-effect level (LOAEL) for acute neurological effects in humans. Recently, our studies in the guinea pig found that an acute disruption of otoacoustic emissions occurs for 8 hr/day, 5 day/wk inhalation exposures as low as 250 ppm. Interestingly, after 4 weeks of exposure, the disruption did not develop into a permanent hearing loss but an acute decrease in succinate dehydrogenase activity was seen. Published data suggest that 50% of toluene is metabolized within the first 60 min post-exposure. This rapid elimination of toluene may be related to the lack of a permanent hearing loss at low toluene concentrations. Subjects were exposed to 500 ppm for 8 hr/day and monitored for auditory disruption and succinate dehydrogenase activity at multiple points during the exposure. Additional subjects were exposed concurrently for determination of blood concentration. Data shows the low-moderate toluene inhalation effects on acute DPOAE shift, toluene blood concentrations, and succinate dehydrogenase activity. (This work was supported in part by NIEHS grant ES08082 and NIOSH grant OH03481 to L.D. Fechter and by NSF EPSCoR Cooperative Agreement OSR-9550478.)

849 DOSE RESPONSE OF CARBOPLATIN-INDUCED OTOTOXICITY IN RATS.

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Carboplatin, a platinum containing anticancer drug, currently being used against sarcomas of head and neck. However, a single high dose of carboplatin is ototoxic in cancer patients. This is the first study to show the ototoxic potential of carboplatin in a rat model. Male Wistar rats were divided into five groups and treated as follows: 1) control (saline, i.p.); 2) carboplatin (64 mg/kg, i.p.); 3) carboplatin (128 mg/kg i.p.); 4) carboplatin (192 mg/kg, i.p.) and 5) carboplatin (256 mg/kg, i.p.). Animals in all groups were sedated with ketamine/xylazine and auditory brain-evoked responses (ABER) were recorded then they were treated as above protocol. Post treatment ABER were measured four days after dosing. The animals were sacrificed on the fourth day and cochleae were harvested and analyzed. Carboplatin dose dependently decreased body weight (82%, 75%, 70% and 54% of control) respectively. No significant changes were noted on ABER threshold at 64 mg/kg and 128 mg/kg dose of carboplatin. However, at higher doses of carboplatin (192 mg/kg and 256 mg/kg), there was a significant elevation of hearing threshold shifts at clicks, 4, 8, 16, and 32 KHz tone burst stimuli. The cochlear antioxidant enzyme activities (SOD, CAT, GSH-Px, GR and GST) were decreased (90%-51% of control) with increasing dose of carboplatin. Cochlear lipid peroxidation was significantly increased (111-146% of control) in a dose dependent manner. The data suggest that higher dose of carboplatin induces oxtotoxicity as evidenced by a significant elevation of hearing threshold shifts, enhanced cochlear lipid peroxidation and depletion of cochlear antioxidant defense system in rats. (Supported by SIU School of Medicine and National Organization of Hearing Research.)

850 TOPOGRAPHIC ANALYSIS OF FLASH VISUAL EVOKED POTENTIALS IN DOGS.

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A visual evoked potential by flash stimulation (flash VEP) indicates an electrophysiological response from the retina to the visual cortex and is expected to be applied in the evaluation of neurotoxicity. Although flash VEPs have

been recorded in dogs, the relation between waves and the visual pathway is not well known. We analyzed the flash VEPs in dogs using a topographic method. On the topographic mapping, a negative response area indicated that evoked potentials were observed in the frontal region of the brain in the stimulated site followed by the shifting of the area to the contralateral frontal region and occipital region, during the first 100 msec. The negative response area in the frontal region in the stimulated site, contralateral frontal and/or temporal region, and occipital region were observed at latencies of N1, P2 and N2 of the flash VEP, respectively. On the ERG, the latencies of a- and b-waves were 12 and 30 msec, respectively. The latencies of a- and b-waves on the ERG were coincident with the latencies of P1 and N1 on the flash VEP. In the dogs with experimentally impaired right lateral geniculate bodies, the latency of P2 was prolonged and the N2 and P3 components of the flash VEPs disappeared after stimulation in the left eye. Only the early negative response area was detected at the stimulated site of the frontal region on the topographic mapping. Therefore, it is concluded that P1 and N1 are referred to ERG, P2 is referred to the potentials from the retina to the brainstem and N2 is referred to those from the brainstem to the visual cortex, respectively.

851 MORPHOMETRIC MEASUREMENT VALIDATION STUDY COMPARING DAY 9 AND DAY 11 SPRAGUE-DAWLEY RATS.

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This study validates an approach to the morphometric component for the US EPA OPPTS 870.6300 Developmental Neurotoxicity Study. Rather than subjecting pregnant rats to a variety of neurotoxic agents, the decision was made to initially perform the morphometric measurements on Day 9 and 11 Sprague Dawley (Crl:CD®BR VAF/Plus®) rat pups. Days 9 and 11 were chosen to demonstrate that the measurement data from the selected neuroanatomic areas would reveal age-related developmental difference and might, therefore, be predictive of either an *in utero* neurotoxic effect or a delay in brain development. Nine gross and microscopic morphometric measurements were made on the five brains/sex/timepoint: anterior-posterior length of the cerebrum and cerebellum; thickness of the frontal and parietal cortex, corpus callosum, hippocampal gyrus and external granular layer (cerebellum); width of the caudate/putamen; and height of the cerebellar cortex. As expected, the mean values for all of the neuroanatomic measurements were greater for the 11 day-old rat pups than for the 9 day-old pups, but there was overlap between the Day 9 and Day 11 rat pup data with the greatest measurement for at least one 9 day-old pup being greater than the lowest measurement for one of the 11 day-old pups. However, in spite of moderate data overlap between the two age groups and the presence of moderate intra-age group variability in the measurements for each neuroanatomic location, statistically significant increases were found for the following brain regions: anterior-posterior length of the cerebrum and cerebellum, thickness of the frontal cortex, width of the caudate/putamen, and height of the cerebellar cortex. Control morphometric data from six Developmental Neurotoxicology Studies will also be presented.

852 NEUROBEHAVIORAL EVALUATION OF RESIDUAL EFFECTS OF ACUTE CHLORINE INGESTION.

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Rationale: Chlorine exposure can lead to respiratory dysfunction resulting in brain cell hypoxia, injury and neurobehavioral disorders. **Assessment Approach:** Comprehensive neurobehavioral toxicity evaluations were performed on 2 cases, including WAIS, Neurotoxicity Screening Survey, Trailmaking (TT), and memory tests including Benton Visual Retention test (BVRT), Expanded Paired Associates Tests (EPAT), a logical memory test (LM), Selective Reminding Test (SRT), and tests for malingering and distortion. **Summary of Findings:** Case 1, 59 year old Ph.D., pre-exposure IQ 95th %, was served water contaminated with chlorine disinfectant, went into respiratory distress and treated at the local emergency room. He was examined 3 years post-exposure, and found to have vocal cord dysfunction and sleep apnea, central-type. BVRT showed 9 errors (strong indication of brain impairment); EPAT was at 34th and 17th %; *Neurotoxicity Screening Survey* was elevated at 236; SRT was at the 8th %; TT was at the 50th and 31st %; and LM < 1st %. Case 2, 40 year old male nurse, pre-exposure IQ at the 63rd%, was examined 1.5 years post-exposure to iced tea contaminated with chlorine. He developed reactive airway disease. Current IQ was at the 32nd %, with specific deficits in immediate memory, visual logic and psychomotor speed, all at the 16th %. BVRT showed 14 errors; EPAT was at 5th and 1st%; *Neurotoxicity Screening Survey* was elevated at 332; SRT was at 2nd %; TT was at 2nd and 24th %; LM < 1st. Follow-up interviews at 1 and 2 years post-exam indicated continued symptoms. In both cases, all test results of malingering were negative, and medical record review found no other explanations for the declines. **Conclusion:** Chlorine ingestion neurotoxicity was found which lasted many years after exposure.

853 USE OF TWO AROCLOR* LOTS TO EVALUATE TEQ AND OXIDATIVE STRESS PREDICTORS.

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In order to characterize risk associated with exposure to polyhalogenated aromatic hydrocarbons (PHAHs), the toxic equivalency factor (TEF) approach was created. Mixtures of polychlorinated biphenyls (PCBs) can thus be compared against dioxin. Two commercial mixtures of Aroclor 1254, Lot 6024 and Lot 124-191, were analyzed and Lot 6024 has approximately ten times the dioxin toxic equivalents (TEQ) of Lot 124-191. The purpose of this study was to determine if the TEQ for the two lots is predictive of any oxidative responses seen on a weight basis. Previous studies in our laboratory indicate that the TEQ is not predictive of all induced enzyme levels in lots with high non-dioxin like congeners. The TEQ works for EROD and MROD, not for PROD or T4 tests. Cytochrome c reduction, a measure of superoxide anion production, was in evidence only at the highest dose of Lot 124-191. Ascorbic acid, an antioxidant, was elevated at the highest doses for both lots. Uric acid, an antioxidant, showed a significant decrease over control at the highest dose for Lot 124-191. This suggests that multiple mechanisms that can lead to higher than expected levels of oxidative stress. In the present study, it is apparent that TEQs for mixtures can explain the variances in dioxin-like effects, but non-dioxin like congeners cause other responses that are not associated with the Ah receptor. The TEQ approach works well when only dioxin-like PHAHs are present. However, when non dioxin-like PCBs are present, application of the TEF approach must be used with caution. This is due to both synergistic and antagonistic interactions that have been observed with PCB mixtures. For antagonism, the TEF approach would overestimate the toxicity of a PCB mixture. For synergism, the TEF approach could significantly underestimate the toxicity of a mixture. For protection of human health and the environment, current regulations rely on the toxicity of individual congeners and do not take into account possible interactions. (This abstract does not necessarily represent EPA policy. DEB supported by EPA CT902908.)

854 HUMAN INTERINDIVIDUAL VARIABILITY IN THE EXPRESSION OF CYTOCHROME P450 FORMS CRITICAL TO XENOBIOTIC METABOLISM.

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Estimates of human interindividual differences in susceptibility to toxic insult may have their basis in pharmacokinetic (PK) and/or pharmacodynamic (PD) variations. Although the US EPA does consider separating uncertainty factors into PK and PD components, as done for perchlorate, there are no guidelines to inform such a process, and critical data are often absent. However, other agencies have separated the animal to human uncertainty factor (UF_A) into PK and PD components. To quantify human interindividual differences and produce results which might be used to support data-derived uncertainty factors for human interindividual variability (UF_H) with respect to the PK component, we have quantified and determined the distribution of several cytochrome P-450 (CYP) forms in a group of 141 human hepatic microsome samples. Mean and SD values (pmol CYP/mg microsomal protein) of CYP1A, CYP2E1 and CYP3A forms were 33.6±28.1, 59.4±18.2, and 141.9±104.5, respectively. Further, activity (pmol/min/pmol microsomal protein) towards substrates characteristic for a total of 7 CYP forms demonstrated standard deviations of 46 to 111% of mean values and normal distributions.Immunologically-detected CYP1A, CYP2E1 and CYP3A forms were also normally distributed. When data on enzyme selectivity for a given chemical exist, the specific activity of that enzyme can be combined with data describing the varaibility of enzyme expression to determine the extent of human interindividual variability in the intrinsic metabolism of the chemcial. Variations in intrinsic metabolism can be included in physiologically based PK models as has been done for trichloroethylene (*Toxicol Appl Pharmacol* 152:376-387, 1998). This improves estimates of human PK variability by including physiologic constraints such as solubility in blood and delivery to the liver.

855 PROSPECTS FOR IMPROVING RISK ASSESSMENT USING DNA-BASED MUTATION DETECTION.

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The sensitivity associated with the DNA-based detection of specific, somatic mutations, termed genotypic selection, continues to improve. We have developed an assay, MutEx/ACB-PCR with a sensitivity of 10-7 in reconstruction experiments. Application of MutEx/ACB-PCR to the detection of specific point mutations in genomic DNA requires isolation of genomic DNA fractions enriched for particular DNA sequences and this methodology is currently being developed. Extrapolation from other mutational targets suggests that the frequency of spontaneous basepair substitution mutation is on the order of 10-7 per basepair. Thus, a genotypic selection with a sensitivity of 10-7 may be sufficient to detect spontaneous mutation as well as any chemically induced mutations at particular oncogene and tumor suppressor gene sites. The potential to use genotypic selection to improve chemical risk assessment should now be explored. We propose that specific mutations known to predominate the human tumor spectrum (i.e., ras and p53 hotspot mutations) be developed as targets for genotypic selection. With such assays, induced mutant frequencies could be measured in situations of known mutagen/carcinogen exposure. For example, tissues of patients who had received chemotherapeutics could be analyzed and compared to those of untreated individuals and similar analyses, using equivalent doses, could be performed in rodent models. In so doing, the relevance of the rodent model for human risk assessment could be established in a quantitative manner with regard to a particularly relevant endpoint for cancer risk assessment, oncogene and tumor suppressor gene mutation. Replacing the default safety margins used to account for uncertainty in species extrapolation with precise measures of chemical-specific, cancer gene mutation induction is an example of how mechanistic data could be incorporated into and improve chemical risk assessment. (Sponsored by the SOT Regulatory and Safety Evaluation Specialty Section.)

856 ESTIMATION OF ACUTE INHALATION THRESHOLD CONCENTRATIONS FOR HUMAN EXPOSURE FROM LIMITED ANIMAL DATA.

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Data on safe concentrations for human exposure are lacking for many potentially hazardous chemicals that may be accidentally released to the atmosphere. We reviewed available sublethal and lethal animal and human toxicity data on 50 chemicals that are produced and transported within the United States. Inhalation exposure limits were derived for 30 min to 8 hr timepoints representing airborne concentrations 1) at or above which most individuals would experience irreversible or disabling effects and 2) which represent a threshold for lethality among humans. These concentration limits were based on the toxicity data and use of appropriate uncertainty factors. Ratios between these levels were generally 10 (92%), and most were 5 (80%). Only four chemicals had ratios that were >10 (8%). The mean ratio for the fifty chemicals analyzed was 5.04±6.22, the median was 3, and the range was 1.7-40. The 95% confidence limits were 3.27-6.80. An analysis of toxicity data utilized for development of these levels revealed a significant difference between ratios for chemical-specific values that were based on human and animal data, values based on animal data from different species ($p<0.05$), or values derived from data using the same animal species (n.s., but an obvious difference). Independent t-tests were used to determine statistical significance between groups of ratios. In summary, when data are lacking for determination of exposure concentrations associated with disabling or irreversible effects of a chemical in humans, a five-fold reduction of the lethality threshold from animal data would encompass ~ 80% of these situations, and a ten-fold reduction would encompass ~92% of these cases.

857 GUIDELINES FOR APPLICATION OF DATA-DERIVED UNCERTAINTY FACTORS IN RISK ASSESSMENT.

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A meeting of investigators of the data-derived uncertainty factor approach for estimating tolerable intakes, acceptable daily intakes or reference doses/concentrations is described. This meeting was designed to move beyond the default uncertainty factors of the IPCS (1994) scheme, by providing an initial description of criteria for the sufficiency of data for use as the basis of data-derived uncertainty factors. The results of this meeting are that animal and human data must be compared on the basis of the measurement of similar endpoints that are relevant to the critical effect. This comparison should consider all available toxicity studies, including all available studies on potential mechanisms of toxicity, modes of action, and biological plausibility. One should recognize that *in vivo* toxicity studies can often give results that reflect both kinetic and dynamic differences, and that some information on either, or both, can be wrought from such studies. Specifically, for consideration of adequacy of data to replace the default value for the toxicokinetic component of data within humans, or between humans and animals, a good PBPK model was considered the best basis for a quantitative interspecies comparison, followed by a good kinetic study in both experimental animals and humans where issues of chemical species, route of administration, amount of dose or concentration, and length of exposure are all carefully evaluated. Specifically, for dynamics comparison of animal to human or within human data, the basis of dynamic comparison must be relevant to the critical endpoint used as a basis of the RfD or TI. The quantitative determination of human toxicodynamic (and perhaps toxicokinetic) data-derived factors should compare mean values to 2 or 3 standard deviations away (as appropriate for endpoint). Comparison with sensitive subgroups should compare mean values in general population with 2 or 3 standard deviations away from the sensitive population mean value. Means values between these populations should not be used as the data-derived factor.

858 COMPARISON OF A SINGLE POINT ESTIMATE METHOD TO BENCHMARK DOSE RESPONSE MODELING FOR ESTIMATING POTENCY OF CARCINOGENS.

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The T25 method of evaluating the carcinogenic potency of a chemical, which is currently used by the European Economic Council, is based on the selection of a single dose in a chronic bioassay with an incidence rate that is significantly higher than the background rate. The T25 is determined from that single point by a linear extrapolation or interpolation to the chronic dose, in mg/kg/day, at which it is expected that there would be a 25% increase in the incidence of the specified tumor type above the background rate. Another method of deriving a carcinogenic potency value based on a 25% increase in incidence above the background rate is the estimation of an ED25 derived from a benchmark dose response model fit to the chronic bioassay data for the specified tumor type. A detailed comparison was made between the two methods using more than 250 chronic bioassays conducted by the National Toxicology Program. In each of the two-year bioassays, a tumor type was selected based on statistical and biological significance, and both T25 and ED25 benchmark estimates were determined for that endpoint. In addition, simulations were done using underlying cumulative probability distributions to examine the effect of dose spacing, number of animals per dose group, the possibility of a dose threshold, and variation in the background incidence rates on the T25 and ED25 estimates. The simulations showed that in the majority of cases the T25 method underestimates the true T25 dose and overestimates the carcinogenic potency. The ED25 benchmark estimate is generally less biased and has less variation about the true T25 value.

859 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR A BIOLOGICALLY-BASED DOSE-RESPONSE ASSESSMENT FOR DIMETHYL SULFATE.

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A biologically based dose response analysis was developed to derive appropriate control strategies for dimethyl sulfate (DMS). Dimethyl sulfate is an oily, colorless liquid with a wide variety of industrial applications. Inhalation studies have shown that repeated exposure to DMS leads to upper and lower respiratory tract tumors in rats and mice. The weight of experimental evidence suggests that DMS possesses genotoxic and carcinogenic potential. Here we present a quantitative dose-response assessment for inhaled DMS using a physiologically based pharmacokinetic (PBPK) model which is used to complete the necessary interspecies (rat-human) dosimetry extrapolation. The model is designed to predict the number of DNA adducts in the nasal mucosa following DMS exposure. The model predictions are compared to experimentally measured N7-methyl guanine (N7mG) DNA adduct levels in the respiratory and olfactory mucosa of rats following repeated daily exposures. The model predicts a steady increase in adduct levels during exposure and a rapid removal of adducts following cessation of exposure, similar to what is seen experimentally. The existing cancer bioassay data on DMS was modeled to determine the point of departure for low dose extrapolation using N7mG DNA adducts as the dose metric. Using the LED10 value from the rat cancer bioassay analysis and the pharmacokinetic model, human equivalent concentrations (HEC) were determined for various risks and exposure scenarios. These HECs were used to support development of appropriate control strategies for DMS exposure.

860 A TEST FOR REASONABLENESS OF DEFAULT VALUES FOR ENVIRONMENTAL CHEMICALS USING MONTE-CARLO SIMULATIONS AND PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS.

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Often the toxicity of environmental chemicals has to be estimated, based on certain assumptions. Some assumptions such as uncertainty factors are well recognized while others are not. Uncertainty factors (UF) of 10 have been routinely used for various types of extrapolations by governmental agencies, advisory bodies, and the private sector, and have been widely accepted by the scientific community. This study was undertaken to evaluate the appropriateness of using of such UFs for two key areas of uncertainties namely, intra- and inter- species extrapolations. This was achieved through the application of advances in computational techniques linking Monte-Carlo simulations with physiologically based pharmacokinetic (PBPK) animal and human models. Simulations were run for dichloromethane (DCM), a major environmental pollutant, at a NOAEL level to derive a distribution for total amount metabolized in animals. The output of these simulations were then applied to the human PBPK model to obtain a distribution for human equivalent NOAELs. The MRL value of 6x10-2 mg/kg/day, derived by applying the UFs approach to the DCM NOAEL, is about the 95 percentile of the simulated exposure distribution in humans. These results show that the use of the default values of 10 for DCM are reasonable. We are in the process of studying other environmental chemicals such as trichloroethylene and vinyl chloride to understand the universality of this trend and determine that the use of these values provide adequate protection to the public.

861 BIOLOGICALLY BASED DOSE-RESPONSE (BBDR) MODELS AS A MECHANISTIC FRAMEWORK FOR ORGANIZING THE EXTANT LITERATURE AND DATA.

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Biologically based dose-response (BBDR) models integrate what is known about a toxicant into a simulation of the toxicant's kinetics and dynamics. Constructing a BBDR model requires a review and organization of the literature and data needed to estimate parameters and to mathematically describe interactions among biologically relevant compartments. As the BBDR model begins to take shape, the model itself provides a useful mechanistic-based framework for further organizing the literature and the supporting data. Recent advances in web-site and database software support this approach and are particularly helpful with two key challenges in constructing BBDR models: 1) an efficient means by which to identify, extract, and organize data especially for compounds with an extensive literature base; and 2) access to the information needed to perform quality assessments. Presented here is an example of a hypertext web-site based upon a BBDR model for the developmental effects of 5-fluorouracil (5-FU). The 5-FU BBDR framework provides an efficient template for organizing and accessing the relevant abstracts, articles, and extracted information from the literature as well as access to a full audit trail of the data used to develop and validate the model. This audit trail provides the information needed to perform quality assessments of the model and the parameter estimates. With appropriate quality controls and peer review for incorporated information, wide-spread access to such a web-site would reduce duplication of effort among researchers and agencies needing to summarize the extant literature, would facilitate collaboration in the construction of a BBDR model, and would provide a mechanistic framework for identifying data needs. (This abstract does not necessarily reflect EPA policy.)

862 PROPOSED NEW RISK ASSESSMENT FOR ACETALDEHYDE.

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EPA is in the process of updating the risk assessment for acetaldehyde. Animal studies have demonstrated irritant effects of acetaldehyde following both oral and inhalation exposure, with the critical effect for both routes of exposure being hyper- and metaplasia of the tissues along the portal of entry. While oral carcinogenicity data are unavailable, chronic inhalation studies have demonstrated tumor formation in the nasal cavity, with tumors being located in the same areas that the noncancer effects are seen. The proposed RfD (Oral Reference Dose) is based on a published NOAEL of 125 mg/kg-day for hyper- and metaplastic responses in the forestomach of male Wistar rats. An uncertainty factor (UF) of 3000, (10 for intraspecies variation; 10 for interspecies extrapolation; 10 for use of a subchronic study; 3 for database insufficiencies), was applied to derive an RfD of 4x10-2 mg/kg-day. The proposed RfC (Inhalation Reference Concentration) is based on a published NOAEL of 390 ppm for hyper- and metaplastic responses in the posterior nasal region of male and female Syrian golden hamsters. Following conversion to a NOAEL[HEC] of 5.45 mg/m3, an UF of 1000 (3 for interspecies variation; 10 for intraspecies variation; 10 for the use of a subchronic study; 3 for database insufficiencies) was applied to derive a proposed RfC of 5x10-3 mg/m3. Oral carcinogenicity data are unavailable. The inhalation unit risk was based on the incidence of upper respiratory tract tumors in male Wistar rats following a 28-month exposure to acetaldehyde. An LEC10 of 7.43 mg/m3 was calculated from the incidence data using a polynomial curve-fitting program (Global86), and used to derive an inhalation unit risk of 1.3x10-5 (µg/m3)-1. Acetaldehyde is presently classified as a group B2 chemical (probable human carcinogen) under EPA's 1986 guidelines for carcinogen risk assessment. (This abstract does not necessarily reflect EPA policy.)

863 CARCINOGENIC POTENCY OF ENVIRONMENTAL TOBACCO SMOKE (ETS) IN STRAIN A/J MICE.

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While directly inhaled tobacco smoke has long been recognized as a human carcinogen, concerns about environmental tobacco-smoke exposure (ETS) as a human cancer risk factor have mounted considerably over the last 15 y. Epidemiological studies suggest a relative risk of about 1.3 for the incidence of lung cancer among nonsmoking spouses, but the statistical and biological significance of this risk remains contested. Recent studies involving exposure of strain A/J mice to well defined ETS atmospheres examined various toxic endpoints, including 5 independent 10-month studies in which rates of lung-tumor occurrence in groups of mice exposed to ETS alone were compared to those of groups kept in filtered air. Detailed data obtained on chamber concentrations of TSP (total suspended particulate matter) throughout exposure allowed traditional multistage estimates of ETS carcinogenic potency in A/J mice using combined sex-specific data sets indicating statistically significant ETS-related elevations in either animals with lung tumor(s) or total lung tumors per animal. Best estimates of ETS potency based on these data range from 0.4 to 1 per (mg TSP per kg body weight per d), implying a corresponding increased lifetime lung-tumor risk of 0.3% to 0.7% in humans

chronically exposed to typical ETS concentrations in U.S. smoking households (assuming ETS is equally potent in A/J mice and humans). The mouse-based ETS risk estimates overestimate lung-cancer mortality risk in human smokers by a factor of ~20 to ~60, which is consistent with A/J-mouse hypersusceptibility to lung tumorigenesis

864 ALTITUDE AS A FACTOR IN CARBON MONOXIDE RISK ASSESSMENT.

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The National Ambient Air Quality Standard for carbon monoxide (CO) is set at 9 ppm for 8 hrs based on studies in subjects with exercise-induced angina exposed to CO during exercise at sea level. It has been estimated that over 35 million people live or visit at altitudes above 5,000 ft in the United States; worldwide, the figure is closer to 80 million. Estimates indicate that nearly 60% of those going to altitude are older than age 40 and 15% are older than age 60. In addition to a higher prevalence of cardiopulmonary disease, older people may have reductions in ventilatory capacity, efficiency and hypoxic ventilatory drive that impair the normal adaptive response to altitude. The population at altitude comprises both residents, fully adapted to altitude, and short-term visitors or sojourners. It is the sojourner at altitude who is at greater risk from exposure to CO. The decreased oxygen tension (hypoxia) in the inspired air, the most prominent feature of the altitude environment, causes physiological changes that affect dosimetry and the potential toxicity of CO. The hypoxemia resulting from ascent to altitude increases with increasing elevation and could be expected to intensify the pre-existing hypoxemia in cardiopulmonary impaired patients. Thus, the increasing altitude would affect dosimetry as well as exacerbate the pathophysiological effects produced by CO in subjects with cardiopulmonary disease. Therefore, cardiopulmonary impaired subjects exposed to CO in the more stressful altitude environment could be expected to suffer adverse health outcomes at lower ambient CO concentrations than subjects exposed at sea level.

865 NONCANCER ASSESSMENTS OF PHENOL.

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Phenol is a component of several consumer products, including analgesics, ointments, throat lozenges, and tobacco smoke, and has been reported as a contaminant in drinking water and at Superfund sites. The current phenol RfD on IRIS was adopted in 1989, based on reduced fetal weight in a gavage developmental toxicity study in rats. A 1986 review found the data inadequate for the development of a RfC. A number of new studies have been conducted since those assessments. New oral studies include a 13-week neurotoxicity study, a 2-generation reproductive study, and a rat developmental toxicity study, as well as a study comparing toxicokinetics via different routes and a number of other toxicokinetics studies. These studies improve the quality of the database, although no database uncertainty factor was used for the RfD currently on IRIS, despite the problematic nature of some of the studies. In light of the new studies, no database uncertainty factor would be required now. A 2-week inhalation study in rats is newly available, and supplements the earlier database of low-quality 2-week and 90-day studies, including a 2-week study that evaluated some neurotoxicity endpoints. The adequacy of the overall inhalation database for develop of a RfC is questionable. The hazard identification of this chemical is focussing on decreased fetal body weight, neural effects, hematological effects, and immune effects; the critical effect is chosen from these endpoints. In addition to the new data, EPA methods for noncancer risk assessment have been updated. An updated RfD and an evaluation of the appropriateness of deriving an RfC, will be presented, based on current EPA methods. (This work was conducted under contract to EPA, but the opinions expressed in this abstract are those of the authors and do not necessarily represent EPA policy.)

866 CALIFORNIA PUBLIC HEALTH GOALS FOR CARBOFURAN, DIQUAT, SIMAZINE, AND THIOBENCARB.

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The California Office of Environmental Health Hazard Assessment conducts risk assessments, based exclusively on public health considerations, for chemicals in drinking water in support of Public Health Goals (PHGs). PHGs are concentrations of chemicals in water that are not expected to produce adverse effects in humans consuming water over a lifetime. The development of PHGs for the pesticides carbofuran, diquat, simazine, and thiobencarb involved a critical review of the available data submitted in support of pesticide registration as well as from the open scientific literature, and the use of state-of-the-art risk assessment methods. The proposed PHG of 1.7 ppb for carbofuran is based on a no-observed-adverse-effect-level (NOAEL) of 0.1 mg/kg-day for observed changes to the testes, particularly to the sperm itself, at higher doses in male rats. For diquat, the proposed PHG of 15 ppb is based on an estimated NOAEL of 0.22 mg/kg-day for lens opacities from a chronic dietary study in male and female rats. The proposed PHG for thiobencarb is 70 ppb, which is based on a NOAEL of 1 mg/kg-day for noncarcinogenic effects in rats. The proposed PHG of 0.4 ppb for simazine is based on mammary gland carcinomas observed in female rats in a chronic dietary study. The cancer slope factor was derived by linear extrapolation from the lower 95% confidence limit on the dose associated with a 10% increased risk of cancer (LED_{10}). Once adopted, PHGs are intended to guide California risk managers in establishing and interpreting state drinking water standards.

867 ENVIRONMENTAL CONTAMINANTS AND POTENTIAL HUMAN RISK ASSOCIATED WITH SELECTED BOTANICAL DIETARY SUPPLEMENTS.

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Botanical dietary supplements have a long history of use in Europe and China, but they are becoming increasingly popular in the United States. However, little data is available regarding environmental contaminants in botanical dietary supplements and the health risk posed to those ingesting contaminated supplements. To investigate this data gap, Ginseng, St. Johns Wart, Passion Flower, Echinacea, and Valarian were purchased from the United States, Europe and China. Samples were analyzed for metals (arsenic, cadmium, nickel, chromium, and lead) and chlorinated pesticides (PCNB, DDT and metabolites, aldrin, etc.). Flame and furnace atomic absorption spectrophotometry were utilized for analysis of metals, while gas chromatography was utilized for analysis of chlorinated pesticides. Since no formalized guidelines exist for the determination of risk and hazard in botanical dietary supplements, U.S. Environmental Protection Agency guidelines for the protection of human health were utilized. Metals and chlorinated organics were present in all samples analyzed, but these concentrations pose a low noncarcinogenic hazard (<0.2); however, chlorinated pesticides presented 1x10-6 risk in Ginseng (24%), Passion Flower (40%), Valarian (45%), Echinacea (29%) and St. John's Wart (58%) samples at a dose of 1000mg/day (a medium to high dose depending on the supplement). If a low dose of supplement is taken daily (100 mg), then only one of the samples exceeded 1x10-6 risk. In addition, a reduction in exposure duration (from 350 d to 42 d) significantly reduced the calculated risk.

868 CORRELATION OF THE UTEROTROPHIC ACTIVITY AND THE EXPRESSION OF ESTROGEN SENSITIVE GENES IN THE RAT UTERUS IN RESPONSE TO XENO- AND PHYTOESTROGENS.

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Uterine response to estrogens involves the activation of a large number of estrogen sensitive genes. We have investigated the estrogenic potency of xeno- and phytoestrogens and the mechanisms of their molecular action by a correlation of the uterotrophic activity and their ability to modulate the expression of estrogen sensitive genes. The expression of androgen receptor (AR), progesterone receptor (PR), estrogen receptor (ER), clusterin (CLU), complement C3 (C3), and GAPDH mRNA in the rat uterus following oral administration of ethinylestradiol (EE), bisphenol A (BPA), o,p'-DDT (DDT), tert-octylphenol (OCT) and daidzein (DAI) was analysed. A significant stimulation of the uterine wet weight could be observed in response to high doses of all substances. Their overall potency was low compared to EE. DDT exhibited a comparatively high potency to stimulate uterine weight whereas BPA and DAI were less potent. The analysis of gene expression revealed a very specific profile of molecular responses which are unpredictable from the uterotrophic response alone. A dose dependent analysis revealed that C3 mRNA is already modulated at doses where no uterotrophic response was detectable. Although DAI and BPA only weakly stimulate uterine growth these substances led to a strong alteration of the expression of AR, ER and C3. In conclusion, according to their response pattern the analyzed compounds can be subdivided into distinct classes of substances, characterized by their

potency to induce a compound specific fingerprint of uterine gene expression. This in turn represents a very sensitive tool to investigate estrogenicity of natural and synthetic compounds and to get information in regard to their molecular mechanisms.

869 TRANSFERABLE FUR RESIDUES AND CHOLINESTERASE INHIBITION OF DOGS TREATED WITH FLEA CONTROL COLLARS CONTAINING ORGANOPHOSPHORUS INSECTICIDES.

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Dogs were treated with a commercial over the counter flea and tick control collar containing chlorpyrifos (Dursban® - 8.0%, 2.54 g) or tetrachlorvinphos (Rabon® - 14.55%, 4.8 g) using the recommended guidelines. The residues available for transfer to humans from the fur of the dogs and inhibition of plasma cholinesterase in the dogs was determined over the study as a biomarker. Twenty-four dogs were used for each study and the fur was sampled by rubbing with cotton gloves for 5 minutes in three 10 x 4 inch areas: along the lower back (back), along the neck without the collar in place (neck), and along the neck with the collar in place (collar). Gloves were extracted by the Accelerated Solvent Extractor. Tetrachlorvinphos and chlorpyrifos residues were analyzed by GC/ECD. The residue and blood samples were taken at 8 time points after placement of the collar: 4 hrs, and 3, 7, 14, 28, 56, 84, and 112 days (and also 140 and 168 days for chlorpyrifos). For tetrachlorvinphos, the average values for these eight time points for the collar were 14, 24, 24, 19, 13, 13, 4, and 3 mg; for the neck were 4, 8, 8, 6, 4, 3, 1, and 1mg; for the back were 0.2, 0.3, 0.2, 0.2, 0.1, 0.1, 0.03, and 0.02 mg. Preliminary results (12 dogs) of the chlorpyrifos samples through 140 days averaged 0.2-0.5 mg for the collar, 0.1-0.25 mg for the neck, and 0.003-0.005 mg for the back. Plasma cholinesterase levels were measured as butyrylcholinesterase using butyrylthiocholine and as acetylcholinesterase using acetylthiocholine. In the preliminary results, chlorpyrifos inhibited the dog plasma butyrylcholinesterase and acetylcholinesterase but tetrachlorvinphos did not. Tetrachlorvinphos showed a peak at 3 to 7 days and decreased throughout the study. In contrast, chlorpyrifos showed constant and significantly lower transferable residue levels throughout the study. (Supported by EPA R 825170.)

870 CHRONIC PATHOLOGICAL EFFECTS FROM EXPOSURE OF JAPANESE MEDAKA (*ORYZIAS LATIPES*) EMBRYOS TO BROMOFORM.

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The purpose of this study was to determine the chronic toxicity of bromoform in an alternative test species, Japanese medaka (Oryzias latipes). In this study, we exposed medaka embryos at 64-cell stage (early high blastula) to 0, 5, 10, 25 and 50 mg bromoform /L (nominal concentrations) and DMSO (solvent carrier) for 10 days. The embryos then transferred to clean embryo-rearing solution until they hatch, followed by six to twelve month grow-out in spring water. At 6 and 12 months post hatch, up to 60 fish were killed by overdose of anesthesia, fixed in 10% neutral buffered formalin, and evaluated histologically. Immature gonads and cystic ovaries were found in all groups, but the incidence was higher in bromoform-exposed fish. The incidence of thyroid follicular hyperplasia was higher at twelve months in bromform-exposed fish. A low incidence of a variety of renal degenerative lesions occurred in both control and bromoform- exposed fish. However, the incidence of renal cystic tubules was higher in bromoform-exposed fish. A low incidence of degenerative and non -neoplastic proliferative lesions were observed in the livers of both control and bromoform- exposed fish. There was an increased incidence of hepatic cysts in the six-month old medaka exposed to bromoform. Dose-response of histopathological endpoints will be discussed. Critical endpoints from this study will be compared to the chronic toxicity of bromoform in rodent risk assessment models. (Abstract may not reflect USEPA policy. Funded in part by USEPA Cooperative Agreement.)

871 CHRONIC TOXICITY OF CHLOROFORM IN THE JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Short-term *in vivo* toxicological methods are needed to provide data for the assessment of health risk of disinfection by-products such as chloroform. Japanese medaka embryos (N=500) at early high blastula stage were exposed to nominal concentrations of 25, 50 or 100 mg/L chloroform for 10 days, transferred to embryo-rearing solution until they hatched, and then were grown-out for six to twelve months in spring water. Sixty fish were killed at 6 months post hatch and the remaining were killed at 12 months post hatch, and histologically evaluated. Hepatic, renal, gonadal , and thyroid lesions were associated with chemical exposure. A low incidence of hepatic neoplasms [cholangioma (25 and 50 mg/L) and lipoma (50mg/L)] was observed in chloroform-exposed fish. The hepatic non-neoplastic proliferative lesions were clear cell foci, vacuolated cell foci and altered hepatocellular foci. The hepatic degenerative lesions observed were excessive accumulation of substances (glycogen and or lipid), fatty change, necrosis, hepatic cysts, and spongiosis hepatis. Renal lesions were primarily degenerative in nature except one case of a neoplasm, renal lipoma, in one 12-month-old fish exposed to 25 mg/L chloroform. Ectopic ovarian follicles were found in one fish exposed to 100 mg/L chloroform and a female control fish had ectopic testis in the anterior abdominal cavity. Immature ovaries were found in fish exposed to 25 (16/46) and 50 mg/L (10/47) chloroform and one control fish examined after 12 months. Thyroid follicular hyperplasia was observed in all groups of fish including controls but the incidence was higher in fish exposed to 25 and 50 mg/L chloroform. Higher mortalities in the 100 mg/L group probably resulted in a lack of expected dose-response. Potential utilization of this research for risk assessment is presented. (Abstract may not reflect U.S. EPA policy. Funded in part by USEPA Cooperative Agreement.)

872 SAR ANALYSES AS PART OF THE HIGH PRODUCTION VOLUME CHEMICAL CHALLENGE INITIATIVE.

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Within the next five years chemical manufactures are required, as part of the EPA's High Production Volume (HPV) Chemical Challenge Initiative, to make available basic toxicological information on approximately 2800 chemicals. These chemicals are all produced or imported into the US in over 1 million pound quantities per year. The data required consist of the Organisation for Economic Cooperation and Development Screening Information Data Set (SIDS). These data are designed to provide an initial minimal set of information for hazard identification. The purpose of our experiment is to assess the utility of structure-activity relationship (SAR) modeling as a prescreening technique for hazard identification. This will allow chemicals to be rationally prioritized for more comprehensive testing. We used the MCASE SAR program and its contingent of approximately 60 validated SAR models for a variety of toxicological endpoints. For analysis, we randomly chose two sets of 100 organic chemicals (i.e, inorganic and chemical mixtures were excluded) from the HPV list. These chemicals have been assigned to undergo SIDS testing in the early phase of the program. This will allow us to assess and improve our methods as the HPV Initiative progresses. Based upon SAR models of *Salmonella* mutagenicity and the *in vivo* micronuclei assay, 8% of HPV chemicals have the potential for being *in vivo* genotoxicants and therefore represent a cancer risk to humans. On the other hand, between 11-31% (depending upon protocol) of the chemicals were predicted to be rodent carcinogens providing that the protocol included testing at the maximum tolerated dose. Also, 3% of HPV chemicals may be developmental toxicants. Our results so far indicate that SAR approaches to hazard identification of widely used chemicals represent a viable alternative to traditional methods.

873 DERIVATION OF A SPECIFIC DERMAL CANCER POTENCY FACTOR FOR BENZO(A)PYRENE.

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A review of recent site-specific risk assessments revealed that in many cases much of the risk was due to dermal exposure to polynuclear aromatic hydrocarbons (PNAs), especially benzo(a)pyrene (B[a]P), in soil. For B(a)P, a dermal cancer potency factor (CPF) is normally derived from an oral CPF adjust-

ed for absorption. This common practice introduces uncertainties due to inherent differences in the absorption, pharmacokinetics, and target organ specificity of chemicals following different routes of exposure. Little or no consideration is given to the bioavailability and degradation of B(a)P in soil. These conservative and arguably erroneous assumptions give disproportionate weight to PNAs at or near urban soil background levels. Using B(a)P as an example, published data from several dermal carcinogenicity studies of B(a)P were evaluated in combination with pharmacokinetic parameters to derive a specific dermal CPF for exposure to B(a)P. Soil, weather, and time also influence the amount of B(a)P that is available to be absorbed from the skin and need to be considered. The major target organ for B(a)P following dermal exposure is the skin. Numerous rodent dermal toxicity studies show that B(a)P absorption into the epidermis produces tumors. Published literature on dermal absorption of B(a)P, skin permeability, the influence of a soil matrix, and aging was used to derive a refined estimate of the amount absorbed through the skin from soil. A comparison of the risks derived from the default and the refined approach demonstrates that the risks associated with dermal exposure to soil bound B(a)P are likely lower than previously assumed.

874 ORGANIC TIN EXTRACTION RESULTS FROM POLYVINYL CHLORIDE (PVC) AND CHLORINATED POLYVINYL CHLORIDE (CPVC).

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NSF International is a third party, independent, not-for-profit organization, which is dedicated to public health safety and protection of the environment. Since 1944, NSF International has developed product standards and provided third party conformity assessment services to government, users, and manufacturers/providers of products and systems. NSF has been developing standards for the testing and certification of plastics plumbing components since 1965. ANSI/NSF Standard 61: Drinking Water System Components-Health Effects establishes requirements for the testing and evaluation of contaminants that are extracted from water, which has been exposed to a material or product that is intended to convey drinking water. Dimethyl and dibutyl organotin stabilizers are added to PVC/CPVC formulations to protect the resin from thermal degradation during processing and usage. Stabilizers protect the PVC/CPVC by inhibiting the dehydrochlorination reaction by exchanging their anionic group, X, with the allylic chlorine atoms in the polymer. In addition, stabilizers can react with the HCl produced, producing compounds which may inhibit other side reactions. There are more than 595 PVC/CPVC products produced by 88 companies at 148 manufacturing sites currently certified by NSF International to the health-effects requirements of ANSI/NSF Standard 61. NSF tests approximately 350-500 samples annually based upon the protocol described in ANSI/NSF Standard 61, Appendix B. The samples tested at NSF are new products which are tested at pH 5, pH8 and pH 10. Before testing, the samples are conditioned for 14 days at 30°C in water. Immediately following conditioning, the samples are exposed for one final 16-hour exposure. The quantitative measurement of organo-tin is an indirect measurement. Our laboratory analyzes the extractant water for total tin content, which is mathematically adjusted for molecular weight to reflect the maximum potential organo-tin extraction value. The lab results are then normalized for the pipe size based on its intended end use. NSF began collecting extraction results from PVC/CPVC material products testing in 1992. The normalized concentrations of organic tin averages 6.4 μg/L with a range from 0.0005 to 46 μg/L for PVC. The normalized concentrations for CPVC averages 11 μg/L with a range from 0.00013 to 140 μg/L. There are occasions when the concentration extracted of organotin exceed the Maximum Drinking Water Level (MDWL) for chronic exposure based on a single time point analysis. In those cases, a follow up multiple time point analysis is performed, followed by a regression analysis of the data to evaluate the decay of tin overtime. The day 1 normalized concentration (acute exposure) is compared to the Short Term Exposure Level (STEL) and the extrapolated Day 90 concentration (chronic exposure) is compared to the MDWL. Based on our current database, more than 99% of the products tested with a multiple time point analysis have met the pass/fail criteria for acute and chronic exposure as defined under ANSI/ NSF Standard 61, Annex A.

875 BIOAVAILABILITY OF CAPTAN IN HUMAN BLOOD.

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Captan is a broad-spectrum fungicide that is commonly used throughout the world to protect food crops and other plants from fungal diseases. Chronic feeding studies have shown captan to cause duodenal tumors in mice (but not rats) at high doses (>800ppm). Mechanistic data suggest that duodenal tumor formation is a result of chemical irritation to the intestinal villi, a non-genotoxic event. The risk of developing duodenal cancer from captan depends upon an "irritation-producing" concentration of captan to the duodenum. The bioavailability of captan to the duodenum is crucial for the formation of tumors. For oral exposure, the extrapolation from mouse bioassays to humans is direct; for dermal exposure, however, the stability of captan in blood becomes an important factor. A freshly drawn human blood sample was used in an *in vitro* degradation study at 37°C with ^{14}C-captan. It demonstrated that captan degrades rapidly in human blood with a measured half-life as short as one second. Evolution of the imide ring, tetrahydrophthalimide, was measured, confirming captan's degradation versus its possible loss through absorption to blood constituents. Following dermal exposure, captan will not be available at the target organ (duodenum) since multiple half-lives would have taken place during the systemic transit time (averaging one minute). This finding is relevant in the risk characterization of captan via dermal route. Farm workers such as strawberry harvesters who contact captan are without measurable risk of duodenal cancer. Current regulatory paradigms do not consider this degradation and thus overestimate the risk to farm workers. The addition of this blood degradation factor to the USEPA and California DPR human risk assessments for dermal exposure will eliminate the cancer risk concern following worker exposure.

876 DERMAL EXPOSURE TO BENZO[A]PYRENE (BAP): COMPARATIVE DOSIMETRY IN THE MOUSE AND HUMANS.

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Chronic rodent bioassays, by either the oral or inhalation routes of exposure, have been used to derive cancer potency values. For dermal exposures, route to route extrapolation is done either by adjusting the orally-derived potency value by the comparative absorption (oral versus dermal) or, less frequently, by pharmacokinetic models that include the skin as an active compartment. When the skin is the target tissue, it may appear preferable to use a chronic dermal study, e.g., mouse skin painting study, to derive a dermal cancer potency factor. In the absence of a kinetic model, significant adjustments to the applied dose are required when the chemical of interest is a PAH. Extrapolations must consider differences in absorption of the compound, metabolism to the active component, and the sensitivity of the tissue to that active component. The human equivalent dose of BaP applied to the skin that would result in the same tissue dose, is more than a 1000 times greater than the dose applied in the mouse skin painting study. Using the applied dose from a mouse skin painting study without dosimetric adjustments will result in a significant overestimation of the dermal cancer potency for BaP.

877 EVALUATION OF THE DERMAL BIOAVAILABILITY OF SOIL-AGED NAPHTHALENE.

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Assessment of health risk and the establishment of remediation levels for chemicals in soil can be improved significantly by determining the effects of chemical "aging" on bioavailability. Studies were conducted on ^{14}C-labeled naphthalene (NAP) administered to dermatomed male pig skin in flow-through diffusion cells. A comparison of dermal penetration was made between pure chemical, NAP added immediately to soil, and aged for 3 months in sandy and clay soils. After aging, the bioavailability of NAP was decreased by 96-99% compared to chemical without soil. However, the bioavailability of NAP in newly contaminated soil was similar to pure compound. Decreased NAP bioavailability with aging indicates that the potential risk to health and the environment will also be reduced. (Supported in part through funding from the Hazardous Substance Management Research Center and the New Jersey Commission on Science and Technology.)

878 COMPARATIVE ABSORPTION OF ALUMINUM NITRATE IN SOIL AND AQUEOUS SOLUTIONS.

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There is little data available concerning the effect of soil on the bioavailability of aluminum. Therefore, complete absorption is usually a default assumption used for assessing environmental exposure. The purpose of this study was to evaluate the possible attenuating effects of soil on the bioavailability of aluminum. The effects of soil and aqueous matrices on the oral absorption of aluminum were compared. An animal model using Sprague-Dawley rats was developed. Test groups were gavaged daily with 300 and 600 mg/kg of aluminum in soil or water for fifteen days. Blood, liver, brain, and bone samples were collected. All samples were digested and analyzed for aluminum using Inductively Coupled Plasma-Atomic Emission Spectroscopy. No clinical signs of adverse effects were observed during this study. Mean body weights of all treated groups were within five percent of the controls for each matrix. Bone aluminum levels were significantly lower for the group exposed to aluminum mixed with soil. However, there were no significant differences in aluminum levels in blood, liver, kidney and brain. Thus, soil may affect the levels of aluminum in some tissues but not others. Therefore, in assessing environmental exposures associated with aluminum in soil, the target organ level should be considered and may be affected by soil adsorption. (Supported in part by NIOSH grant 412874-03.)

879 FACTORS AFFECTING THE ACTIVITY AND EXPRESSION OF HUMAN CYTOSOLIC GLUTATHIONE S-TRANSFERASE (GST) ISOFORMS.

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Understanding the factors that influence the levels of expression of GST enzymes in humans is important to identifying sensitive subpopulations who may be at risk for adverse health effects when exposed to certain environmental contaminants. The GSTs are phase II enzymes involved in the detoxification of many chemicals in mammals. Alterations in the levels of GST alpha, mu, pi and theta isoform expression have been associated with differential susceptibility to some toxic contaminants, such as polycyclic aromatic hydrocarbons, aflatoxin B1, and heterocyclic amines. GST isoforms expression is determined by an interplay between many genetic and environmental factors, which is important in understanding their detoxification activities. Generally, higher levels of the GST isoforms are associated with an increased tolerance to some chemical insults. Conversely, the loss or reduced expression of most GSTs is often associated with an increased susceptibility. For example, research has shown that individuals with the GSTM1 null genotype and greater than 50-pack years of cigarette smoking had a two-fold greater risk of bladder cancer when compared to equivalent smokers who were not homozygous for the GSTM1 null allele. In addition, those individuals with no identified exposure to toxicants, who are deficient in GST theta are more likely to have chromosomal abnormalities than those who are heterozygous. (The scientific views expressed are solely those of the authors and do not necessarily reflect those of the U.S. EPA.)

880 DNA REPAIR GENES-POTENTIAL CANCER SUSCEPTIBILITY GENES.

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In 1999, approximately 175,000 US women will be diagnosed with breast cancer. About 60% of breast malignancies do not have identifiable risk factors such as a first-degree relative with breast cancer. Studies suggest an association between deficient DNA repair and breast cancer with odds ratios (ORs) ranging from 1.6 to 10.0. Therefore, suboptimal DNA repair is hypothesized to be an important breast cancer susceptibility factor. DNA repair systems maintain a cell's genetic integrity and prevent DNA lesions from being replicated during DNA synthesis. DNA repair genetic polymorphisms have been identified in a normal human population. Some of the variant alleles may affect DNA repair capacity as the sites of polymorphism lead to amino acid substitutions. Thus, DNA repair genotypes have the potential to be classified as cancer susceptibility genes and may be associated with individual's risk for cancer. We have completed a breast cancer case-control study to evaluate the role of genetic polymorphisms of DNA repair genes in breast cancer risk. The study population consisted of 123 cases and 185 controls recruited at Georgetown University Medical Center from 8/95 to 11/96. The genotypes examined include 5 polymorphisms in 4 DNA repair genes: XRCC1, XRCC3, APE, and XPD. Genomic DNA isolated from whole blood was used for PCR-RFLP genotype analysis. The allele frequency of individual genotypes was not significantly different between cases and controls. However, a potential association was observed between breast cancer risk and XRCC1 (exon 6) and XRCC3 (exon 7) variants with age-adjusted ORs of 1.6 (95% confidence interval (CI)=0.8,3.3) and 1.3 (95% CI=0.8,2.2), respectively. For both cases and controls, the data suggested a possible linkage between the variant allele of XRCC1 (exon 10) and the wild-type allele of XRCC1 (exon 6). The most interesting finding of this study is that women with both XRCC1 (exon 6) and XRCC3 (exon 7) variant alleles have a 4.5-fold increased risk for breast cancer (95% CI=1.4,16.0). This study supports the hypothesis that DNA repair genetic polymorphisms may serve as potential biomarkers for human breast cancer risk. (Supported by grants from ACS and NCI.)

881 USE OF PHARMACOKINETIC MODELING TO RELATE HYDRAZINE LUNG CANCER POTENCY ACROSS GAVAGE AND DRINKING WATER BIOASSAYS.

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Gavage dosing has frequently been used in cancer bioassays, but questions exist regarding how best to apply gavage results to human risk assessment. Carcinogenic potency was compared across gavage and drinking water routes of administration using a variety of dose-metrics as derived from a pharmacokinetic model of hydrazine (HZ). HZ was selected because adequate bioassay data exist by relevant dose routes in mice. The cancer database encompassed eleven gavage and eight drinking water (DW) studies conducted in mice of various strains. Dose-response relationships for lung cancer frequency suggest that the rate of nitrogen exhalation is a predictive biomarker of exposure to carcinogenic metabolites of HZ, while other dosemetrics (e.g.; total amount of nitrogen exhaled, or AUC or peak HZ in blood) are not. Use of nitrogen exhalation rate to extrapolate cancer potency across dose routes is consistent with evidence that metabolic pathways leading to nitrogen exhalation involve oxidation to radical species. Results with HZ indicate that gavage and DW cancer potency is similar when dosemetrics are expressed in terms of peak rate nitrogen exhalation. To determine if the results of the HZ analysis also pertain to the structurally similar carcinogen, isoniazid (INH), the gavage and drinking water bioassay INH database was evaluated. This comparison indicates that on an administered dose basis, INH is equi-potent to HZ for gavage exposure. However, for DW exposure INH was considerably less potent than HZ. This larger gavage/DW differential for INH is due to the lower DW potency of INH as compared to HZ. This difference can be explained by INH having detoxification pathways not available to HZ that are saturated during gavage dosing.

882 DEVELOPMENT OF PROPOSED OCCUPATIONAL EXPOSURE LIMITS FOR SELECTED GLYCOL ETHERS USING PBPK MODELING AND MONTE CARLO SIMULATION.

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Certain glycol ethers are known to be teratogenic in rodents. A safe working environment may be assured by establishing and achieving appropriate occupational exposure limits for these compounds. Physiologically based pharmacokinetic models for methyoxyethanol, ethoxyethanol and ethoxyethyl acetate in pregnant rats and humans have been developed. The models were used to calculate human-equivalent no observed effect levels (NOELs), based on internal concentrations in rats exposed at previously determined NOELs for developmental toxicity. The human-equivalent NOELs were derived from modeling using average values for physiological, thermodynamic, and metabolic parameters. The uncertainty in the point estimates for the NOELs was estimated from the distribution of internal dose estimates obtained by varying key parameter values over expected ranges and probability distributions. Key parameters were determined through sensitivity analysis. Distributions of the values of key parameters were sampled using Monte Carlo techniques and internal doses calculated for 1200-2000 parameter sets. The 95th percentile was used to calculate interindivual uncertainty factors (UFs). NOELs were divided by these UFs to calculate proposed occupational exposure limits.

883 COLGATE-PALMOLIVE'S PROGRAM - VALIDATE THE EPIOCULAR™ HUMAN TISSUE CONSTRUCT MODEL (OCL-200).

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The objective of this corporate-based program was to formally validate the EpiOcular™ model's ability to predict the eye irritation potential of surfactants and surfactant-based formulations. As defined by the Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No. 97-3981), a validated method is one for which the reliability and relevance for a specific purpose have been established. EpiOcular™ is a commercially available three-dimensional *in vitro* model of the human corneal epithelium composed of normal human-derived epidermal keratinocytes. Four laboratories (Institute for In Vitro Sciences, MatTek, 3M and Kimberly Clark) conducted a range finding and three definitive assays on each of 20 blinded materials (6 formulations, 14 surfactants) in compliance with FDA GLPs using a formal and detailed test protocol. EpiOcular results for the test materials were compared to previously published eye irritation studies in animals. In terms of reliability, the results were reproducible within and between the laboratories. In terms of relevance, the EpiOcular™ model correctly predicted the Draize score for a majority of the samples tested. Results from five of six formulations were in agreement with the *in vivo* data. In addition, the eye irritation potential for anionic and cationic surfactants was correctly predicted. For those materials not predicted, the majority seemed to be alcohol ethoxylate surfactants that were under-estimated or amphoteric surfactants that were over-estimated for eye irritation. A complete statistical analysis of the data will be presented. These data indicate that, for certain classes of surfactant and surfactant-based formulations, the EpiOcular™ model has met the critical validation criteria for assessing ocular irritation.

884 THE EPIOCULAR TISSUE MODEL: *IN VIVO* VERSUS *IN VITRO* DRAIZE SCORES FOR CONSUMER PRODUCTS.

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EpiOcular™ (OCL-200) is an organotypic tissue model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. H&E stained histological cross-sections show that the structure of EpiOcular closely parallels that of the HCE. Previously, a prediction equation for eye irritation, Draize score (MMAS) = $-4.74 + 101.7/\sqrt{ET\text{-}50}$, was developed by correlating the *in vitro* ET-50 with Draize rabbit eye scores for 19 water-soluble chemicals from the ECETOC database and 41 cosmetic or personal care products/ingredients (ET-50 refers to the time of exposure which reduces the tissue viability to 50%, as determined by the MTT assay, in minutes). The current study reports *in vitro* results for an additional 24 consumer products, including shampoos, hand soaps, laundry detergents, dishwashing liquids, and skin lotions. A plot of the *in vivo* and calculated *in vitro* Draize scores, when correlated to the line *In Vivo Draize (MMAS) = In Vitro Calculated Draize*, gave the correlation coefficient, $r = 0.85$; when a single outlier was excluded $r = 0.93$. The useful range of the EpiOcular test was evaluated by testing surfactants at concentrations at which the Draize test is insensitive (MMAS scores < 2.0). EpiOcular was able to distinguish between surfactants at concentrations 3-10 fold below this point. Thus, the EpiOcular tissue model appears to be a sensitive, accurate *in vitro* means of predicting *in vivo* ocular irritancy for a range of consumer products and raw materials.

885 AN HISTOPATHOLOGICAL ANALYSIS OF DAMAGE TO BOVINE CORNEAS IN VITRO BY SELECTED OCULAR TOXICANTS.

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The Bovine Corneal Opacity and Permeability (BCOP) assay has proved a useful tool for the *in vitro* assessment of potential ocular irritation of various chemicals and formulations. In this assay viable corneas excised from normally discarded bovine eyes are directly exposed to test materials for various lengths of time and then monitored for changes in corneal opacity and permeability. However, analysis of a major validation program jointly sponsored by the European Commission and the British Home Office (EC/HO Study) indicated that, although the BCOP assay was arguably the best performing assay in the study, the ocular irritation potential of several materials in that study was not correctly predicted. We investigated whether histopathological evaluation of the bovine corneas would reveal damage that was not suggested by the traditional *in vitro* endpoints of opacity and permeability. We found that the three most severely underpredicted materials, parafluoroaniline, quinacrine, and sodium oxalate, showed diverse cellular damage throughout the epithelium and extending into the stroma. This was also true of a fourth underpredicted material, aspartic acid, although after exposure in a non-buffered medium opacity and permeability increased to the point where they were also predictive of the *in vivo* injury. Three moderately underpredicted materials, potassium cyanate, methyl cyanoacetate and 2-ethyl-1-hexanol, showed multi-focal separation of the basal epithelial cells from the basal lamina, as well as other changes which were more reflective of the *in vivo* scores than were the *in vitro* opacity and permeability measurements. It is clear that the excised bovine cornea does respond appropriately *in vitro* to the above test materials and that it may be possible to develop a histopathological scoring scale which extends the utility and further optimizes the BCOP assay beyond its current capacity.

886 THE CORNEAL ENDOTHELIUM IN THE BOVINE CORNEA OPACITY AND PERMEABILITY (BCOP) ASSAY.

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The corneal endothelium is essential for maintenance of corneal transparency. Damage can result in corneal opacity and may be irreversible since corneal endothelial cells do not proliferate. Examination of the corneal endothelium has not been included in the BCOP assay. The purpose of this study was to evaluate effects of irritants on the corneal endothelium. Corneas were treated according to the BCOP protocol following exposure to acetone, isopropanol, 1% NaOH, 30% sodium lauryl sulfate (SLS), or 30% trichloroacetic acid (TCA). The endothelium was stained with alizarin red S and trypan blue, and analyzed by light microscopy and image analysis. Exposure to acetone, SLS, isopropanol or NaOH for 1 min caused damage to 10-15% of endothelial cells. Exposure to TCA for 1 min caused death of up to 50% of endothelial cells. Corneas treated with acetone for 10 min had extensive areas of damage and isopropanol treatment for 10 min caused death of 50-100% of endothelial cells. A 10 min exposure to NaOH or TCA, caused death of all endothelial cells. Interpretation of these data is complicated, however, by the observation that simply mounting the cornea in the BCOP assay holders without further treatment damaged to 15-20% of the endothelial cells. This damage corresponded to folds in the cornea caused by clamping the aspherical bovine cornea into the circular chamber of the corneal holder. Examination of the corneal endothelium should be added to the BCOP assay; however, optimization will require modification of the cornea holders.

887 EVALUATING THE EYE IRRITANCY POTENTIAL OF ETHANOLIC TEST MATERIALS WITH THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY.

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Organic solvents can play a vital role in consumer product formulation. They can solubilize and deliver ingredients as well as provide desirable product attributes such as skin feel or fragrance. Experience has shown that the actual irritation induced by such formulations *in vivo* can be quite different than what might be expected on the basis of individual ingredient concentrations. This study was undertaken to evaluate the role of ethanol, as a formulation vehicle, in the ocular irritancy of insect repellent formulations. The concentration of the active repellent ingredient was held constant while the type and concentrations of organic solvents including ethanol, were varied. Ocular irritation potential was measured was measured using the Bovine Corneal Opacity and Permeability Assay. Abattoir-derived corneas were exposed to test materials for ten minutes and opacity and permeability end points measured. Selected tissues were fixed for histological evaluation. Concurrent positive and negative controls were tested along with a severely irritating benchmark formulation. Limited *in vivo* data were also available. Experience showed that the permeability endpoint of the assay, which measures disruption of the corneal epithelium, correlated better with the *in vivo* results than did the combined opacity and permeability scores. It proved useful to normalize the permeability scores against the established benchmark, thus yielding a P-ratio and enabling the rank-ordering of the test formulations for ocular irritation potential. Ethanol concentration alone was not predictive of irritation potential. However, certain non-irritating formulations could be ren-

dered irritating by the addition of low levels of ethanol. Histopathology and judicious use of *in vivo* testing confirm the utility of this *in vitro* approach as a product development tool.

888 OPTICAL DETERMINATION OF CORNEAL OCULAR IRRITANCY AND POTENTIAL FOR RECOVERY.

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As the function of the cornea is to focus light entering the eye, corneal transparency is a clinically well-established indicator of its health. Disease or injury leads to opacification. In this study, light transmission through the cornea was used to predict ocular irritancy potential of chemicals. We examined differential changes in transparency of ex vivo rabbit corneas, human eye bank corneas and human corneal equivalents constructed from cell lines, in response to different classes of potentially irritating and/or toxic chemicals. We measured transmission of light through each cornea on a custom-designed instrument. Then each cornea was treated with 100 µL of test substance for 5 min, washed and transmission was re-measured. At 30 sec post treatment, all three types of corneas showed similar changes in transmission in response to the culture medium control and the different test substances. However, rabbit corneas (the current standard for animal toxicity testing) and corneal equivalents showed greater sensitivity to chemical exposure than human eye bank corneas. The latter was most likely due to the poorer conditions of human corneas available for research. At 3 and 7 days post-treatment, decreased levels of light transmission were maintained, indicating a lack of recovery of corneal transparency in this time period. These results indicate that the custom-designed instrument was able to reliably and quantitatively measure responses of *in vitro* rabbit, human and reconstructed corneas to surfactant-based substances. The ability to measure responses up to at least one week also indicates potential of the approach to assess recovery following chemical exposure.

889 USE OF CONFOCAL MICROSCOPY TO MEASURE CORNEAL RESPONSES TO SURFACTANTS IN AN *EX VIVO* RABBIT EYE MODEL.

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An *ex vivo* method was developed to assess the area and depth of injury to rabbit corneas following application of surfactant based substances to the corneal surface of abattoir-derived rabbit eyes. Previous *in vivo* confocal microscopic analysis indicated area and depth of injury are quantitative measures of corneal response to surfactant-based substances *in vivo*. In the current work, surfactants and surfactant-based product formulations previously characterized *in vivo* were applied to the corneal surface of *ex vivo* rabbit eyes for 30 seconds. Then, the corneas were washed and incubated with Syto10 and Dead Red, fluorescent nucleic acid stains which differentiate live and dead cells. These dyes provided clear visualization of individual cell status by laser scanning confocal microscopy in serial images. Depth of injury was determined to be the focal plane of the cornea where no further staining of dead cells was seen, and live cells were present. The endpoint of the test, the normalized depth of injury (NDI), was expressed as a percent of overall corneal thickness. These methods were developed based on responses to 4 prototype anionic and cationic surfactant-based formulations and ingredients, demonstrating it was possible to distinguish substances and test conditions producing slight (epithelium alone), mild to moderate (epithelium and superficial stroma), and severe (deep stroma to full-thickness) damage to the corneas based on comparison to *in vivo* confocal measurements. Following methods development, 7 additional coded nonionic, anionic and cationic surfactants previously characterized *in vivo* were used to further validate the *ex vivo* test. The *ex vivo* NDI results agreed quantitatively with previous *in vivo* measurements, and agreed also with *in vivo* classification of responses as slight, mild to moderate, and severe. Thus, measurement of area and depth of injury in *ex vivo* rabbit corneas provides mechanistically-based quantitative data to assess the eye irritation of surfactant-based substances.

890 MÜLLER CELL CULTURES IN EVALUATIONS OF RETINAL TOXICITY.

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Retina is one of the most metabolically active tissues in the body, and maintaining the neuronal microenvironment by Müller cells is of vital importance to vision. Müller cells are predominant glial cells in the neural retina. They regulate the ion balance and e.g. glutamate concentration in the retina. Too high glutamate concentrations have been shown to cause excitotoxic neuronal damage and death. Pure Müller cell cultures would serve a good model for studies of the regulatory functions of Müller cells, and for evaluations of glutamate toxicity in the neural retina. There are several methods for culturing Müller cells. Many of them use avascular retinas like that of a rabbit. The advantage is that relatively pure cultures free of astrocytes, endothelial cells and pericytes can be obtained, if the area near the medullary ray is not used. In vascular retina, blood vessels cover the whole retina area, and cultures free of contaminating cell types are not easy to set up. The retina can be dissociated by enzymatic digestion (e.g. with trypsin or papain), and then cultured for definite times. Neurons do not survive long in this type of coculture, but other cell types from blood vessels can survive. A more refined method uses additional gradient centrifuging steps in order to separate purer Müller cells. Another method, where Müller cell cultures are initiated from floating pieces of retina, from which Müller cells drop out, has also been presented. These methods rely on the fact that activated Müller cells are highly viable, and degenerating or necrotic retinas trigger the activation. We have used the above methods for initiating Müller cell cultures from porcine retinas. In characterization of Müller cells antibodies to glial fibrillary acidic protein (GFAP), cellular retinaldehyde binding protein (CRALBP), carbonic anhydrase II and glutamine synthetase have been used. The usual problem with all these methods seems to be that pure cultures cannot be obtained or cultures do not survive many subcultures. Some Müller cell lines have been presented, but they are not freely available. A continuous cell line would be necessary for toxicological large-scale evaluations.

891 SAFETY EVALUATION OF INTRAVITREAL ADMINISTRATION OF RHUFAB VEGF IN CYNOMOLGUS MONKEYS FOR 1 MONTH.

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A recombinant antigen binding fragment of a humanized monoclonal antibody to VEGF (rhuFab VEGF) is being developed as a therapeutic for age-related macular degeneration. The toxicity of intravitreal administration of rhuFab VEGF to cynomolgus monkeys, given once every two weeks for 1 month, was assessed. Monkeys (n=16) were assigned to 3 groups and received either: Vehicle, (n=4); 500 µg/eye, (n=4); or 2000 µg/eye, (n=8) rhuFab VEGF, in a 50 µL volume. The 4 additional animals in the high-dose group were designated recovery animals and were observed for 1 month after cessation of dosing. The first intravitreal administration of rhuFab VEGF produced a dose-dependent anterior chamber (AC) inflammation consisting of only flare and cell observed 24-hours after dosing. Inflammation was transient with only anterior vitreal cell persisting at 7 days postdose. After the third dose, AC inflammation in 500 and 2000 µg treated eyes was equivalent to that of vehicle treated eyes. Repeated administration in the same location resulted in scleral weakening. Two high-dose treated animals developed transient retinal perivenular infiltrates that were reversible during the recovery period. rhuFab VEGF administration did not alter posterior segment as evidenced by changes in electrodiagnostics, fundus imaging or ocular histopathology. Additionally, rhuFab VEGF induced no changes on any systemic parameters evaluated. Intravitreal administration of rhuFab VEGF once every two weeks was well tolerated at doses up to 500 µg/eye and produced no lasting effect on ophthalmic parameters.

892 A NOVEL APPROACH FOR IRIS COLORIMETRY DETERMINATIONS.

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Prostaglandin derivatives are used as promising agents for glaucoma in eye drops because of their ocular hypotensive activity. With Latanoprost, the phenyl-substituted prostaglandin F2a, iris color changes were observed in cynomolgus monkeys (Macaca fascicularis) and in multi-center clinical trials. To document these changes, the standard approach up to now was that photographs were taken and compared with color plates. The disadvantage of this method is that the color intensity varies between the measurements of different months due to changes in the developer and experimental conditions. Furthermore, one person has to judge if there are any changes or not which includes the subjective opinion of this person.Therefore, a computerized method (patent pending) using a video camera (3-CCD) attached to the slit lamp was developed. The signals are transferred to a computer and single frames which are "frozen" by means of a "grabber card" can be stored. In advance the equipment is calibrated and color plates are measured to check the standard conditions. The pictures are evaluated by a software program which displays the average color (as red, green and blue values) of the selected area as a basis for statistical evaluation. This inexpensive method provides a fast and accurate means to quantify color changes in the iris of experimental animals and humans. In addition the stored photographs could be evaluated visually.This is the first time that iris color changes are demonstrated in a publication based on this new method which produces objective values.

893 A METHOD FOR ASSESSING VISUAL ACUITY IN UNANESTHETIZED RATS USING THE OPTICOKINETIC REFLEX.

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An efficient, noninvasive procedure to assess visual function in adult and developmental rodent toxicity studies would enhance the safety evaluation of drugs and the hazard identification of chemicals. We are developing a technique that utilizes the opticokinetic reflex (OKR) that has been used successfully in the past for measurement of visual function in several species including rodents and children, but has only rarely been applied to toxicological assessment. For assessment of visual acuity, the method involves observation of a reflexive lateral nystagmus (OKR) in response to vertical black and white stripes of equal width that move across the visual field of an unanesthetized subject. An acuity threshold is determined by varying the widths of the stripes until the OKR is no longer elicited and then calculating the threshold in minutes of visual angle. Male Long Evans rats were restrained individually on a stationary platform inside of a rotating cylinder (45.7 cm diameter, 29.2 cm high) with the eyes unobstructed 20cm from the inside surface of the cylinder. Stimulus sheets of black and white stripes were placed sequentially inside of the cylinder which was then rotated between 1-1.5 rpm. The eyes were observed with an illuminated magnifier for the presence or absence of the OKR. In initial experiments, stripes 10mm wide were presented to confirm the presence of OKR and then stimulus stripes from 5-1mm were presented in 0.5mm increments by a psychophysical tracking procedure. A threshold was considered to be midway between a stimulus that elicited the OKR and a narrower stimulus that did not. The presumed threshold was crossed at least twice. Later experiments used stimulus width increments of 0.25 mm to provide finer resolution. Test-retest and interobservor reliabilities were found to be high with this method and the time required to test a rat was usually less than 20 minutes. Automation of stimulus presentations and objective recording of the OKR would improve efficiency and are being pursued. Visual acuity of male Long Evans rats weighing 300-400 grams under bright illumination was determined to be in the range of 37 to 54 minutes of visual angle, which agrees very well with reported findings from other laboratories using behavioral and electrophysiological methods.

894 HUMAN SENSORY IRRITATION AND ODOR TESTING ON A COMPLEX AROMATIC HYDROCARBON.

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A complex aromatic hydrocarbon (CAH) is a C10-C12, high boiling hydrocarbon composed predominantly of alkylated naphthalenes, with < 0.4 ppm benzene and is used primarily as a carrier solvent for agricultural pesticide spray applications. Previous studies in the mouse suggested that CAH fluid might be a potent sensory irritant. Therefore, we initiated testing to determine human odor and sensory (nasal and ocular) irritation response thresholds on CAH and its major vapor phase constituents.Serial dilutions of CAH, naphthalene, 1-methylnaphthalene (1-MN), 2-methylnaphthalene (2-MN) and a low naphthalene CAH product (CAH-LN) were placed in 2L glass vessels equipped with portals for the eye or nose. Using a 2- or 3-alternative forced-choice test to detect nasal and ocular response, respectively, headspace vapors from dilution series of each material were presented to subjects (n=22) in 14 trials with a 2-min. rest period between trials. The respective mean detection thresholds for ocular and nasal responses in ppm were 39 and 54 for CAH, 37 and 50 for CAH-LN, 88 and 90 for naphthalene, 66 and 85 for 1-MN, and 109 and 149 for 2-MN. Odor detection thresholds for these materials were < 3.0 ppb. Results from this study showed, in humans, CAH and CAH-LN do not appear to be potent sensory irritants as previously suggested by the mouse studies. These materials have odor thresholds well below sensory irritation thresholds. The eyes appear to be more sensitive than the nose. These results contribute to the database on CAH.

895 FROM EPIDEMIOLOGY TO THE GENE: MECHANISMS BY WHICH PARTICULATE MATTER (PM) INDUCES ADVERSE EFFECTS.

D. L. Costa and R. Devlin. *USEPA, Research Triangle Park, NC.*

Epidemiology studies have shown a relatively consistent association between outdoor PM concentrations and various adverse health effects, including premature mortality, exacerbation of asthma and other respiratory diseases, and decreased lung function. The biological basis of these associations is unknown, although epidemiology, toxicology, and clinical studies suggest asthmatics, young children, elderly people, and those with pre-existing cardiopulmonary conditions might be particularly susceptible. Little is known of the contributory factors specific to PM or the host. Consequently, several laboratories are earnestly examining the pathophysiology and toxicological mechanisms that underlie PM effects. The attention being focused on understanding potential physiological linkages between PM and acute mortality has led to the perception that PM may directly or indirectly (via inflammation of the lung) affect the heart. The sequence of biological steps from target dose of PM within the lung to cardiac alterations are likely to be complex. This symposium will provide an update attempting to link the latest cross-discipline research on PM addressing the importance of altered intrapulmonary target dose in those with lung disease, the correlative evidence from field study cardiac monitoring to new findings in experimental animals, and the fundamental molecular signaling events that may well trigger the biological cascade from the cell to the whole organism. The attendee will gain an appreciation for the complexity of the PM response, the role of target tissue dose, responses from the cellular to organismic level, and the potential for cardio-pulmonary interactions in the clinical impact of PM on health.

896 POPULATION STUDIES EXAMINING PM-INDUCED CHANGES IN CARDIAC FUNCTION.

D. W. Dockery. *Harvard Public Health, Boston, MA.* Sponsor: D. Costa.

A large and growing body of epidemiologic studies have reported that daily mortality increases following acute increases in particulate (PM) air pollution. Although the relative increase is greatest for respiratory deaths, the increase in numbers is greatest for cardiovascular associated deaths. Analysis by location shows that most of these acute deaths occurred out of hospital, suggesting sudden cardiac deaths. Time series studies of hospital admissions have also consistently shown increased daily admissions for cardiovascular disease (ischemia, coronary heart failure, and dysrhythmias) associated with increased PM concentrations. The mechanisms underlying these associations are not understood. A recent study assessed the potential linkage through acute hypoxemia in a panel of elderly subjects. No association was found between PM exposures and twice daily pulse-oximetry measurements. However, heart rate was observed to increase with increasing PM concentrations. In addition in a subset of these subjects with ambulatory electrocardiographic monitoring, heart rate variability was found to decrease with increasing PM concentrations. Decreased heart rate variability, a measure of impaired autonomic control, is associated with a poor cardiovascular prognosis. In another recent study, the incidence of cardiac arrhythmias in patients with implanted cardioverter defibrillators have been reported to increase with air pollution exposures. This rapidly developing body of epidemiologic studies of cardiovascular effects of PM exposures will be reviewed with particular attention to possible mechanisms.

897 DOSIMETRY OF INHALED PARTICLES IN SUSCEPTIBLE POPULATIONS.

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Particulate air pollution has been linked to acute increases in mortality/morbidity primarily in the elderly, children and those with preexisting cardiorespiratory disease. While these individuals may be predisposed to acute toxic effects, they may also receive an increased dose of particles to their lungs compared to healthy, young adults. In a series of studies we have been measuring deposition (both total and regional) and clearance of inhaled particles in these susceptible subpopulations for comparison to healthy young adults. While healthy elderly subjects appear not to receive enhanced deposited doses of fine particles compared to younger individuals, those with chronic obstructive pulmonary disease (COPD) receive 2.5 times on average the deposited dose at rest of their age-matched healthy cohort. The deposited dose in COPD increases (as much as 5 times normal) with increasing airway obstruction. Furthermore, the deposited dose in COPD is very nonuniform in the lung, i.e. "hotspots" associated with airway deposition, and the insoluble particle clearance from the airways in COPD is about half the rate of normal. These effects greatly enhance the airway tissue dose in COPD to >> 10 times normal. Similar studies are being conducted in children. It's likely that children also receive an increased dose to their bronchial airways relative to healthy adults. Much of this effect may be associated with children's higher activity levels and ventilation rates that shift deposition of particles to proximal, airway surfaces. Exercise is also associated with a shift from nasal to oral breathing, thereby diminishing the filter capacity of the nose and exposing the lung to higher particle concentrations. Studies are currently ongoing to compare oronasal switching patterns in children vs. adults. Results from these dosimetry studies in susceptible populations may prove useful in determining relative risks associated with the inhalation of airborne particulates. (Supported by USEPA Cooperative Agreement CR817643.)

898 CARDIAC AND THERMOREGULATORY EFFECTS FOLLOWING EXPOSURE TO PARTICULATE MATTER IN HEALTHY AND COMPROMISED RATS.

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Extensive epidemiological evidence suggests that exposure to ambient particulate matter (PM) may be responsible for an increased incidence of cardiopulmonary-related morbidity and mortality. A series of animal studies conducted in our laboratory examined this reported association. All studies used male rats (60d) implanted with radiotelemetry transmitters to monitor electrocardiogram (ECG), heart rate (HR), and core body temperature (Tco) throughout the experimental time period (48h pre-exposure–96h postexposure). In the first study, healthy Sprague-Dawley rats (SD) were intratracheally instilled (IT) with one of four doses of residual oil fly ash (ROFA; 0.0, 0.25, 1.0, or 2.5 mg). These rats demonstrated a biphasic, dose-related response consisting of an acute and delayed bradycardia, accompanied by an increase in cardiac arrhythmias, profound hypothermia, and pulmonary inflammation. This protocol was repeated in three models of cardiopulmonary stress/disease, induced by subjecting rats to 1) cold-stress (10°C), 2) ozone-induced (1 ppm×6h) pulmonary inflammation, and 3) monocrotaline-induced (MCT; 60 mg/kg) pulmonary vasculitis/hypertension. Compromised rats exhibited an exaggerated response with lethality observed only in the MCT-treated group. Subsequent studies examined the effects of inhaled (IH) ROFA (15 mg/m3×6h/d×3d) in both MCT-treated SD and Spontaneously Hypertensive (SH) rats. These animals demonstrated similar effects as above (SH>SD) but of lower magnitude with no lethalities. Studies using aged SH rats (15m) investigated the differential responses to prototypical ambient (OTT; Ottawa dust; 2.5 mg), combustion (ROFA; 0.5 mg), and natural source (MSH; Mt. St. Helens volcanic ash; 2.5 mg) particles. Adverse effects, as measured by changes in ECG, HR, and arrhythmia incidence, in order of severity were OTT>ROFA>MSH. Other studies examined the effects of the specific metal constituents of PM (e.g., Fe, V, and Ni), either alone or in combination, in awake MCT-treated SD rats. These studies showed significant acute and delayed decreases in HR and Tco, and increased arrhythmogenesis and lethality. In sum, these results demonstrate substantial cardiotoxicity in rats after both IT and IH exposure to PM or its acidic metal constituents and suggest that the adverse health effects observed following PM exposure may represent a summation of effects of individual metals, as modulated by host susceptibility. (This abstract has been internally cleared and does not reflect EPA Policy.)

899 THE ROLE OF CALCIUM AND ANTIOXIDANTS IN ULTRAFINE PARTICLE-MEDIATED PULMONARY INFLAMMATION.

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Evidence to support the hypothesis that ultrafine particles mediate adverse health effects of PM is still limited. However, toxicological studies support the contention that ultrafine particles have the potential to cause lung inflammation. We have utilised a number of surrogate particles to investigate the generality and mechanism of the pro-inflammatory effects of ultrafine particles. In inhalation studies, exposure to ultrafine carbon black (ufCB, 14nm primary particle diameter; 1000μg/m3 for 7 hours) we detected low level inflammatory change plus evidence of oxidative stress; no such effects were seen with fine carbon black (CB, primary diameter 260nm) at identical exposure. Instillation studies at low dose (<125μg) confirmed that ufCB is substantially more inflammogenic that CB and that antioxidants blunted ufCB inflammation. Monodispersed latex particles with primary particle diameters 525, 202 and 64nm (ufLatex) show a distinct size-related ability to cause inflammation following instillation with ufLatex particles being substantially more inflammogenic than the other size particles. In vitro studies have highlighted the oxidative stress-inducing properties of ufCB and the ability of both ufCB and ufLatex to cause rapid alterations in intracellular Ca^{2+} in a macrophage cell line. These effects appear to involve the plasma membrane Ca^{2+} channels. These allow rapid ingress of extracellular Ca^{2+} in response to a signalled release of endoplasmic reticulum stores of Ca^{2+} within the cell, leading to cell activation for gene expression. could lead to cell activation and gene expression. Enhanced cytokine secretion by macrophages and other target cells in the lungs of compromised individuals where the Ca^{2+} signalling pathways are already 'primed' may therefore be anticipated and this may help in understanding susceptibility.

900 DIFFERENTIAL ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASE (MAPK) PATHWAYS AND TRANSCRIPTION FACTORS BY SPECIFIC PARTICULATE (PM) COMPONENTS.

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Numerous epidemiological studies have shown associations between ambient levels of PM and respiratory-related morbidity and mortality. The mechanisms responsible for these effects are not understood, but may involve an inflammatory response to metallic compounds present in PM. Our studies have sought to identify signaling mechanisms through which combustion-derived metals associated with PM, such as As, Cu, V and Zn, induce the expression of inflammatory proteins in human airway epithelial cells (HAEC). Specifically, we have examined the effect of metal exposure on protein phosphotyrosine metabolism and the activation of the phosphorylation-dependent MAPK cascades. Western blotting and activity assays showed that acute, non-cytotoxic exposures to As, Cu, V or Zn result in rapid activations of the MAPKs ERK, JNK and P38, as well as the MAPK kinase MEK-1, the EGF receptor tyrosine kinase, and the MAPK-dependent transcription factors Elk-1, JNK and ATF-2 in the human bronchial epithelial cell line BEAS. In addition, we have obtained evidence suggesting activation of Ras in BEAS cells treated with As, V or Zn ions. Phosphotyrosine accumulation and MAPK activation is also evident immunohistochemically in rats intratracheally instilled with the metallic mixture residual oil fly ash. In order to identify possible mechanisms that account for the activation of intracellular signaling elements in HAEC, we studied the effect of metal exposure on tyrosine phosphatase activity in whole and fractionated HAEC homogenates. These results showed that V and Zn ions, but not As ions, are potent inhibitors of tyrosine phosphatases, such as PTP1B, present in HAEC. Taken together, these studies demonstrate that ambient PM metals differentially activate intracellular signaling in HAEC.

901 INTERACTION WITH IONOTROPIC NEUROTRANSMITTER RECEPTORS BY ENVIRONMENTAL TOXICANTS: CONSEQUENCES FOR NEURONAL FUNCTION.

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Ionotropic receptors comprise one of two major classes of ligand-gated ion channels. The ligand binding site(s) are directly coupled to the ion conductance pathway in these macromolecules, as opposed to being coupled to a GTP-binding protein or intracellular second messenger. As such, ionotropic receptors provide the simplest and fastest way of transducing an extracellular stimulus into a change in neuronal excitability. Ionotropic receptors comprise the major class of transmitter receptors involved in "fast neurotransmission," and mediate both excitatory and inhibitory synaptic function. Neurotransmitter receptors of the ionotropic class comprise a major gene family which is unrelated to the specific class of neurotransmitter, but rather to the mechanism of signal transduction. Included in this class are the nicotinic receptors for acetylcholine, gamma amino butyric acid (GABA) A-subclass, several types of glutamate receptors (kainate/AMPA, NMDA) glycine and serotonin ($5HT_3$) receptors. This class of receptors shares common structural characteristics, typically consisting of multimeric macromolecules, with similar overall hydrophobicity of the subunits, and significant homology in their primary structure. The receptors play crucial roles in functions such as learning and memory, nervous system development and synaptogenesis, and regulation of overall neuronal excitability. Additionally, these receptors collectively serve as targets of a number of important therapeutic agents, natural toxins and xenobiotics. As such, these receptors serve as important targets of some major classes of toxicants. Mechanisms by which toxicants may act include direct block of the binding sites for the stimulating ligand, direct effects on the ion channel itself, or effects on the modulatory processes associated with these receptors. (Supported by NIEHS grants ES03299 [wda] and ES06365 [jbs].)

902 PERTURBATION OF EXCITATORY AND INHIBITORY GABAA RECEPTOR RESPONSES IN CORTICAL NEURONS *IN VITRO* BY POLYCHLORINATED BIPHENYLS (PCBS).

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$GABA_A$ receptors are the major inhibitory receptor in the central nervous system of mature animals and mediate excitatory responses which underlie trophic actions of the neurotransmitter GABA in the developing nervous system. Because $GABA_A$ receptors are important to nervous system development and are substrates for steroids, hormones and chlorinated chemical pollutants, we examined the hypothesis that disruption of $GABA_A$ receptor function in neurons contributes to the developmental neurotoxicity of PCBs. In cortical neurons maintained in culture, Aroclor 1254 (A1254, 2-20 μM, 1hr) initiated (within 5min) sustained Ca^{2+} oscillations, suggestive of action potential generation. These oscillations in $[Ca^{2+}]_i$ were dependent on Ca^{2+} entry via voltage-sensitive Ca^{2+} channels as they were blocked by 1 μM nifedipine. A1254 (10 and 20 μM) increased basal $[Ca^{2+}]_i$ significantly after 1 hr and reduced significantly (2-20 μM, 1hr) both excitatory and inhibitory responses to GABAA receptor stimulation. In addition, both *ortho-* (2,2'-dichlorobiphenyl, 2,2',3,4,4',5-hexachlorobiphenyl) and non-*ortho-* (4,4'-dichlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl) substituted PCB congeners (5-10μM, 1hr) inhibited $GABA_A$ responses. Thus, effects of PCB congeners on $GABA_A$ receptors appear to have a different structure-activity relationship than reported previously for other endpoints of neuronal function. In addition, only 4,4'-dichlorobiphenyl and 2,2',3,4,4',5-hexachlorobiphenyl significantly increased basal $[Ca^{2+}]_i$. Thus, perturbation by PCBs of $GABA_A$ receptor-mediated responses occurred in the absence of or after only slight PCB-induced increases (2 μM A1254) in basal $[Ca^{2+}]_i$, suggesting that PCB effects on $GABA_A$ receptors may not depend on changes in $[Ca^{2+}]_i$+]. These results demonstrate that GABAA receptor responses are sensitive to PCB effects. Such effects may contribute to the developmental neurotoxicity of PCBs.

903 DISRUPTION OF CEREBELLAR GRANULE CELL GABAA FUNCTION BY METHYLMERCURY INDUCES EARLY ONSET SYNAPTIC EXCITATION.

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Methylmercury (MeHg) has wide-ranging neurological effects including disturbances of motor coordination, an effect mediated primarily in the cerebellum. In both developing and mature organisms, cerebellar granule neurons are primary targets of MeHg-induced neurotoxicity. Studies of effects of acute administration of MeHg to isolated slices of rat brain reveal a striking disruption of synaptic transmission in both hippocampal CA1 region and cerebellar parallel and climbing fiber pathways. A common effect which precedes this block of excitatory transmission is a transient enhancement of synaptic transmission- reflected as increases in amplitude of excitatory postsynaptic potentials (EPSPs). This action, which is most pronounced with low concentrations of MeHg could be due either to direct facilitation of glutamatergic function, or indirectly to disruption of tonic inhibitory synaptic transmission at these synapses. Analysis of $GABA_A$ receptor-mediated inhibitory postsynaptic potential amplitudes revealed that they were blocked with a timecourse which preceded block of EPSPs, but which was consistent with the observed increase in EPSP amplitude. In isolated cerebellar granule cells in culture, MeHg at 1 μM completely blocked $GABA_A$ activated current within 6-10 min and generated an inactivating inward current which persisted in the presence of TTX, TEA and Cd^{2+}. When the MeHg-induced effect on GABA-mediated nonspecific current was maximal, GABA no longer induced an inward current, but rather an outward current, which was blocked by bicuculline and activated by muscimol. Thus $GABA_A$ receptor responses are sensitive targets for MeHg. Due to the importance of synaptic inhibition to neuronal function, these effects of MeHg could have important implications for cerebellar function in both developing and mature organisms by inappropriately activating certain neuronal circuits. (Supported by NIEHS grant ES03299.)

904 NEURONAL NICOTINE ACETYLCHOLINE RECEPTORS: A NOVEL TARGET OF INSECTICIDES.

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The development of insect resistance to insecticides is one of the most serious problems in pest control since insects could not only gain resistance to any insecticides but also gain cross resistance to a new insecticide that shares the same target site with the existing insecticides. Therefore, the development of insecticides that act on a new and novel target site is urgently required, and neuronal nicotinic acetylcholine receptors (nnAChRs) hold promise as a new target. Nitromethylene heterocycle insecticides such as imidacloprid and the oxadiazine insecticide indoxacarb (DPX-MP062) have recently been demonstrated to act on nnAChRs. Imidacloprid and cartap suppressed the activity of nnAChRs of PC12 cells, yet significant differences in their mechanisms of action could be unveiled only by single-channel analyses. Indoxacarb suppressed the α7 type ACh-induced current in rat cortical neurons while augmenting the α4β2 type current. On the contrary, the metabolite DCJW had little or no effect on both types of current. Thus indoxacarb's action is subunit specific. (Supported by NIH grant R01NS14143.)

905 INTERACTIONS OF PB^{2+} WITH NICOTINIC RECEPTORS AND WITH GABAERGIC AND GLUTAMATERGIC SYNAPSES: IMPLICATIONS FOR SYNAPTIC PLASTICITY IN THE DEVELOPING HIPPOCAMPUS.

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Recently we demonstrated that Pb^{2+} (≥100 nM), by interacting with targets within presynaptic terminals, facilitates the action potential-independent release of GABA and glutamate from hippocampal neurons in culture. With an IC50 of approximately 65 nM, Pb^{2+} was also shown to block the action potential-dependent release of both GABA and glutamate. This Pb^{2+}-induced inhibition of evoked transmitter release was likely due to block of voltage-gated Ca^{2+} channels. Further, Pb^{2+} causes a Ca^{2+}-dependent, biphasic block of whole-cell currents mediated by activation of α7-like neuronal nicotinic receptors (nAChRs) in hippocampal neurons in culture. In the subsecond time scale Pb^{2+}(≥1 μM) associates with a site on the extracellular domain of α7-like nAChRs to cause a reversible block of the receptors. This is evidenced by reduction of the amplitude and acceleration of the decay phase of the α7-nAChR mediated type IA currents, and by shortening of the open time and reduction of frequency of openings of α7 nAChR channels. Within 1-2 min, Pb^{2+}, via an intracellular mechanism, causes an additional irreversible reduction of the amplitude of type IA currents. It is noteworthy, that at lower concentrations (10-100 nM), Pb^{2+}, by activating protein kinase C (PKC) in hippocampal neurons, effectively blocks the ability of acetylcholine to control, via nAChRs, release of GABA and glutamate. Thus, given that synaptic activity is critical for the establishment of stable synaptic connections early in development, Pb^{2+}-induced long-lasting effects on neuronal maturation and plasticity could be accounted for by changes in mechanisms that control transmitter release. Support: USPHS grant ES05730.

906 LATEX ALLERGY IN THE WORKPLACE.

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The United States Occupational Safety and Health Administration (OSHA) estimates that 8-12% of healthcare workers are sensitized to natural-rubber latex. In addition, approximately 0.5-1% of the general population is reported to be sensitized. Clinical signs and symptoms of latex induced disease range from simple irritation to immunologic manifestations such as urticaria, asthma, and anaphylaxis. The mechanisms of latex allergy are complex, and are induced by exposure to numerous allergenic proteins found in natural-rubber latex as well as other chemicals used in latex products manufacturing. For example, there are 250+ proteins containing multiple epitopes in latex of which at least 30 have allergenic potential. Several latex proteins have been epitope mapped. Sequencing demonstrates both unique epitopes and sequences commonly found in other plant proteins. These common epitopes result in cross-reactivity to other plant allergens found in pollens and foods. A further complication arises from the ability of latex proteins to associate with glove powder. This enhances the potential for respiratory sensitization from aerosolized powder associated proteins, and both humans and experimental animals have demonstrated hypersensitivity following exposure to latex via the respiratory route. The diagnosis of latex allergy is complicated by these variables, which in turn hinders the development of intervention strategies. Further epidemiological assessment can more explicitly define the scope, trends, and demographics of latex allergy. Diagnostic accuracy can be improved through greater knowledge of proteins involved in the development of latex allergy, and factors analysis of presently available diagnostic tests. *In vivo* and *in vitro* models can elucidate mechanisms of sensitization and provide an understanding of the role of exposure route in latex associated diseases. Combined, these efforts can lead to intervention strategies for reducing latex allergy in the workplace.

907 LATEX ALLERGY: CLINICAL AND EPIDEMIOLOGICAL DATA.

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Natural rubber latex (NRL) allergy has become an important occupational health concern particularly among healthcare workers (HCWs). Clinical manifestations include irritant contact dermatitis, allergic contact dermatitis (type 4), and type 1 immediate hypersensitivity responses. Type 1 (IgE mediated) NRL allergy includes contact urticaria, systemic urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm and anaphylaxis. Diagnosis of type 1 latex allergy is made by accurate history including questions on atopic status, food allergy, reactions to latex devices (gloves, balloons, condoms), and reactions during medical and dental procedures. To confirm a diagnosis either *in vivo* skin prick testing (SPT) or *in vitro* assays for latex specific IgE are performed. The major source of workplace exposure has been the powdered NRL gloves used by HCWs. Surveys of HCWs have demonstrated that the prevalence of sensitization to latex ranges from 2.9% in Finland to 4.7% in Belgium among hospital workers as a group, and from 6.2% in operating room staff in Finland to 9-10% in Canada and France. We recently observed that the prevalence of latex sensitization based on SPT was 12.1% with a minimum prevalence of 9.5% among 1351 HCWs in two hospitals. There were significant associations with atopic status, positive SPT to certain foods, work related symptoms, and departmental use of gloves per HCW. In this study about 60% of the latex SPT positive participants had work related symptoms. It has been reported that 2.5% of HCWs have latex induced occupational asthma confirmed by specific inhalational challenge. Sensitization to latex has extensive cross-reactivity with certain foods and leads to clinical allergic reactions. Positive food SPTs occurred in our patients with the following frequency: avocado (53%), potato (40%), banana (38%), tomato (28%), chestnut (28%), and kiwi (17%). As well we reported that dental school students were at high risk for latex sensitization with prevalence rates of 0% for year one and two students, 6% of year 2 students, and 10% of year four students having a positive latex SPT. The prevalence of latex sensitization in occupationally unexposed groups is quite low, generally less than 1%.

908 MOLECULAR CHARACTERIZATION OF LATEX ALLERGENS.

D. Beezhold. *Guthrie Research Institute, Sayre, PA.* Sponsor: M. Toraason.

Type 1 latex allergy is an IgE mediated response to antigenic proteins found on products made from natural rubber latex. Natural rubber latex is harvested from trees and ammoniated to prevent coagulation resulting in the hydrolysis of the latex proteins. Prior to use in manufacturing, the latex is formulated by the addition of multiple chemicals. Thus, human exposure is to a mixture of residual chemicals and hydrolyzed latex peptides. The hydrolyzed nature of the allergens has complicated identification of major allergens and the development of reagents for *in vivo* and *in vitro* diagnostic tests. Multiple investigators, using a variety of methodologies, have succeeded in identifying the major IgE binding allergens (Hev b 1-8). More recently, these proteins have been cloned, sequenced and expressed as recombinant proteins. Skin testing with recombinant Hev b 2, 3, 5, 6, 7, and 8 revealed Hev b 5, 6 and 7 to be the most common allergens for healthcare workers. Using overlapping synthetic peptides, IgE binding epitopes have been determined for these allergens. Hev b 5 contained at least 6 epitopes, while Hev b 7 contained 12 epitopes all with limited sequence homology to other known plant proteins. Hev b 6 however, contained six epitopes with sequence homology to defense proteins common to many different plants. Sequence homology helps to explain the cross reactivity to a variety of foods experienced by latex allergic individuals. The development of recombinant allergens provides reagents which should greatly improve the diagnostic accuracy of tests for latex allergy.

909 COMPLICATIONS IN INTERPRETATION OF DIAGNOSTIC TESTS FOR LATEX ALLERGY.

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The diagnosis of natural rubber latex allergy begins with a clinical history and often involves a confirmatory test. While the puncture skin test (PST) has been regarded as a primary confirmatory test for the assessment of patients for IgE-mediated disease, the absence of an FDA-licensed Hevea brasiliensis latex extract in the USA has restricted its use in the diagnosis of latex allergy. Serological tests have therefore become critically important as alternative diagnostic tools. Three manufacturers have currently obtained 510K clearance from the FDA for their latex *in vitro* reagents: the CAP System (Pharmacia-UpJohn); the AlaSTAT, (Diagnostic Products Corporation) and the HY-TEC assay (HYCOR Biomedical). Although all of these commercial assays are based on non-ammoniated latex (NAL) as their primary allergen, there are differences in their solid or soluble supports and detector systems. Paired-comparisons of the three assays indicates that they disagree on the antibody status of an individual serum. This leads to patient's sera being "positive" by one or two tests and negative in (an)other(s). It is speculated that the disagreement among tests is due to IgE antibody assays detecting different subsets of IgE antibody of a given specificity, possibly as a result of differential specificities of their allergen-containing reagents. Reasons for this include variability in allergen content in different batches of source latex; sensitized individuals producing specific IgE antibody to at least 8 *Hevea* allergens, Hev b 1-Hev b 8, each of which differs in its structure, size, net charge (pI), relative allergenicity and abundance in natural rubber latex. The relative content and ratios of Hevs in the final allergen preparation most probably could enhance the diagnostic accuracy of a specific test. Other potential causes of allergen-containing reagent heterogeneity include variable stability during storage and variable binding of allergen to labels (e.g., biotinylated co-polymer in AlaSTAT) or solid supports (sponge in CAP; cellulose disc in HYTEC). Using receiver operating characteristic (ROC) curve analysis, and positive PST to NAL as the discriminator, the HY-TEC system has significantly greater ($P<0.01$) area under the curve (AUC, 0.924 + 0.017, standard error) than CAP (0.869 + 0.024) or AlaSTAT (0.858 + 0.024), suggesting it may be more accurate under the comparison conditions evaluated.

910 ANIMAL MODELS AND MECHANISMS OF LATEX ALLERGY.

B. J. Meade. *NIOSH, Morgantown, WV.*

Animal models are frequently used to conduct mechanistic studies that due to test article toxicity or sensitization potential would be unethical to perform in humans. Several laboratories have developed models to study latex allergy and although these include rabbit and guinea pig models, most animal research has been conducted in murine models. Although clinical and exposure data have been gathered on the factors affecting the elicitation of responses in latex allergic individuals, less is known regarding the development of sensitization. Coupled with *in vitro* dermal penetration studies, murine models were established to investigate the role of the route of exposure in the development of latex sensitization. Time course and dose response

studies have shown multiple subcutaneous (s.c.) administrations of as little as 0.19μg non-ammoniated latex proteins (NAL) elicited IgE production in 5 weeks. Animals exposed s.c., intratracheally (i.t.) or topically to 12.5μg NAL demonstrated IgE production within 2, 3 and 5 weeks, respectively. Although elevations in total and latex specific IgE have been observed following topical, intranasal (i.n.), i.t. and s.c. exposures, pulmonary responses (as measured by plethysmography) following respiratory challenge was only seen in mice sensitized by the topical or respiratory routes. Both *in vitro* penetration and *in vivo* studies highlight the importance of skin condition in the development of latex allergy with enhanced penetration and earlier onset of IgE production seen with experimentaly abraded skin. These models are also being used to investigate the role of concurrent exposure to other chemicals and agents in the workplace on the development of latex allergy. I.n. co-exposure to endotoxin with recombinant Hev b 5 was reported to increase the Hev b 5 specific IgE response without altering total IgE. In our model, i.n. co-exposure of endotoxin with a mixture of NAL proteins was found to suppress the elevation in total IgE seen in response to NAL alone. These observations underscore the complexity of the mechanisms involved in the development of an allergic response to a complex mixture.

911 HARMONIZATION OF CANCER AND NON-CANCER RISK ASSESSMENT: MOVING BEYOND THE NRC BOOK.

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The publication of the 1983 NRC report, Risk Assessment in the Federal Government: Managing the Process established the framework and principles for risk assessment that are still applied today, essentially unmodified. At that time, the primary focus was on cancer risk assessment with the consequence being the emergence of a somewhat dichotomous approach to assessing cancer and non-cancer risks. The rapid increase in our understanding of toxicokinetic and toxicodynamic mechanisms is now challenging the validity of these assumptions and the value of retaining such a disparate approach. Evidence suggests that carcinogens may work initially through a variety of less direct targets/processes, some of which may have thresholds. Similarly, because of factors such as differences in individual susceptibility and existing background rates, certain non-cancer effects may not exhibit a threshold. In this workshop, a more consistent and integrated approach to human health risk assessment will be discussed. In exploring the future directions that risk assessments will take, mechanistic commonalties between cancer and non-cancer effects will be examined as well as issues related to differences in disease expression (e.g. exposure parameters, disease latency). Discussions in this workshop will include an historical perspective, the central roles of mode of action and tissue dosimetry, and stochastic vs. tolerance distribution models in integrated approaches to cancer and non-cancer risk assessment.

912 HARMONIZATION IN RISK ASSESSMENT—OVERVIEW HISTORICAL ASSUMPTIONS AND PRACTICES.

G. Kimmel and V. Vu. *USEPA, Washington, DC.* Sponsor: H. Zenick.

In 1983, the National Research Council published Risk Assessment in the Federal Government: Managing the Process. Since then, much has been done in the way of producing guidance for carrying out risk assessments, as well as developing new approaches. Recently, the risk assessment field has begun to challenge the traditional approaches to risk assessment. This is especially true in the separation of cancer and non-cancer risk assessments. Historically, cancer has been considered a non-threshold response, while other health endpoints were considered threshold effects. However, in 1994 NRC, in Science and Judgement in Risk Assessment, noted the importance of an approach that is less fragmented, more consistent in applications of similar concepts, and more holistic than endpoint-specified guidelines. The report questions the application of a non-threshold quantitative approach as a default in all cancer risk assessments. Conversely, the use of a threshold concept as a default for agents that cause nerve, reproductive and developmental toxicity or that act on various systems through receptor-medicated events is also questioned. The EPA has begun to address this changing philosophy, and its revised cancer risk assessment guidance has proposed departing from the assumption that all cancer effects show linear dose-response relationships. The Agency is also developing an over-arching framework for human health risk assessment. The Agency is seeking the input of the risk assessment community as evidenced by a recent SOT Contemporary Concepts in Toxicology workshop, cosponsored by several governmental and non-governmental groups, on the harmonization of risk assessment approaches including incorporation of mechanistic information. The present talk will briefly review the historic assumptions underlying current approaches to health risk assessment and will summarize EPA's current interest in a more integrated approach to risk assessment in the future.

913 MODE OF ACTION AND TISSUE DOSIMETRY—LINCHPINS OF HARMONIZATION OF CHEMICAL RISK ASSESSMENT.

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Chemical risk assessments have evolved as knowledge of toxicological and biological processes has matured. However, there is divergence of risk assessment practices among agencies and offices within the same agencies. As chemical risk assessments become more objective, there is increasing emphasis on more consistent guidelines for these assessments. Increasingly, the emphasis has been on how the chemical interacts with the biological system to produce the adverse outcome, the so-called mode of action. Mode of action is especially helpful in defining approaches for low dose extrapolation. Comprehensive mode of action statements, however, should convey both the biological consequence of the exposure and the form of chemical that initiates changes in the biological system. The form of chemical relates to proper tissue dosimetry. Quantitative integration of dosimetry and mode of action occurs through pharmacokinetic (PK) and pharmacodynamic (PD) models. Biologically based dose response (BBDR) models link dosimetry from PK models quantitatively to adverse response through mode of action. Presently and perhaps well into the future, our confidence in PK and precursor effect models will be much higher than that in complete BBDR models. Harmonization will be accelerated by consistent use of mode of action data to infer the nature of low dose curves and dosimetry/precursor effect models that allow more confident prediction of the shape of dose response curves over a wide range of exposure conditions. This talk illustrates mode of action and dosimetry concepts qualitatively and quantitatively by reference to harmonization of cancer and non-cancer risk assessment and efforts to create BBDR models for carcinogens.

914 MODE OF ACTION AS A GUIDE TO QUANTITATIVE ANALYTICAL APPROACHES.

L. Rhomberg. *Gradient Corporation, Cambridge, MA.* Sponsor: H. Zenick.

Harmonization of quantitative risk assessment methodologies should aim at achieving logical consistency among methods. Mere imposition of similarity in the operational steps of calculation can actually work against this more fundamental harmony by hiding important differences in interpretation. The same growing insight into underlying mechanisms of toxicity that suggests some commonalities between cancer and noncancer toxicities also reveals an impressive diversity of fundamental mechanisms within these two classes. Some aspects of underlying modes of action relevant to choosing quantitative analytical approaches are discussed, including action at different levels of biological organization, functional redundancy of targets, and the distinction between stochastic processes and tolerance distributions as a basis for the existence of a dose-response relationship. The pursuit of biologically based mechanistic models for characterizing dose-response relationships will engender more diversity as each endpoint comes to be analyzed in a way appropriate to its underlying biology. Too rigid a notion of what "harmonization" means, therefore, may stultify development of models yielding deeper and more reliable insight into toxicity and may cloud proper interpretation of risk analysis results.

915 INTEGRATIVE APPROACHES TO RISK ASSESSMENT BASED ON MODE OF ACTION: CANCER AND NON-CANCER ENDPOINTS.

W. Farland, A. Jarabek and V. Vu. *USEPA, Washington, DC.*

There is general agreement in the scientific community that increased use of more and different information will be required to improve our understanding of risks from environmental toxicants. Recent advances in approaches to risk assessment are designed to accommodate such information. Among other things, these advances focus on breaking down the traditional dichotomy between approaches to evaluating cancer and non-cancer endpoints. The focus on better metrics for dose, understanding the basis for outcomes within

the range of observation and use of these improvements to inform judgment below the range of observation (extrapolation) will result in better characterizations of hazard and risk. To be most valuable in risk assessment, incorporation of more information should allow the identification of hazards by providing data on measurable biochemical or cellular endpoints which can serve as biomarkers of response for more complex adverse biological effects such as cancer, or developmental disorders. Ideally, these measurable endpoints should be mechanistically linked to the effect of concern rather than simply being correlated with it. Identification of key events leading to toxicity can provide insights into the conditions necessary for response, and the shape of the dose-response relationship for effects of concern as one goes from high to low dose. Developing means for the incorporation of such *in vitro* and *in vivo* data should allow the extension of the dose response relationship established by more traditional toxicology studies to lower levels, based on increased ability to detect responses in individual cells or using more sensitive biochemical or molecular biolological techniques. The determination of an appropriate exposure or dose metric to link these data will be critical. Incorporation of such information into risk assessments may also suggest targets of opportunity for further study in human populations by identifying potentially sensitive populations based on molecular or cellular characteristics or on sensitive stages in development and aging. Several examples including recent assessments of perchlorate and vinyl acetate will be used to illustrate the positive direction of the field of risk science and the role of new methods and data in improving assessments.

916 FINDING OUR WAY THROUGH THE GENE EXPRESSION DATA MAZE USING A DYNAMICAL MODEL OF OXIDATIVE STRESS.

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Gene expression array technology is creating a flood of data on gene expression responses by cells and tissues exposed to toxicants. Oxidative stressors, such as GSH depletion, oxidation, or exposure to oxidants, underlie many disease states and oxidative mechanisms have been demonstrated for a wide range of chemicals. Computer modeling provides a means to integrate our understanding of cellular biochemistry, signal transduction pathways, gene expression and transcriptional regulation in 'normal' cells and cells undergoing oxidative stress. Such models also provide a means to investigate the dynamics of interactions between these factors. Exposure of HepG2 cells to diethyl maleate, paraquat, and potassium bromate altered gene expression, detected using Clontech Expression Arrays and TaqMan, and GSH:GSSG values. Our experimental data and published literature were used as a basis for the development of a dynamical model (Biofusion Modeling on an Extend software platform) to study gene expression responses to oxidative stress. The model simulated aspects of energy metabolism (ATP:ADP), redox balance (GSH:GSSG), generation of superoxide and hydroxyl radicals, antioxidant responses (SOD, CAT, gammaGCS, GPX1, GR, HO-1), and transcription regulation (AP-1, NF-κB, p53) over 24 hrs in 1 min time steps. The model was found to (1) assist in the storage, integration and interpretation of these complex information/data sets while providing a dynamic rendition of their interrelationships, (2) permit hypothesis development and 'in silico' testing, and (3) provide a basis for interpretation of data derived from gene expression array technology. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

917 TOXICOGENOMICS: UNDERSTANDING THE USE OF MICROARRAYS FOR TOXICOLOGY STUDIES *IN VIVO*.

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Toxicogenomics has recently emerged as the science of interpreting gene expression patterns elicited by toxicants. However, it is critical to define the usefulness and potential limitations of any new technology before it is applied as part of a human safety assessment in drug development. The current study focused on these types of issues; 1) defining the assay reproducibility, 2) understanding the heterogeneity between samples, and 3) the ability to identify similarities or differences in gene expression patterns obtained from animals treated with several different toxicants. Groups of Sprague-Dawley rats were treated with either peroxisomal proliferators (clofibrate, gemfibrozil, or Wyeth-14,643) or an enzyme inducer (phenobarbital) for 2 weeks by oral gavage. After treatment the animals were sacrificed, hepatic RNA was isolated, and low-density gene array analysis (about 200 genes/slide) was conducted by a dual labeling technique using the Cy3 and Cy5 fluorescent probes. A pooled sample of RNA, isolated from the liver of untreated rats, served as the control for normalization between arrays. Results indicate that the assay is highly reproducible. When each RNA sample was assayed on 2 separate arrays, there was a high correlation in the gene expression profiles ($r > 0.900$). Likewise, when groups of rats were treated with the same compound, animals responded uniformly with correlations in the gene expression pattern again greater than 0.900. Since these initial data clearly established that the assay was reproducible and animals responded uniformly, comparisons were made between different compounds. As expected, similarities in gene expression were obtained when comparing the clofibrate, gemfibrozil, and Wyeth-14,643 whereas, phenobarbital produced a pattern dissimilar to that obtained by the peroxisomal proliferators. Together these data provide an early indication for the reproducibility and potential usefulness of Toxicogenomics in the field of Safety Assessment.

918 DIFFERENTIAL GENE EXPRESSION IN RATS FOLLOWING CHRONIC SODIUM VALPROATE EXPOSURE.

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Sodium valproate (SV) is clinically employed as an anti-convulsant and, recently in the USA, as a mood stabiliser in manic-depressive illness (bipolar disorder). While the incidence of toxicity associated with the clinical use of valproate is lower than with many other anticonvulsant/mood stabilising drugs, it is becoming increasingly clear that serious hepatotoxicity makes up a significant percentage of these adverse effects. Jeavons1 reported 60 cases of death due to hepatic failure within the first six months of multidrug treatment, including valproate, with fatalities occurring mainly in children. Previous studies have highlighted the different pharmacological effects of acute SV exposure, a combination of which are likely to underpin its observed broad-spectrum anticonvulsant efficacy. However, limited studies have been undertaken to study the chronic effects of this compound, and how genomic effects may underlie the observed mood stabilising and hepatotoxic effects. Suppression PCR Subtractive Hybridisation (SSH) is a PCR-based method for isolating differentially expressed genes, enabling the identification of genes implicated in the modes of action of drugs. This study uses SSH, along with RT-PCR, to identify and quantitate mRNA species regulated in response to chronic SV exposure in rats. Male Sprague Dawley rats were dosed with vehicle (saline), 250 or 500mg/kg/day sodium valproate i.p. for four days, a dose previously shown to elicit similar effects to those seen in humans (e.g. microvesicular steatosis, mitochondrial damage). Twenty-four hours after the final dose, (Day 5) animals were killed, mRNA extracted from control and treated livers and subjected to SSH. Resultant regulated species were screened to remove false positives, sequenced and changes quantified using RT-PCR. 10 up-regulated and 15 down-regulated mRNA species were identified. Six novel mRNA species were identified, for which no functional data is known, and these provide new lines of investigation which we are currently under investigation. Several mRNA species were identified that may play a role in the observed hepatotoxicity. These include a down regulation of species involved in cellular energetics (i.e. succinate dehydrogenase, aldolase B) which may be involved in the mitochondrial damage, possibly leading to the observed loss of Acyl CoA (Ponchaut et al., 1992). Cellular proliferation (i.e. NAP4) and protein synthesis (i.e. ribophorin II) were also down-regulated, suggesting a general loss of cell viability. Such data forms the basis for further studies into the genomic changes associated with chronic SV exposure.

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919 THE GENE EXPRESSION SIGNATURE OF TCDD IN HUMAN HEPG2 CELLS.

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One of the most puzzling aspects of the biological impact of *2,3,7,8*-tetrachlorodibenzo-p-dioxin (TCDD), the prototype dioxin, is that it causes a profusion of apparently unrelated biological effects. In humans, exposure to TCDD and to many other chlorinated phenolic agents causes chloracne, a long-lasting skin disease characterized by the hyperkeratinization of follicular sebocytes. Long-term epidemiologic studies have also established a strong

link between exposure to high doses of TCDD and certain types of cancers and cardiovascular disease. TCDD exposure during mouse embryogenesis causes hydronephrosis and craniofacial anomalies, whereas in adult rodents, TCDD causes an elevated incidence of hepatic carcinoma and pulmonary and skin tumors. Other consequences of TCDD exposure include disturbances of lipid metabolism, cardiovascular and craniofacial abnormalities during fish development, and immunotoxic, reproductive and endocrine effects, some of which appear to be present also in exposed humans. The effects of TCDD at the cellular level are just as diverse and often contradictory. In human keratinocytes, dioxin induces proliferation as well as terminal differentiation. Immature thymocytes from rats and mice treated with TCDD *in vivo*, but not *in vitro*, show increased apoptosis. In rat hepatocytes, TCDD has also been reported to induce apoptosis as well as to inhibit uv-induced apoptosis and to increase, in some reports, and to decrease, in others, proliferation rates. TCDD also abrogates the estrogen-dependent proliferation of human breast cancer cells. Many of the effects of TCDD exposure have been known for many years to result from the activation of the Ah receptor, a ligand activated transcription factor that, through dimerization with ARNT, is responsible for the up-regulation of several well-characterized genes; however, a more complete signature of regulatory mechanisms affected by TCDD, which might shed light on the mechanisms responsible for its many biological effects, has never been obtained. We have used a microarray hybridization approach to characterize the response of the human HepG2 hepatoma cell line to TCDD exposure. We find that TCDD disregulates by at least a factor of 2 the expression of 412 known genes in addition to that of several hundred more expressed sequence tags (ESTs). Treatment with TCDD in the presence of cycloheximide (CX), allowed us to distinguish primary effects of exposure from secondary effects, which are blocked by inhibition of protein synthesis. Of the 412 known genes, 25 are up-regulated regardless of CX treatment, and 125 other are up-regulated only when protein synthesis is allowed to proceed. Similarly, 80 genes are down-regulated independently of CX treatment, whereas down-regulation of an additional 137 is blocked by CX. The remaining 46 genes are either induced (10) or repressed (36) by TCDD only when protein synthesis is blocked. Regulatory patterns induced or repressed in the presence of CX by benzo(a)pyrene, another Ah receptor ligand, are by and large the same as those resulting from TCDD exposure. These results suggest that the development of a sound understanding of the molecular mechanisms governing the outcome of TCDD exposure promises to be orders of magnitude more complicated than might have been previously imagined. (Supported by NIH P30 ES06096 and R01 ES06273.)

920 THE USE OF MICROARRAYS TO IDENTIFY AND CHARACTERIZE POTENTIAL HEPATOTOXICANTS BY PATTERN RECOGNITION.

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Transcript profiling using microarray technology provides new approaches to hazard characterization and determination of modes of action. Here we describe the use of transcript profiling to determine changes in gene expression associated with compounds that cause hepatotoxicity. A custom designed nylon microarray comprising of 2400 cDNA clones representing 600 genes, selected to represent pathways related to cell growth, differentiation or death, has been employed to investigate gene expression in the human HepG2 cell line exposed to hepatotoxicants. Cells in replicate flasks were exposed to high or a low toxic doses, as determined by LDH release, of ethanol (EtOH: 500mM, 300mM), carbon tetrachloride (CCl4: 20mM, 5mM), hydrogen peroxide (H_2O_2: 10mM, 1mM) and acetaminophen (APAP: 10mM, 2.5mM) for 24 h. Preliminary data reveal that a number of genes with roles in many cellular functions including apoptosis, cell cycle, transcription and stress were upregulated (up to 6.5 fold) or downregulated (up to 7.2 fold) by treatment with selected hepatotoxicants. In addition, the regulation of certain genes was common to more than one treatment, for example; prolactin was induced and p27Kip1 was repressed in both low EtOH and low H_2O_2 treatments. These results suggest that it may be possible to associate gene expression patterns with specific forms of hepatotoxicity.

921 GENE EXPRESSION PROFILE OF PRIMARY HUMAN HEPATOCYTES TREATED WITH A PEROXISOME PROLIFERATOR, WY-14,643.

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Peroxisome proliferator-activated receptor alpha (PPAR-α) is a nuclear hormone receptor thought to mediate the generation of peroxisome proliferation and non-genotoxic hepatocarcingenesis in rodents. In humans, however, clofibrate and other PPAR-α ligands are used safely for the treatment of hyperlipoproteinemias. The mechanisms underlying the differences in species responsiveness to peroxisome proliferators are not clear, but could involve different expression levels of the receptors or differences in the regulatory sequence of PPAR-α target genes. To help understand this species difference we have used oligonucleotide arrays (Affymetrix GeneChip probe arrays) to generate gene expression profiles of primary human hepatocytes exposed to the PPAR-α ligand Wy-14,643. Primary human hepatocytes treated for 24 hours with up to 100 mM of Wy-14,643 showed many changes in gene expression when compared to vehicle-treated cells. In addition to increasing the expression of certain stress-associated and growth-regulated genes, Wy-14,643 decreased the expression of a variety of drug- and fatty acid-metabolizing genes. These results provide insight into previously reported findings regarding the responses of human hepatocytes to peroxisome proliferators and demonstrate the utility of gene expression profiling for characterizing cellular responses to xenobiotics.

922 ANALYSIS OF THE DRUG PHARMACOLOGY TOWARDS PREDICTING DRUG BEHAVIOR BY EXPRESSION PROFILING USING HIGH-DENSITY OLIGONUCLEOTIDE ARRAYS.

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An important aspect of the drug development process is prediction of efficacious and toxic side effects. Many lead compounds for drugs fail due to unexpected toxicity or lack of efficacy in late stage of the expensive and lengthy drug development process. We hypothesize that pharmaceutical compounds targeted to cells will induce characteristic changes in mRNA expression patterns, as a result of affecting specific pathways downstream to the molecular targets of the compounds. Thus, the mode of action and efficacious or toxic side effects of the compounds may be reflected in these characteristic changes in mRNA expression. We attempt to explore the approach of using expression profiling to identify patterns or fingerprints that can help predicting specific drug effects or behaviors. Specifically, we aimed to correlate known drug effects and properties with expression patterns that are likely to be the signature for a specific drug effect or property. A large-scale experimental study was performed to discover if there are changes in expression levels and patterns that can be correlated with drug activation mode and target specificity. We treated human aortic smooth muscle cells with a large number of agonists and antagonists targeted at adrenergic receptors to produce 100 expression profiles from 50 treatments. The resulting data sets were analyzed by two statistical analysis methods. The hierarchical clustering analysis based on the behavior of the most variable genes showed that these compounds can be classified into separate groups correlated with their activation mode and target specificity as well. By principal component analysis, a set of genes have been identified whose expression changes correlated with the activation properties of the compounds targeted at adrenergic receptors. Agonists and antagonists can be classified primarily into two different groups based on the behavior of this set of genes. These two sets of genes can be potentially used as fingerprints for predicting the activation or target specificity properties of the compounds. This approach may be extended to identifying expression patterns correlated with specific toxic property of compounds, which may be used as fingerprints for prediction of drug toxicity. This study suggests that specific gene expression patterns correlated with certain drug effect and behavior can be identified through expression profiling.

923 USING TRANSCRIPT PROFILING TECHNOLOGY TO GAIN A BETTER UNDERSTANDING OF TOXIC MECHANISMS.

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Microarray technology would appear to offer great potential for toxicology - in either a predictive context or helping to unravel mechanisms of toxicity at the molecular level. As a first step in evaluating the true potential of toxicogenomics, we have utilised an *in vivo* approach to gain new perspectives on the molecular events triggered by a range of liver toxicants, including peroxisome proliferators (PPs). The key objective of these experiments has been to identify important gene expression changes associated with toxic events ie. which genes are switched on/off and what is the magnitude of induction/repression? To enable such meaningful data interpretation, it is

important to recognise the challenges presented by adopting an *in vivo* approach, in particular the extent of background variability in gene expression patterns between animals, as well as examining dose response and time course effects. In this regard, we have conducted experiments to gauge the extent of blot to blot, probe to probe and animal to animal variability. These data have clearly shown that variability decreases with increasing intensity of hybridisation signal. However, our data have confirmed that it is important to characterise the degree of variability within each *in vivo* experiment. Our data have also shown marked differences in gene expression patterns dependent on dose and/or time. Set against this background however, we have shown that administration of compounds such as DEHP to male rats have not only altered the hepatic expression of genes known to be induced by PPs (e.g., CYP4A1) but have induced a number of unexpected changes that may provide important mechanistic information.

924 CONSTITUTIVE AND INDUCIBLE EXPRESSION OF CYP1B1 IN VASCULAR SMOOTH MUSCLE CELLS: DIRECT INVOLVEMENT OF THE ARYL HYDROCARBON RECEPTOR IN BENZO[A]PYRENE-MEDIATED DEREGULATION OF *C-HA-RAS*.

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Previous studies in this laboratory have found that transcriptional deregulation of *c-Ha-ras* in vascular smooth muscle cells (vSMCs) by benzo(a)pyrene (BaP) involves oxidative metabolism to intermediates that generate reactive oxygen species. Interestingly, *c-Ha-ras* gene activation by BaP (3μM) is absent in aryl hydrocarbon receptor (AhR) null ($AhR^{-/-}$) vSMCs, suggesting that the AhR participates in the regulation of cytochrome P450-mediated metabolism of BaP, or is directly involved in the transactivation response. The present studies were conducted to examine constitutive and inducible mRNA expression of cytochromes P4501A1 (*CyP1A1*) and 1B1 (*CyP1B1*) in vSMCs from $AhR^{+/+}$ and $AhR^{-/-}$ C57/Bl6J mice. Constitutive *CyP1B1*, but not *CyP1A1*, expression was observed in $AhR^{+/+}$ and $AhR^{-/-}$ vSMCs, while inducible expression of both genes was only seen in $AhR^{+/+}$ vSMCs challenged with BaP (3μM). Aryl hydrocarbon hydroxylase (AHH) activity was upregulated to comparable levels in $AhR^{+/+}$ and $AhR^{-/-}$ vSMCs challenged with BaP (3μM). This response, however, was not mediated by *CyP1A1* since ethoxyresorufin-o-deethylase activity (EROD) activity was not detected. Actinomycin D (2μg/ml) or cycloheximide (10μM) did not block BaP-induced AHH activity, indicating that increased transcription, or de novo protein synthesis, were not responsible for AHH induction. Modulation of growth-related signaling in vSMCs was not dependent on the AhR phenotype since BaP (3μM) inhibited DNA synthesis in both $AhR^{+/+}$ and $AhR^{-/-}$ vSMCs. Collectively, these data suggest that AhR is directly involved in the deregulation of *c-Ha-ras* gene by BaP in vSMCs, but that this role is independent of *CyP1B1*-associated AHH activity. (Supported by NIH Grants ES 04849 and ES 09106.)

925 FURTHER CHARACTERIZATION OF OXIDANT-ACTIVATED PROTEIN BINDING TO THE ANTIOXIDANT/ELECTROPHILE RESPONSE ELEMENT IN VASCULAR SMOOTH MUSCLE CELLS.

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Previous reports from this laboratory have established that xenobiotic-induced deregulation of gene expression in vascular smooth muscle cells (vSMCs) involves binding of nuclear proteins to the antioxidant/electrophile response element (ARE/EpRE). Studies using mutant *c-Ha-ras* oligonucleotides have shown that maximal protein binding in response to oxidant challenge requires a TMA box 5' of the ARE/EpRE core region, as found in the mouse *GST-Ya* ARE/EpRE (5'TAATGGTGACAAAGCAACTT3'). Our first experiment was conducted to determine if the length of chemical treatment influences *GST-Ya* ARE/EpRE protein binding in intact cells. Randomly cycling vSMCs were tested for ARE/EpRE protein binding following challenge with hydrogen peroxide (25μM) for 0.25-48 hr. Increased binding in response to oxidant challenge was observed as early as 15 minutes, and remained elevated for up to 48 hr. We next evaluated ARE/EpRE protein binding in isolated nuclei or intact murine vSMCs challenged with hydrogen peroxide (25 or 40μM) to determine if direct macromolecular oxidation, or cellular coupling of a stress response, is required for activation of protein binding. Enhanced binding to the ARE/EpRE was observed in both intact cells and isolated nuclei, suggesting that direct chemical modification of macromolecular structures is sufficient for activation of protein binding to the ARE/EpRE. Addition of hydrogen peroxide (25 or 40μM) to nuclear preparations isolated from peroxide-treated cells (25μM) did not modify the binding response profile. On the basis of these findings we conclude that activation of ARE/EpRE protein binding by hydrogen peroxide is a rapid and sustained response which does not appear to require intracellular coupling of a stress signal. (Supported by NIH Grants ES 04849 and ES 09106.)

926 ACTIVATION OF *L1MD* RETROTRANSPOSON IN MOUSE VASCULAR SMOOTH MUSCLE CELLS BY BENZO[A]PYRENE METABOLITES AND HYDROGEN PEROXIDE: IMPLICATIONS IN CHEMICAL ATHEROGENESIS.

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We have previously shown that the *L1Md* retrotransposon is activated by acute exposure of vascular smooth muscle cells (vSMCs) to benzo(a)pyrene (BaP). Retrotransposons are mobile DNA elements that propagate through the genome by the process of transcription, reverse transcription, and reintegration. The mechanism of *L1Md* retrotransposon activation by BaP is currently unknown and therefore, the present studies were conducted to determine if BaP metabolites (7, 8-diol, 3-hydroxy, 3, 6-quinone) and/or hydrogen peroxide mimic the ability of the parent compound to activate retrotransposons in mouse vSMCs. RT-PCR and Northern analyses were used to evaluate *L1Md* expression in murine vSMCs following an 8 hr chemical challenge. Our results showed that BaP 7, 8-diol was the strongest inducer of L1Md retrotransposon activation relative to BaP itself, or the other metabolites examined. A low concentration (25μM) of hydrogen peroxide also activated *L1Md*. These results demonstrate that BaP induces activation of retrotransposons and that activation is mediated, at least in part, by cytochrome P450-derived metabolites and reactive oxygen species. Because retrotransposon activation is associated with genomic instability, our results implicate *L1Md* in the acquisition of proliferative vSMC phenotypes during the course of the BaP-induced atherogenic response. (Supported by NIH Grants ES 04849 and ES 09106.)

927 CHARACTERIZATION OF THE NZB/NZWF1 MOUSE MODEL FOR IDENTIFICATION OF DRUG INDUCED AUTOIMMUNE DISEASE.

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The female NZB/NZWF1 mouse is a well recognized model of human systemic lupus erythematosus (SLE). Autoimmune parameters such as anti-DNA antibody formation occur spontaneously and progress with increasing age. The goal of these studies is to identify drugs with the potential to induce autoimmune disease. An autoimmune syndrome can be induced in NZB/NZWF1 mice at a young age following an acute exposure to mercuric chloride. Following exposure to mercuric chloride (1.0mg/kg), total serum IgG (mg/ml) was increased 67% and the anti-DNA IgG titer was increased by 87%. Spleen cells were obtained from mice exposed to mercury and treated *in vitro* with concanavalin A (Con A) or lipopolysaccharide (LPS). The Con A induced production of IL-2, IL-4, and IL-6 was increased by 46%, 39%, and 36% respectively in spleen cells from mercury treated mice. The LPS stimulated production of IgG was increased by 77% in splenocytes from mercury treated mice. Additionally, splenocytes from naïve NZB/NZWF1 mice were treated *in vitro* with mercuric chloride. IL-2 and IL-4 production were increased by 10mM mercuric chloride by 70% and 77% respectively. Further experiments with drugs known to induce a lupus like symptoms will determine the ability of this model to detect compounds that may induce autoimmune disease.

928 A COMPREHENSIVE MODEL TO TEST FOR IMMUNOMODULATION: THE LISTERIA IMMUNIZATION CHALLENGE ASSAY (ICA).

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Because of the complexity of the immune system a large battery of different assays has been developed over the years to test its functionality. To investigate 'overall' effects of immunomodulating compounds on the immune system varying numbers of experiments are necessary to demonstrate wanted (modulation of the immune functions by drugs or nutrients) or unwanted

(toxic or sensitizing compounds) effects. A good example is the tiered approach that has been developed to test for immunotoxic properties of xenobiotics. Although on itself a sensible approach, it requires often more than one study to allow a proper evaluation with regard to possible immunomodulating characteristics of the test compound. The development of the immunization Challenge Assay enables the evaluation of various aspects of the specific and non-specific immune response in one study. The Listeria Immunization Challenge Assay (Listeria ICA) is divided into two phases: an immunization phase with Listeria monocytogenes bacteria followed by a challenge phase in which the animals will be intravenously infected with the live, virulent bacterium. This approach offers the possibility to investigate (possible) effects on various compartments of the immune system. It may be adapted for either a broad general screening of compounds or to investigate in more detail the mechanism behind already established immunomodulating effects. Compound interactions with the immune system are tested during the immunization phase i.e., during a time-period the immune system is forced to respond to a stimulus and is expected to be more sensitive for interference. Effects on one or more immune parameters measured may be related to effects on the overall response of the animals during the challenge (infection) phase, i.e., the overall protection of the animals. In this way, the relevance of the observed immunomodulating effects with respect to the ultimate susceptibility for infection can be evaluated. By applying just one antigen, effects measured on all separate parameters may be evaluated within the same context, i.e., a comprehensive test model is used. The choice of the intracellular surviving Listeria monocytogenes bacterium as an antigen not only enables the investigation of humoral responses but also, and especially, cellular specific and non-specific responses. In the first set of experiments an optimal immunization procedure has been developed. It was found that (sc) immunization with viable bacteria, but not dead (irradiated) bacteria, resulted in a dose-dependent protection against the experimentally (iv) induced listeria infection. Depending on the immunization dose up to 100% protection was obtained. In contrast, immunization with dead, irradiated, bacteria did not result in a measurable protection against the listeria infection. However, when using an adjuvants (aluminium hydroxide) the responses induced by the immunization with dead bacteria could be enhanced sufficiently to obtain some level of protection. Parameters which were amongst others measured in these studies were antigen specific delayed type hypersensitivity responses, *in vitro* proliferative responses and antibody levels, lymphocyte subpopulations and cytokine m-RNA transcription. The level of protection was assessed by determining the number of remaining Listeria monocytogenes bacteria in liver and spleen two or four days after infection. So far the model has been developed to a stage that immunomodulating effects of compounds can be tested. A sub-optimal immunization procedure, i.e., resulting in a level of protection which may be influenced by the test compound in either a positive or a negative way, has now been defined. The next step will be the application of known immunomodulating compounds to validate the immunization challenge assay (ICA) and to determine the sensitivity of this approach.The use of dead bacteria for the immunization procedure offers special application possibilities with respect to the investigation of new adjuvants.

929 USE OF DENDRITIC CELLS FOR IMMUNOTOXICOLOGICAL SCREENING: EFFECTS OF IL-16 AND RSV-INFECTION.

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Dendritic cells (DC) are the most important cells in regulating the cellular immune response by recruiting and activating naive T-cells. Therefore, they play an important role in allergic inflammation and viral infections. The aim of our studies was to generate human dendritic cells and to use these cells as tool for immunotoxicological screening. Human dendritic cells were generated from monocytes by cultivation in IL-4 and GM-CSF. Having characterized optimal serum-free culture conditions by measuring T cells responses (MLR) and surface phenotype (flow cytometry) the effects of recombinant IL-16 (mediator of the late phase of allergic reactions) and the effect of an infection with the respiratory syncytial virus on DC function were assessed. IL-16 induces the expression of different chemokines/ cytokines both on protein and on mRNA level (e.g. MIP-1a, RANTES, IL-8 and IL-10) and accelerates the maturation of DC as measured by increased expression of surface antigens e.g. CD83, CD86. In the case of RSV infection it could be shown that the virus infects DC and induces maturation, production of IL-10 and at higher MOI apoptosis. These studies demonstrate that DC are a suitable target for immunotoxicological screening *in vitro*.

930 UTILITY OF THE LOCAL LYMPH NODE ASSAY FOR THE IDENTIFICATION OF IMMUNOSUPPRESSIVE DRUGS.

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The Local Lymph Node Assay (LLNA) is a robust tool for the identification of compounds producing contact sensitization. Some investigators have suggested that the popliteal LLNA could be used to identify immunosuppressive drugs. The purpose of these studies was to explore this possibility by using one model immunosuppressive agent and several test compounds with known or suspected immunosuppressive effects to inhibit the LLNA response elicited following exposure to the contact sensitizer oxazolone. Female CBA/J mice were exposed dermally to test compound or oxazlone (0.05% and 0.1%) alone or the combination of the two. Following the exposures, radiolabeled thymidine incorporation into lymph node cells was determined or lymph node phentotypic analysis was performed. Dermal exposure to dexamethasone (0.05%) was capable of attenuating the radiolabel incorporation induced by oxazolone by 80% (0.05%) and 86% (0.1%). Dexamethasone reduced the oxazolone induced increases in the number of lymph node T-cells by 92% and B-cells by 97%. The oxazolone induced alterations in lymph node percentage of T-cell and B-cells was inhibited by approximately 70%. In contrast, dermal exposure to indomethacin, thalidomide, pentoxifylline, or chelerythrine were unable to alter the effects of oxazolone on proliferation and lymph node cell phenotypes. The absence of activity of these compounds may be dependent on the route of exposure. Parenteral exposure to these compounds is under evaluation. These results suggest that the LLNA is not a sensitive model for detecting compounds with weak to moderate immunosuppressive activity.

931 SUBERYTHEMAL UVB EXPOSURE AFFECTS AIRWAY HYPERRESPONSIVENESS INDUCED BY TH1 AND TH2 MEDIATED PULMONARY HYPERSENSITIVITY.

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One of the major immunomodulatory effects of UV exposure is suppression of cellular immune responses that can be evoked by skin contact with contact sensitizers (e.g., picryl chloride, DNCB, and oxazalone). The Th1 mediated contact hypersensitivity response was suppressed by low doses of UV exposure if the animals or human volunteers were exposed to UV prior to skin sensitization. This immunosuppressive effect was detectable if mice were exposed to UV at the site of first contact with the chemical or even at a distant site; the first referred to as local and the latter as systemic immunosuppression. We demonstrated that in a model for contact hypersensitivity in mice the Th1 associated cytokine IFN-γ production was impaired whereas the Th2 cytokine interleukin 4 remained unaffected after exposure to low doses of UV light prior to skin-sensitization. Based on these observations, the hypothesis was put foreward, that UV exposure could have an influence on respiratory allergy. For this reason, the effect of UV exposure was studied in two different murine models, i.e., for type I (IgE mediated) and type IV (delayed type) pulmonary hypersensitivity, respectively. In both animal models inflammatory reactions were present in the lungs of mice that were challenged with the specific antigen via the airways. In the picryl chloride type IV allergy model, inflammatory reactions consist of especially mononuclear cells, whereas in the ovalbumin type I allergy model also eosinophylic granulocytes were present. In both models non-specific airway hyperreactivity to bronchoconstricting stimuli such as acetylcholine was induced. Prior studies have indicated that IFN-γ is involved in the induction of airway hyperreactivity both in models for Th1 mediated and Th2 mediated pulmonary hypersensitivity. If mice were exposed to UV light for 5 consecutive days prior to sensitization with picryl chloride or ovalbumin, airway hyperreactivity to cholinergic stimuli was significantly impaired. In the picryl chloride model, IFN-γ production was suppressed; in the ovalbumin model, specific IgE production was suppressed. This study indicates that immunomodulation induced by exposure to low doses of UV might have consequences for respiratory allergy.

932 ACTIVATION OF GUINEA PIG COMPLEMENT BY TRIMELLITIC ANHYDRIDE CONJUGATED TO GUINEA PIG SERUM ALBUMIN (TMA-GPSA).

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Actively sensitized guinea pigs challenged intratracheally with TMA-GPSA

respond with eosinophil, neutrophil and mononuclear cell infiltration into the lungs and lung hemorrhage. Previously, using Cobra venom factor to deplete complement, we demonstrated a role for the complement system in TMA-induced cell infiltration into the lung and lung hemorrhage (J Pharmacol Exp Ther 273:793-801, 1995). In addition, we detected the complement activation product C3a in the bronchoalveolar lavage fluid of these animals. To determine if TMA GPSA could cause complement activation *in vitro*, varying concentrations of TMA-GPSA and GPSA (0.5 to 500 μg/ml) were incubated at 37°C for 15 min with complement from either unsensitized guinea pigs or guinea pigs sensitized with TMA. Complement activation was assessed by measuring cleavage of the third component of complement (C3 conversion). After agarose gel electrophoresis of samples, C3 conversion products were visualized in the gel by immunoprecipitation with antibody to guinea pig C3 and Coomassie blue staining. TMA-GPSA caused significant C3 conversion when incubated with complement from unsensitized animals. This is the first demonstration that TMA-GPSA can activate the complement system in the absence of specific antibody. Significantly more C3 conversion occurred when TMA-GPSA was incubated with complement in the presence of TMA specific antibody than without antibody. GPSA itself caused minimal C3 conversion. Thus, TMA-GPSA causes cleavage of C3 *in vitro*, consistent with previous evidence that TMA induced respiratory hypersensitivity in the guinea pig is, in part, dependent on the complement system. (Supported by NIH ES 07406.)

933 IMMUNOLOGIC AND BIOCHEMICAL ABNORMALITIES IN WORKERS EXPOSED TO POLLUTANTS AT AN EGYPTIAN COPPER COMPANY.

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For this project we studied: a) biochemical changes; b) serum immunoglobulin (Ig) levels, and c) mitogenicity of peripheral blood lymphocytes (PBL) in workers (experimentals) **directly** exposed to high concentrations of pollutants in several sectors of a major copper company in Alexandria. These sectors included the aluminum utensils area, semicontinuous aluminum casting, brass foundries and steel furnaces. Toxicants in these sectors included aluminum hexachloroethan, silica, cadmium, copper, mercury, lead, asbestos, nickel, zinc, silver, carbon, iron and sulphite detected in high concentrations in the sectors where workers are directly exposed. Administrative personnel in the plant not directly exposed were included as positive controls; negative controls were people living in areas of Alexandria where the concentration of these toxicants was relatively low. Significant variations occurred in liver and lung functions, in lipid profiles, vitamins E and C, glucose-6-phosphate dehydrogenase and other enzymes including superoxide dismutase, glutathione, and anti-oxidants. All personnel of the aluminum utensils area showed reduction in the Ig's gamma, mu, and alpha assayed by ELISA, while workers directly exposed in the other sectors showed elevated Ig's. Mitogenic activity in cultured PBL assayed by ^{3}H-thymidine uptake was impaired in all plant personnel. However, experimentals showed increases in the cytokines IL-2, IL-6, IFN-gamma, TNF-alpha and beta assayed by ELISA, but not in IL-4. Changes were directly related to duration of exposure. Some workers (16.2%) showed symptoms of arthritis and spondylitis. Thus, abnormalities were greatest in directly exposed workers, while other plant personnel showed some form of toxicity in the parameters studied. Clinical significance of the immunologic abnormalities seen is under further study.

934 AFB_1-INDUCED SUPPRESSION OF MACROPHAGE-DERIVED CYTOKINE PRODUCTION IS DUE TO INCREASES IN PGE_2.

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Previously, we showed that male F344 rats given a single, acute intratracheal dose of Aflatoxin B_1 (AFB_1) and subsequently challenged with *Listeria monocytogenes*, demonstrated impaired clearance of bacteria from the lung, liver and spleen. In these animals, splenic macrophage phagocytic activity and TNFα production were suppressed whereas T cell derived cytokine (IFNγ) production was not altered. As TNFα and IL-12 are required for host resistance against *L. monocytogenes*, the mechanisms by which AFB_1 impairs macrophage-derived cytokine production was investigated *in vitro* by exposing RAW 264.7 macrophages to AFB_1 and quantitating cytokine production. LPS-inducible TNFα and IL-12 production was significantly suppressed (50 and 60% of controls respectively) in cells exposed to 30 μM AFB_1. In contrast, IL-1 production was not affected, thereby suggesting a specificity for cytokines targeted by AFB_1. In addition, AFB_1 exposure increased LPS-inducible PGE_2 4-fold as compared to cells treated with medium alone. TNFα levels were restored in AFB_1-exposed cells treated with 1 nM indomethacin, thus blocking the AFB_1-induced increase in PGE_2. Therefore, it appears that AFB_1 is increasing COX2 activity. This finding is significant, as the likely mechanism by which AFB_1 is suppressing TNFα and IL-12 is through increased production of PGE_2, as PGE_2 is known to downregulate macrophage-derived TNFα and IL-12 production. In conclusion, the data provide a potential mechanism by which *in vivo* exposure to AFB_1 may be suppressing host resistance against *L. monocytogenes*.

935 ALTERATIONS IN THE PRE-B CELL POPULATION OF THE BONE MARROW AFTER *IN VIVO* EXPOSURE TO A MIXTURE OF HERBICIDES.

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Chemical mixture risk-assessment extrapolated from single treatment data does not account for interactions between mixture components. Previous research has demonstrated that the herbicide propanil (3,4-dichloropropionanilide) is immunotoxic to the thymus, spleen and bone marrow affecting specific T and B cell responses and progenitor differentiation. Propanil is sold as a mixture with the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) in commercial preparations such as Nox-D and Herbanil. The goal of our research is to determine the immunotoxic effects of *in vivo* intraperitoneal exposure to a mixture of propanil and 2,4-D in C57Bl/6 mice. Single herbicide treatments include 50 mg/kg or 200 mg/kg doses. Mixtures were composed of either 50 mg/kg + 50 mg/kg or 50 mg/kg + 200 mg/kg combinations of the two herbicides. In the bone marrow, exposure to propanil, 2,4-D, or a mixture of the two herbicides specifically decreased the pre-B cell population as assessed by flow cytometric analysis of B220+ lymphocytes. Mixtures of 50 mg/kg propanil and 50 mg/kg 2,4-D did not cause additive decreases. However, a 50 mg/kg propanil + 200 mg/kg 2,4-D mixture synergistically decreased the percent of pre-B cells as compared to the 200 mg/kg 2,4-D component alone. All mixture concentrations caused significant decreases in pre-B total cellularity. Preliminary experiments indicate that the mechanism of selective lymphocyte depletion by propanil or 2,4-D may be through the release of glucocorticoids in response to chemical stress. This study demonstrates that propanil and 2,4-D are immunotoxic to a developmental immune organ and that exposure to a mixture of the two herbicides can potentiate immune cell loss. (Supported by NIEHS grants ES07460 and ES07512.)

936 DYNAMICS OF THE IMMUNE SYSTEM OF THE RAT IN AN INTERMITTENT DOSING STUDY WITH AFLATOXIN B1 (AFB_1).

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AFB_1 is a known carcinogen and affects immune function in animals. We were interested in the immunotoxic mechanisms relevant to low doses and to intermittent exposure. Fisher-344 male rats were fed intermittently for 40 weeks (4 weeks-"on" and 4 weeks-"off" diet) doses of 0.01, 0.04, 0.4 and 1.6 ppm of AFB_1 and for 40 weeks continuously at the 1.6 ppm level. Groups of animals were sacrificed at 4 week intervals up to 20 weeks and then at 40 weeks. Lymphoid tissues were removed and processed for histopathology and imaging. Splenic cell suspensions were analyzed by flow cytometry for % T (CD3) and % B (CD45R) cells and % helper (CD4) and % suppressor (CD8). Portions of the suspension were stimulated in culture for analysis of the productive capacity for cytokines IL-2 (Con-A), IL-1 and IL-6 [LPS, LPS + γ interferon (INF) and γINF + NGMA, a nitric oxide synthetase inhibitor]. Hematologic and other endpoints were measured also. Animals were 6 weeks of age at the start of dosing and were in growth phase during the 20 weeks of dosing. Dynamic changes were seen in both T-and B-cells for controls, e.g., % T increasing from 27 to 58% and B-cells decreasing from 50 to 28%, both reaching a plateau at approx. 20 weeks of age. The %CD4 and CD8 cells as well as IL-2 increased with time, (CD4, 24 to 50%, and CD8, from 10 to 20% at 14 weeks of age.) A 7-fold increase for IL-2 was seen at 18 weeks of age. After the first 4 weeks of dosing, B-cells decreased from 50 to 32% while T-cells increased from 27 to 43% at 0 to 0.4 and 1.6 ppm AFB_1. Increases in both CD4 and CD8 populations were seen. IL-2 was increased only at lower doses. After the first "off" cycle the trends for %T, %B and %CD4 were not reversed while %CD8 was reversed. After the 2nd "on" cycle shifts in the various cell populations were seen again. Dynamic changes were seen also for IL-1 and IL-6 after the 2nd "on" cycle suggesting a pro-inflammatory response. The significance of these changes in immune parameters in relation

to functional responses and carcinogenicity is of interest and need to be determined.

937 ACUTE *IN VIVO* EXPOSURE TO THE MAMMALIAN IMMUNOTOXICANT, BENZO[A]PYRENE ALTERS IMMUNE RESPONSES OF FISH.

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Increasing public concerns regarding the use of mammals in toxicological studies have led researchers to seek "alternative models" to investigate the biological effects of xenobiotics. Due to its small size and adaptability to a laboratory environment, the Japanese medaka (Oryzias latipes) lends itself well to such studies. Although medaka are used routinely for carcinogenicity testing, little emphasis has been placed upon this species for assessing the immunotoxic effects of chemicals. Given the known effects of benzo(a)pyrene (BP) on the immune system of mammals, studies were conducted using medaka to examine the effects and cellular mechanisms by which exposure to BP might alter the immune status of this laboratory fish model. A single IP injection of BP at doses ranging between 2 and 600μg/g BW had no effect upon medaka survival for up to 7d post-injection. Forty-eight hr after injection of either BP or the vehicle control, fish were sacrificed, and appropriate organs/cells used to assess effects upon: lymphocyte proliferation, oxyradical production, antibody forming cell (AFC) numbers, thymic cellularity and apoptosis, and CYP1A protein level/activity. Results demonstrated that a single, relatively low dose of BP (2μg/g BW) suppressed stimulated T- and B-lymphocyte proliferation and kidney phagocyte intracellular superoxide production, in the absence of increased levels of hepatic CYP1A expression/activity. At higher BP concentrations, suppressive effects were also observed upon AFC numbers and kidney phagocyte extracellular superoxide and hydrogen peroxide production. Thus, similar to that observed in mammalian species, the immune responses of medaka appear sensitive to the immunotoxic effects of BP exposure. The observation that both mammalian and piscine models respond similarly to BP helps to solidify the applicability of the medaka model for use in immunotoxicological research. (Research supported by U.S. Army Augmentation Award.)

938 NON-GENOTOXIC CARCINOGENS (NGC) CAUSE MUTATIONS *IN VIVO* BY OXIDATIVE STRESS.

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The mechanism by which non-genotoxic carcinogens such as oxazepam (OX), phenobarbital (PHE) or Wyeth 14,643 (WY) cause hepatocarcinogenesis is unknown. These compounds do not form DNA adducts, are weak mutagens in bacterial systems and weak clastogens in mammalian cells. One mechanism that has been proposed for tumorigenesis is replication of cells with oxidative lesions. Since chronic feeding of PHE, OX and WY induce specific cytochrome P450's and cause hepatomegaly, we hypothesize that increased oxidative stress could result from enhanced production of superoxide radicals following induction of these enzymes and the resulting lesions could be fixed during hyperplasia. To investigate the role of oxidative stress *in vivo*, Big Blue® transgenic mice were fed carcinogenic doses of OX, PHE or WY for six months. The mutant frequency of the lacI transgene was determined in the liver of the mice and a representative sample of mutants was sequenced. After clonal correction, the MF of lacI in the treated groups receiving OX and WY was significantly increased ($p<0.05$) compared to the control. An intriguing finding was the observation that the mutation spectra recovered from the three treated groups were virtually identical to one another but significantly different ($p<0.02$-0.008) from that of a historic control spectrum. A 2-5-fold increase in the mutation frequency of transversions at guanines, (both G:C>T:A and G:C>C:G) was observed in all the spectra. Although hypertrophy was evident after six months of feeding, clonal expansion of mutations was less than 10% in all the spectra. Our findings suggest that the lesions were caused by oxidative stress and that most of the mutations were unique and did not arise from clonal expansion. (Supported by NIH CA72534.)

939 A ROLE FOR OXIDATIVE STRESS IN THE PROMOTION OF INITIATED CELLS.

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During the process of cancer formation, normal cells appear to exert some control on the growth and/or apoptotic death of preneoplastic initiated cells. The selective clonal growth of initiated cells can occur following stimulatory pressures from endogenous or exogenous agents. This results in an increase in the growth (or decreased death) in the initiated cells compared to surrounding normal cells. This response of initiated cells appears to be an epigenetic process (tumor promotion). In the present study, the promotion effect of oxidative stress on 'initiated' Syrian Hamster Embryo (SHE) cells was investigated. A cell line from a morphological transformed clone (via benzo(a)pyrene treatment) of SHE cells was selected as the 'initiated' cells. Some of the cells were transfected with pEYFP-mito plasmid to develop a cell line with green fluorescence protein (GFP). The cells with GFP were co-cultured with normal SHE cells. The growth of 'initiated' cells was studied following the treatment of t-butyl hydroperoxide (tBH). Without treatment, the growth of the 'initiated' cells was slower than normal SHE cells and appeared to be influenced by the number and proliferation status of the surrounding normal cells. Low concentrations of tBH (0.05-0.1 μM) increased the clonal growth of the 'initiated' cells. The 'initiated' cells were found to be more resistant than normal cells to the toxicity of tBH treatment. This resistance in 'initiated' cells could be explained by increased cellular defenses in the cells. Therefore, the growth of 'initiated' cells following tBH treatment could result from relative growth inhibition of surrounding normal cells. Interestingly, Tea antioxidants (EGCG and tea pigments) suppressed the growth of 'initiated' cells, but had no effect on normal cells. The results suggested that oxidative stress functions during the promotion stage of the cancer process by selectively inducing the growth of initiated cells.

940 ROLE OF ACRYLONITRILE METABOLISM IN THE INDUCTION OF OXIDATIVE STRESS AND MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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Acrylonitrile (ACN), a chemical used in the manufacture of plastics, rubber and acrylic fibers, produces tumors of the brain in rats following chronic oral exposure. The mechanism(s) for ACN carcinogenicity appears to involve the induction of oxidative damage. ACN is detoxified primarily through conjugation with GSH, while oxidative metabolism by cytochrome P450 produces cyanoethylene oxide (CEO), a reactive epoxide metabolite. CEO can be further metabolized to cyanide (CN). In the present study, SHE cell morphological transformation (MT), an assay system that has been shown to be predictive of carcinogenicity *in vivo*, was utilized to further examine ACN metabolism and oxidative stress induction in the transformation process. ACN treatment produced MT in a dose-dependent manner in SHE cells at 50 and 75 μg/ml that was accompanied by increased hydroxyl radical and 8-hydroxydeoxyguonosine (8OHdG) formation after 1 and 3 days of treatment. Similar increases in hydroxyl radical and 8OHdG production were observed following treatment with 500 μM CN. Additionally, the MT induced by CN was blocked by addition of the antioxidant vitamin E. Modulation of GSH by chemical depletion with buthionine sulfoxamine enhanced and co-incubation with the sulfhydryl donor N-acetylcysteine protected ACN-induced MT. These results indicate that ACN induces oxidative stress during metabolism that appears to contribute to SHE cell transformation and further substantiates a role for oxidative stress in the induction of glial cell tumors in the rat.

941 MECHANISMS FOR THE INDUCTION OF OXIDATIVE STRESS IN SYRIAN HAMSTER EMBRYO (SHE) CELLS BY ACRYLONITRILE.

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Chronic administration of acrylonitrile (ACN) to rats resulted in an increased incidence of neoplasms of the brain. Recent evidence from our group and others has suggested that acrylonitrile induces oxidative stress in rat brain and cultured rat glial cells. Additionally, our group has shown that acrylonitrile induces morphological transformation concomitant with an increase in the

formation of oxidized DNA in Syrian Hamster Embryo (SHE) cells in a dose dependent manner. The mechanism of the induction of oxidative stress in SHE and glial cells remains unresolved. The present study examined the effect of acrylonitrile on both enzymatic and nonenzymatic antioxidants in SHE cells. SHE cells were treated with sublethal doses of acrylonitrile (0, 25, 50, and 75μg/ml) for 1hr, 4hrs, 1 day, 2 days, and 7 days. Acrylonitrile (50μg/ml and 75μg/ml) increased the amount of reactive oxygen species in SHE cells. GSH was depleted and catalase and superoxide dismutase was significantly decreased in SHE cells after 4 hours of treatment. The inhibition of this antioxidants was temporal, returning to control values after 1 day of treatment. In the absence of metabolic enzymes acrylonitrile had no inhibitory effect on antioxidant enzymes. In additional experiments, cyanide (a possible metabolite of acrylonitrile) produced a similar inhibition of catalase and superoxide dismutase in the SHE cells. These studies suggest that the induction of oxidative stress by acrylonitrile involves a temporal decrease in antioxidants in the cells possibly through the metabolism of acrylonitrile to cyanide.

942 MODULATION OF FOCAL AND NON-FOCAL HEPATOCYTE DNA SYNTHESIS IN 2-BUTOXYETHANOL TREATED MICE.

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2-Butoxyethanol (2-BE) has been reported to induce liver neoplasia in B6C3F1 liver following chronic treatment. While the mechanism for the observed tumor induction has not been defined, 2-BE induces hemolysis and subsequent hemosiderin deposition in the liver. This has led to a hypothesis that the resulting iron deposition in the liver contributes (via an oxidative stress mechanism) to the formation of the liver tumors. The current study examined the effects of 2-BE treatment on the induction of cell growth in focal lesions and non-focal hepatocytes. Male B6C3F1 mice that contained focal lesions (induced by DEN) were treated with 2-BE (400mg/kg/day; gavage) for 7 and 30 days. Additional mice received 2-BE plus Vitamin E-supplemented diet (450mg/kg diet). Controls included vitamin E supplementation only and untreated mice. Seven days prior to sampling, mice received an osmotic minipump containing BrdU. At sampling livers were harvested and examined for DNA synthesis in focal and non-focal hepatocytes. 2-BE treatment resulted in a decreased hematocrit of 15% in both 2-BE only and 2-BE plus Vitamin E supplementation. A 2 - 4 fold increase in DNA synthesis was seen in non-focal hepatocytes after 7 and 30 days. Supplementation with Vitamin E in the diet ablated this 2-BE induced increase. While no increase in focal labeling index was seen after 7 days of treatment, a 2-BE related increase in DNA synthesis was seen after 30 days of treatment. This increase in focal lesion DNA synthesis was prevented by vitamin E supplementation. These results suggest that the 2-BE produces its carcinogenic effect through indirect means (via iron deposition and resulting oxidative stress).

943 EFFECTS OF 2-BUTOXYETHANOL, 2-BUTOXYACETIC ACID, AND FERROUS SULFATE ON THE MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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The compound 2-butoxyethanol (2-BE), a component in many industrial and consumer cleaning products, has been recently reported to induce an increase in liver tumors in B6C3F1 mice following chronic treatment. Previous studies by our group showed that 2-BE *in vivo* treatment induced oxidative stress in the mouse liver. This effect appears to be through the induction of hemolysis and the resulting increase in iron deposition rather than via the compound or a metabolite of the compound directly. Based on this previous study, we questioned whether 2-BE, its primary metabolite (2-BAA) or iron (ferrous sulfate) was responsible for the observed hepatic carcinogenicity. To help address this issue we used the Syrian Hamster Embryo (SHE) cell morphological transformation assay, an *in vitro* assay with a good correlation to the chronic bioassay results for screening carcinogenic agents. SHE cell was treated with 2-BE (1 - 20 mM), 2-BAA (0.5 - 20mM), and ferrous sulfate (0.5 - 50 μg/ml) for 7 days. The treatments of 2-BE and 2-BAA did not induce any significant morphological transformation in the SHE cells. In contrast, treatment with ferrous sulfate (2.5 and 5 μg/ml) induced a significant increase in SHE cell morphological transformation. Co-treatment of ferrous sulfate with alpha tocopherol or EGCG (green tea antioxidant) resulted in a prevention of ferrous sulfate induction of morphological transformation. These data support the hypothesis that the observed induction of hepatic tumors by 2-BE is through an indirect mechanism via iron deposition which results in oxidative stress in the mouse liver.

944 ROLE OF OXYGEN FREE RADICALS IN PCB AND DIOXIN CARCINOGENESIS.

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In order to explore a role for reactive oxygen species in the carcinogenesis of dioxin-like agents, female Sprague-Dawley rats were exposed to PCB126, PCB153, dioxin (TCDD), and an environmentally relevant mixture of PCBs 126 and 153. Liver microsomes were analyzed for estrogen catechol metabolites by gas chromatography-electron capture and cytochrome P450 1A1 enzyme induction by 7-ethoxyresorufin-O-deethylase (EROD) assay. Experimental groups received control, low, or high doses of each of the chemical groups in corn oil gavage and were sacrificed at 13 or 30 weeks. EROD activity increased in dose-responsive fashion in rats treated with Ah-receptor binding TCDD and PCB126. No changes in CYP1A activities were observed with the non-Ah-receptor binding PCB153. The PCB153/126 mixture produced an unexpected greater-than-additive elevation in EROD activity. Analyses of catechol estrogen metabolites demonstrated a dose-responsive decrease in 2/4-hydroxyestradiol ratio after TCDD and PCB126 exposures (2.2 and 2.4-fold, respectively), while PCB153 exposures resulted in an increased ratio (1.5-fold). PCB126/153 exposures resulted in a 1.7-fold decline in the 2/4-hydroxyestradiol ratio. 4-Hydroxylated catechol estrogens may be metabolized via cytochrome P450 reactions to quinones that undergo redox cycling reactions, producing oxygen radical species that can cause oxidative DNA damage. When combined with the higher levels of endogenous estrogen in females, this may provide an explanation of gender differences seen in carcinogenesis. These results suggest that carcinogenic effects of complex mixtures of chlorinated hydrocarbons are difficult to predict based on their separate effects on P450 enzyme induction and subsequent estrogen metabolism. (This work is supported by NIEHS Grant #E5-97-003.)

945 ANTICARCINOGENIC ROLE OF METALLOTHIONEIN IN THE DMBA/TPA-CAUSED SKIN CARCINOGENESIS.

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Metallothioneins (MTs) are a family of cysteine-rich proteins, inducible by endo- and exogenous stimuli, such as heavy metals and cytokines. They are evolutionally highly conserved, but the full physiological nature of these proteins are largely unknown. As a possible physiological role, MTs are thought to scavenge active oxygen species, mainly hydroxyl radicals *in vivo* and *in vitro*. Utilizing MT-null mice, whose MT-I and -II genes are knocked out by homologous recombination, we studied susceptibility of MT-null mice in the DMBA (7,12-dimethylbenz[a]anthracene) /TPA (12-O-teradecanoylphorbol-13-acetae)-caused two stage carcinogenesis model. Mice on a 129/Ola x C57BL/6J background were subjected to a single topical application of carcinogen (50 or 100 μg DMBA/mouse) and, 1 week later, to promotion with 10 μg TPA twice a week for 20 weeks. At week 20, 90-100% of MT-null mice developed papillomas in the skin whereas only 10-40% of wild-type mice had the tumor. No tumor was observed in wild-type or MT-null mice receiving TPA alone. These results indicate that MT-null mice are extremely susceptible to the two-stage carcinogenesis caused by DMBA/TPA. Investigating mutations, by PCR-RFLP and PCR-SSCP methods, in c-Ha-ras, c-Ki-ras and c-N-ras genes in the papilloma tissue of the wild-type and MT-null mice, we detected transversion of A182 to T of the codon 61 of c-Ha-ras, showing that MT is preventing the occurrence of ras mutation that is activated by DMBA/TPA. We here show that MT, constitutively expressed in the skin, is acting as an anticarcinogenic factor in the chemical carcinogenesis, which is a novel physiological function of MT.

946 EFFECT OF DIETARY VITAMIN E ON OTHER CELLULAR ANTIOXIDANT DEFENSE SYSTEMS IN PHENOBARBITAL-TREATED RATS.

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The purpose of this study was to determine the effect of vitamin E on other cellular defense systems when rats are treated with phenobarbital, a liver

tumor promoter. Upon phenobarbital treatment in rodents, hyperplasia and increased cytochrome P-450 2B1/2B2 activities occur. Since hydrogen peroxide may be released as a by-product of cytochrome P-450, a reactive oxygen environment may be important in tumor promotion by phenobarbital. The oxidative stress-activated transcription factor nuclear factor-κB (NF-κB) is activated in phenobarbital-treated rats, but when dietary vitamin E is increased, NF-κB activation is decreased. In this study thirty-nine male Sprague-Dawley rats were fed a purified diet with varying levels of dietary vitamin E (10, 50, 250 ppm of alpha-tocopherol acetate). After 28 days, 0.05% phenobarbital was added to the diets of half the rats for 10 days. Liver homogenates were made and used to determine catalase activity, and liver cytosol was used to assay total glutathione, glutathione peroxidase, glutathione reductase, and glutathione transferase. The results demonstrate that higher dietary vitamin E levels increase total glutathione and glutathione peroxidase activity and slightly elevate catalase and glutathione transferase activities in both phenobarbital treated and control animals. This study shows that vitamin E does not act solely to scavenge free radicals, but also influences other cellular antioxidant defenses. (Supported by CA74147 and CA01688.)

947 ANALYSIS OF RAT BONE MARROW BY FLOW CYTOMETRY FOLLOWING IN VIVO EXPOSURE TO CYCLOHEXANONE OXIME (CHO) OR DAUNOMYCIN HCL (DAUN).

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The purpose of these studies was to evaluate bone marrow from male CD rats following exposure to known hematotoxins using flow cytometry and a monoclonal antibody to the cell surface antigen CD71. Male rats, 12 per dose group, were treated with either CHO at 300 mg/kg for 10 days or DAUN at 10 mg/kg for 1 day. Corresponding control groups received equivalent volumes of the appropriate vehicle. Half of the animals from each treatment group were sacrificed at the end of the dosing schedule while the remaining animals were sacrificed after a recovery period. Hematology analyses were completed prior to the onset of each study and on the day of necropsy. Marrow was isolated from the tibia of each rat, stained with R-phycoerythrin-conjugated mouse anti-rat CD71 (transferrin receptor on proliferating cells) monoclonal antibody and then analyzed on the flow cytometer for myeloid:erythroid (M:E) ratios. Flow cytometric determinations of myeloid and erythroid population percentages and M:E ratios from untreated rats were confirmed by microscopic examination of marrow cytospins and flow cell sorts. M:E ratios for control animals determined by flow cytometry were not significantly different between the two studies (1.83 vs. 1.89). CHO treated rats showed a significant ($p < 0.01$) decrease in M:E ratios (0.96 - CHO vs. 1.48 - control) at day 11 as a result of an increase in erythroid cells. M:E ratios were significantly increased ($p < 0.05$) for rats treated with DAUN at day 3 (5.07 - DAUN vs. 1.70 - control) and corresponded to generalized depletion of all marrow cell lines with greater loss occurring in erythroid cells. At the end of the recovery period, M:E ratios of CHO and DAUN rats were similar to controls. Hematological values corroborated changes in marrow myeloid and erythroid populations evaluated by this flow cytometric technique. Using flow cytometry and a monoclonal antibody to the cell surface antigen CD71, this study demonstrated the reversible selective toxicity on myeloid and erythroid populations of the marrow following *in vivo* exposure to CHO or DAUN.

948 A PROPOSED APPROACH FOR THE VALIDATION OF A RADIOTELEMETRIC DEVICE TO MEASURE CARDIOVASCULAR PARAMETERS IN THE RAT.

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The measurement of various cardiovascular parameters in laboratory animals can play a very important role in pharmacology and toxicology evaluations. The data from such studies are commonly used as predictors of the safety and efficacy of new chemical entities in humans. Parameters of interest typically include blood pressure, heart rate and an electrocardiogram. Historically, this type of data has been obtained from chemically restrained animals. However, anesthesia affects normal cardiovascular physiology and data from conscious and unrestrained animals is more desirable and relevant. Radiotelemetry provides a method to obtain measurements of various cardiovascular parameters from unrestrained and conscious animals. Before such instrumentation can be used though, a series of procedures must be completed ensuring that the instrument conforms to the defined needs of the user and that the designed output meets the designed input. Testing is conducted under very specifically defined conditions simulating actual use. The validation process is composed of two arms: electronic and physiologic validation. What is reported here is the detailed step-wise electronic and physiologic validation of a Data Sciences International (DSI) remote monitoring system. Six to eight week old, male, Sprague Dawley rats weighing 300 - 350 gm apiece were surgically implanted with type TL11M2-C50 (DSI) transducers. Standards were selected to test the ability of the system to clearly define changes in blood pressure and other cardiovascular parameters in a measurable and interpretable fashion. The agents used were: hydralazine (H; 5 mg/kg), a vasodilator; propranolol (P; 30 mg/kg), a β-blocker, isoproterenol (I; 0.1 mg/kg), a β-agonist and epinephrine (E; 0.25 - 3.0 μg/kg), an α,β-agonist. The vehicle was 0.25% methylcellulose (M). Animals were dosed with M on days 1, 8 & 15; H on days 2 & 5, P on days 9 & 12; I on days 16 & 19 and I + E on days 22 & 23. Days 3, 4, 10, 11, 17 & 18 were washout days. The complete package of electronic and physiologic data represent a regulatorily defensible validation package.

949 RATIOMETRIC ANALYSIS OF NONYL ACRIDINE ORANGE (NAO) FLUORESCENCE IS A SENSITIVE INDEX OF OXIDATIVE LOSS OF MITOCHONDRIAL CARDIOLIPIN.

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Nonyl acridine orange (NAO) is a lipophilic acridine orange derivative with high affinity for cardiolipin, the chief lipid comprising the mitochondrial inner membrane. Because binding of NAO to cardiolipin is membrane potential insensitive, it has been used to measure total cellular mitochondrial mass. NAO fluorescence is usually measured in the green as unbound NAO in solution has an excitation and emission maximum of 495 nm and 519 nm respectively. Petit[1] *et al* have shown, however, that NAO binds cardiolipin with a stoichiometry of 2 dye molecules to 1 cardiolipin, dependent on the diacidic structure of cardiolipin. When bound to cardiolipin in this 2:1 ratio, a red NAO emission fluorescence is detected, presumably from excimer formation. Red NAO fluorescence is more specific for cardiolipin than the green fluorescence. Green NAO fluorescence is lost as a consequence of mitochondrial oxidative stress produced by menadione[2]. We have recently demonstrated that AML12 hepatocytes exposed to actinomycin D and TNF undergo apoptosis which involves oxidative stress, including the loss of cardiolipin (measured as red fluorescence). Coincubation with α-lipoic acid, a potent antioxidant, blocks the loss of NAO fluorescence[3]. We show here that this loss of NAO staining cannot be attributed to a change in cellular pH. Furthermore, we report a method for analyzing NAO fluorescence as the ratio of red:green fluorescence. Cells undergoing oxidative stress-induced loss of cardiolipin can be identified by the fluorescence shift from red to green. This ratiometric approach is useful for both flow cytometric and confocal microscopic analyses and has several distinct advantages over single color fluorescence techniques. Chief among these benefits is that ratiometric data is independent of cell size and cellular variability in dye loading. In addition, for confocal microscopy studies, ratiometric analyses allow one to perform real-time identification of cells undergoing oxidative stress, and to assess heterogeneity among mitochondrial cardiolipin oxidation within individual cells. (Supported by NIH grants ES04696, ES07033, AG01571 to TK and CA 74131 to NF.) [1] Petit JM, *et al* Eur J Biochem 220:871-9. [2] Polyak K, *et al* Nature 389:300-5. [3] Pierce RH, Chaisson M, Stevenson A, Poot M, Campbell J, Franklin CC, Kavanagh T and Fausto N, Glutathione levels regulate TNF-mediated apoptosis in murine hepatocytes. In preparation. The views expressed in this article are those of the author(s) and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the US Government.

950 RELATING MELANIN DEGRADATION PRODUCTS TO ACTUAL MELANIN CONTENT: APPLICATION TO HUMAN HAIR.

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Numerous studies have confirmed that melanin pigments play an important role in the incorporation of drugs into hair. This may lead to a bias in the interpretation of hair testing results. To develop non-biased testing procedures, the role of specific subtypes of melanin in drug incorporation should be characterized. Methods for characterizing and quantitating melanin subtypes from the two types of melanin found in hair-eumelanin and pheomelanin, have been established. 5,6-Dihydroxyindole (DHI) and 5,6-Dihydroxyindole-2-carboxylic acid (DHICA) make up the majority of the eumelanin polymer while benzothiazene units derived from 2-Cysteinyl-S-DOPA (2-CysDOPA)

and 5-Cysteinyl-S-DOPA (5-CysDOPA) comprise the majority of the pheomelanin polymer. To ascertain the true nature of a melanin pigment, the contribution from each of the above monomer units must be determined. Procedures that allow for quantitation of eumelanin and pheomelanin subtypes by HPLC methods have been developed. Our results show that: 1) Pyrrole-2,3-dicarboxylic acid (PDCA) and Pyrrole-2,3,5-tricaboxylic acid (PTCA), markers for DHI and DHICA units, respectively, are produced in 0.37% and 4.5% yields, respectively, when melanins are subjected to alkaline hydrogen peroxide degradation, 2) 3-Aminotyrosine (3AT) and 4-Amino-3-hydroxyphenylalanine (AHP), markers for 2-CysDOPA and 5-CysDOPA, respectively, are produced in 15% and 22% yield, respectively, when subjected to hydriodic acid hydrolysis, and 3) PDCA and PTCA are not specific markers of eumelanin, and that PTCA can be produced from DHI derived units. In spite of these facts, production of degradation products from unexpected sources is consistent and thus can be accounted for. The degradation products PDCA, PTCA, 3AT, and AHP are used to quantitate the amounts of eumelanin and pheomelanin subtypes in hair. These assays will allow deeper investigation into the relationship between melanin composition and drug incorporation into hair. (Supported by NIH grant DA07820.)

951 THE USE OF FLUORO-JADE B AND BLACK-GOLD TO DETECT NEURONAL AND MYELIN DEGENERATION IN THE RAT FOREBRAIN.

K. J. Hopkins and L. C. Schmued. *National Center for Toxicological Research, Jefferson, AR.* Sponsor: W. Slikker, Jr.

Neurological disease and exposure to certain chemical compounds can result in neuronal degeneration and myelin changes in the central nervous system. Two novel compounds, Fluoro-Jade B (FJ B) and Black-Gold (BG), have been developed to histochemically identify such brain lesions. The first compound, FJ B, is the second generation green fluorescent marker specific for degenerating neurons. It is sensitive, simpler and more reliable than traditional supressed silver techniques. Exposure to excitotoxins, like kainic acid, or the electron transport inhibitor 3-nitropropionic acid, results in extensive, neuronal labeling with FJ B, while treatment with ibogaine, MK801, MPTP, d-fenfluramine, or methamphetamine results in a more restricted pattern of cell staining. FJ B has also been used to detect amyloid plaques in human brain tissue and astrocytosis following semi-chronic chemical treatments. The second compound, Black-Gold, is a haloaurophosphate complex used to visualize normal and pathological myelin. Exposure to compounds such as kainic acid or 3-nitropropionic acid results in myelin damage characterized by fragmentation and "bead-like" varicosities. The use of these dyes individually, or in combination with other specialized stains, allows for a rapid, reliable evaluation of disease and chemically induced brain damage. (Supported by FDA protocol #7013.01.)

952 DEVELOPMENT OF AN *IN VIVO* METHOD TO ASSESS SINGLE FIBER ELECTROMYOGRAPHIC JITTER IN JUVENILE AND ADULT RATS.

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A novel *in vivo* technique is presented that uses single fiber electromyography (SFEMG) to measure muscle action potentials elicited by electrical stimulation of the sciatic nerve in rats. The variability in latency from the stimulus to a point on the rising phase of the muscle action potential (MAP) at a selected percent of the MAP amplitude is called the neuromuscular jitter. Although the clinical usefulness of jitter measurement as an electrodiagnostic tool for the study of neuromuscular transmission disorders has been well established, it has only recently been used as an early endpoint to assess the effects of toxicants. In anesthetized animals, stimulating needle electrodes delivered a 50 μsec cathodal pulse near the sciatic nerve. The stimulus intensity was adjusted to produce small twitches in the rat's toes and a MAP greater than -1 mV. The SFEMG recording electrode was inserted into the subject's gastrocnemius muscle. The SFEMG jitter was computed as the mean consecutive difference (MCD) of latencies from a series of 50 consecutive discharges. The variability of the MCD was assessed by recording from 3 different SFEMG electrode placements within the gastrocnemius muscle of each rat and calculating the MCD at 3 different percent amplitude points (10, 25, and 50) of the MAP. There were no significant differences in the jitter from recordings made at the different SFEMG electrode placements within each subject, or at any of the percent amplitude criterion points. Results indicate that using this *in vivo* technique the overall median (and 25-75 percentiles) for SFEMG jitter within the gastrocnemius muscle of juvenile rats was 21.3 μsec (16.7-34.3) and in adult rats was 15.9 μsec (9.0-25.2). These results indicate that direct axonal stimulation and subsequent SFEMG jitter measurements may prove to be a sensitive endpoint for examining xenobiotic effects on neuromuscular transmission. (Funded by the EPA/UNC Toxicology Research Program, Training Agreement CT902908. This abstract does not necessarily reflect US EPA policy.)

953 DEVELOPMENT OF A NEW MECHANICAL IRRITATION TEST.

M. A. Farage. *The Procter and Gamble Company, Cincinnati, OH.* Sponsor: R. S. Nair.

Certain consumer products worn in close proximity to the skin, such as feminine hygiene products and diapers, are sometimes tested for the potential to cause mechanical irritation as part of the overall safety assessment. Historically, this test has been performed using the wrist as the test site. However, a more sensitive test was needed to detect subtle differences in current product candidates. We chose to investigate the behind the knee as an alternative body site. The negative (satin) or the positive (burlap) control materials were covered by either an athletic band on the wrist, or an Ace* band/brace on the knee. Twelve panelists wore the positive control material on one wrist and one knee, and the negative control material on the alternate wrist and knee. The bands were worn 6 hours/day for 4 days. Test sites were scored for irritation on a visual scale twice each day; prior to wrist or knee band application and 30 minutes after removal. The mechanical irritation caused by the positive and negative control materials was significantly different after the first day of wear at both test sites (knee and wrist). However, at the behind the knee these differences were greater, and the effects more prolonged. Based on this initial study we concluded that this protocol, i.e., using the behind the knee as a test site, 4 days of material wear for 6 hours/day, and materials applied using a knee band/brace, is a sensitive test system for evaluating mechanical irritation. Further, satin and burlap are effective negative and positive controls to use in future experiments.

954 EVALUATION OF NMR ANALYSIS OF URINE FOR SCREENING LIVER AND KIDNEY TOXINS.

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The combination of NMR and pattern recognition analysis of urine, "metabanomics", has been successfully used to characterize *in vivo* toxicity. The utility of this technology was assessed by administering Wistar rats (4/dose) single doses of 2 known hepatotoxins, 0.1 and 0.5 ml/kg carbon tetrachloride (CCL4) and 10 and 100 mg/kg α-napthylisothiocyanate (ANIT); 2 known nephrotoxins, 15 and 150 mg/kg 2-bromoethylamine (BEA) and 15 and 150 mg/kg para aminophenol (PAP), and 1 and 5 mg/kg of the diuretic furosemide (FURO). 24-hour urine samples were collected (0°C.) pretest and daily for 4 days postdose. Animals were sacrificed on Day 4 with a control and high dose CCl4 and BEA treated animals maintained for 10 days to assess reversal. Clinical pathology samples were obtained on Days 1, 2 and 4 or Days 1, 4 and 10 (reversal groups). Target organ pathology was evaluated at termination. Standard urinalysis, including urine volume, quantitative protein, creatinine clearance, and electrolyte excretion were also conducted on BEA, PAP and FURO samples. Urine was subjected to 600 MHZ ^{1}H NMR analyses with spectra further evaluated using principal component analyses (PCA). Results indicated that high doses of all toxins produced the anticipated lesions, while the low doses produced minimal if any pathology. PCA data were in agreement with clinical chemical indices of toxicity but were more consistent with regard to onset, duration and reversal of toxicity and potentially more sensitive. Pharmacological doses of FURO had no effect on NMR/PCA. These data suggest that NMR/PCA can serve as a powerful tool for the rapid evaluation of onset, severity and reversal of *in vivo* toxicity using a peripheral sample.

955 EVALUATION OF RESPIRATORY PARAMETERS OBTAINED FROM CONSCIOUS PRIMATES DURING INHALATION EXPOSURE.

P. McDonald and K. McInally. *Inveresk Research, Tranent, United Kingdom.* Sponsor: S. B. Wilson.

During the course of inhalation toxicology experiments it is essential to quantify the volume of aerosol inhaled by the test animal. There is currently limited information in the literature regarding respiratory data for non-human pri-

mates. Published data available tends to be focused on sedated or anaesthetised animals which may not be reflective of the breathing patterns of a fully conscious restrained animal. During the course of a repeat 90 day inhalation toxicity study with a nebulised protein, respiratory frequency, tidal volume, minute volume, expiratory and inspiratory flow times were recorded in 52 cynomolgus monkeys (Macaca fascicularis) using a pneumotachograph and differential pressure transducer incorporated into the face mask dosing system. Animals were fully conscious under light restraint during all procedures. Measurements were obtained from all animals prior to and during inhalation exposure on 5 occasions over the study duration. The results obtained showed a wide degree of variability in both respiratory frequency, tidal volume and minute volume. A significant degree of inter and intra animal variation was noted which was not related to any adverse behavioural events during the dosing procedures. The results indicate that careful appraisal of in situ respiratory measurements are required if the volumetric data is to be used for dosimetry purposes.

956 A STUDY OF THE EFFECTS OF RESTRAINT DURATION FOR NOSE-ONLY INHALATION EXPOSURE ON EMBRYO/FETAL DEVELOPMENT IN RATS.

B. J. Varsho, G. D. Cappon, D. T. Kirkpatrick, D. C. Welter, J. C. Obrecht and T. D. Feikert. *WIL Research Laboratories, Inc., Ashland, OH.*

The current study assessed the effects of various nose-only (N-O) inhalation exposure restraint durations on pregnancy, maternal body weight, food consumption, clinical condition, and embryo/fetal development in Crl:CD®(SD)IGS BR rats. Animals were incrementally acclimatized to restraint tubes for at least 4 days prior to mating. The maximum acclimatization time for each animal matched its designated exposure duration. Following acclimatization, 25 mated female rats per group were exposed to filtered air using N-O exposure techniques for 1, 2, or 6 hours on gestation days (GD) 6 through 17. The control group was exposed to filtered air for 6 hours using whole-body exposure techniques. Acclimatization to N-O restraint had no effects on mating, ovulation, or nidation. N-O restraint from GD 6-17 had no effects on clinical condition or food consumption. Slight, statistically significant reduced mean body weight gains or body weight losses were induced in all N-O groups on the first day of restraint (GD 6). These reductions were sufficiently minor as to create no statistically significant difference in mean body weight throughout the study. The reductions were transient, because much if not all of the differences from the weight gain in the whole-body group was recovered following the last day of restraint (GD 18-20). Net mean body weight (GD 20 weight minus gravid uterine weight) and net mean body weight gains were unaffected by any restraint duration. Gravid uterine weights and fetal viability and weights were comparable across groups. No N-O restraint-related malformations were observed. No significant changes were observed in biological endpoints using these three N-O exposure paradigms; therefore; N-O exposure in a suitable approach for prenatal developmental toxicity studies.

957 USE OF JUVENILE DOGS IN PRECLINICAL INHALATION TOXICITY TESTING.

R. Salame, A. Viau and C. M. Banks. *ClinTrials BioResearch Ltd., Senneville, Quebec, Canada.* Sponsor: G. W. Lulham.

The dog is a standard large animal species used in inhalation toxicity studies. Recent changes in the approach taken in toxicity testing of drugs intended for pediatric population necessitate the use of an animal model at a correspondingly young age. The feasibility of using young dogs in inhalation toxicity studies and the environmental modifications required were investigated in this experiment. Animals were received at approximately 2 to 3 months of age. A period of acclimation to the laboratory environment was allowed before commencement of acclimation to the exposure apparatus. Modifications to the housing units, environmental conditions, watering and feeding regimens as well as additional exercise periods were required. The inhalation exposure mask and restraint systems were modified to accommodate the smaller size of the animals and to ensure efficient containment of the atmosphere. In conclusion, with minimal modifications to the current husbandry and exposure procedures, animals as young as 4 months old can be utilized on inhalation toxicity studies.

958 ELECTRORETINOGRAM (ERG) RECORDING IN THE BEAGLE DOG.

P. Mansell, M. Vezina and G. Washer. *ClinTrials BioResearch Ltd., Senneville, Quebec, Canada.* Sponsor: G. W. Lulham.

Electroretinography is becoming increasingly recognised as an aid in assessing retinal changes in toxicology studies in conjunction with ophthalmology and histopathology. Background ERG data for beagle dogs was obtained using an EPIC 2000 ERG recorder, Grass flash stimulus and Ganzfeld dome. Under ketamine and xylazine anesthesia, following dark adaptation, ERG's were performed to verify the range of scotopic response. The ERG protocol consisted of a series of tests to assess scotopic response utilising neutral density wratten filters to regulate light transmission. Following the filter sequence, the dogs were adapted to background light and exposed white flicker light at 31 Hz to assess photopic cone response. Parameters recorded were a-wave implicit time and amplitude and b-wave implicit time and amplitude for the single flash ERG, and b-wave amplitude and latency for the 31 Hz white flicker. Small differences were observed between left and right eyes in the mean values although occasional, larger variations were noted in certain individual animals. A-wave amplitude and implict time for descending light log units varied between 0 and -65 uV (sd 0.1-23) and 23 and 16 ms (sd 7.3-1.5) respectively. B-wave amplitude and implict time varied between 0 and 165 uV (sd 0.01-56) and 73 and 50 ms (sd 1.8-9.5) respectively. B-wave amplitude and latency for the 31 Hz white flicker were 25 uV (sd 12.7) and 27 ms (sd 0.9) respectively. It is concluded that the reproducibility of ERG data in the beagle dog using this GLP validated method is acceptable and suitable for use as an aid in the overall evaluation of change in the retina.

959 DETERMINATION OF TAMOXIFEN AND METABOLITES IN MOUSE FETAL TISSUE USING CAPILLARY ELECTROPHORESIS (CE) CHROMATOGRAPHY.

J. M. Sanders, K. L. Alford, P. J. Schupp, R. R. Newbold and M. L. Cunningham. *NIEHS, Research Triangle Park, NC.*

Tamoxifen (TAM), an antiestrogen, has been approved for use by women at risk for developing hormone-dependent breast cancer. Although transplacental exposure of TAM has been linked to reproductive tract toxicity in female offspring of CD-1 mice, there is little or no data describing potential TAM-induced fetal toxicity to women who may become pregnant while receiving prophylactic TAM treatment. In support of the National Toxicology Program's characterization of reproductive and developmental effects of TAM, the present work describes a CE-based analytical technique used for detection of TAM and two of its major metabolites, N-desmethyltamoxifen (DMT), and 4-hydroxytamoxifen (4-HT) in CD-1 mouse fetal tissue. Hexane/isoamyl alcohol (98:2, v/v) was used to extract TAM-derived material from CD-1 mouse fetuses 2-12 hr following administration of 100 mg TAM/kg to dams on gestation day 16. The presence of TAM, DMT, and 4-HT was confirmed in fetal extracts by CE chromatography utilizing a 57 cm x 50 μm capillary with a nonaqueous buffer system of 1:1 (v/v) methanol/acetonitrile containing 50 mM ammonium acetate and 1.5% acetic acid. Voltage and temperature were optimized at 15 kV and 40°C, respectively. The limit of detection by UV absorption of TAM was approximately 675 attomoles at a signal to noise ratio of 2:1. This work demonstrates transplacental transport of TAM following exposure to CD-1 mice and a sensitive analytical technique for detecting low concentrations of TAM and similar compounds in biological tissues.

960 THE YEAST DEL ASSAY DETECTS CARCINOGENS.

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We have constructed and used DEL assays, which quantify potentially carcinogenic DNA deletion events in yeast, human cells and *in vivo* in mice. DEL events in all three assay formats are induced by a wide variety of carcinogens, many of which, such as benzene and PCBs, are negative in most other short-term genotoxicity tests. The yeast DEL assay also differentiates correctly between problematic carcinogen/non-carcinogen structural analogs. Of 62 chemicals of known carcinogenicity tested, the yeast DEL assay correctly identifies 85%. In comparison, the widely used Salmonella reverse mutation assay correctly classifies only 33%. The yeast DEL assay is now under development as a fully 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 fur-

ther development have allowed the detection of two-fold differences in DEL frequency, and significant, dose-dependent effects of carcinogens on deletion frequency have been detected. Further assay development (including the addition of cytotoxicity compensation) and full robotic automation is underway. Ultimately we will validate the automated assay with 500 chemicals of known carcinogenic activity. Automated, higher throughput and highly predictive genotoxicity screens like the DEL assay will assist in the identification of undesirable compound characteristics earlier in the drug discovery process, improving pharmaceutical companies' ability to make fast, scientifically-based decisions on early drug leads. More widespread adoption of a validated DEL assay may reduce the number of animals used in carcinogenicity testing of novel pharmaceuticals, and its high predictive value may reduce the failure rate of promising drug candidates due to unanticipated carcinogenic potential.

961 A YEAST ASSAY FOR CHROMOSOME GAIN.

N. G. Howlett and R. H. Schiestl. *Harvard School of Public Health, Boston, MA.*

Aneuploidy is defined as a deviation in chromosome number from an exact multiple of the haploid state. Aneuploidy in germ cells is a significant contributor to genetic disease in man, e.g., Down's and Klinefelter's syndrome. Furthermore, an increased susceptibility to neoplasia has been observed with many congenital aneuploidies, an observation consistent with the increased levels of genomic instability characteristic of tumor cells. Currently there is a notable absence of information on the mechanism and genetic control of both spontaneous and induced aneuploidy. The yeast DEL assay selects for deletion events removing the LEU2 gene that is flanked by his3 repeats. Cells positive for His+ and Leu+ occur after duplication of the chromosome and deletion on one of the duplicated chromosomes. Spontaneous frequencies of chromosomal gain were approximately 2.3 x 10-3 and 1.6 x 10-2 for the haploid strain RSY6 and the diploid strain RS112, respectively. We have also assessed the ability of the yeast DEL assay to accurately detect the known aneugens benomyl, MMS, EMS, and actinomycin D. All chemicals increased the frequency of chromosome gain in a dose-dependent manner. For example the alkylating agent EMS, and actinomycin D induced aneuploidy approximately 8.8-fold and 5.2-fold in RSY6, respectively. FACS-analysis of propidium iodide-stained spontaneous and methotrexate-induced HIS+LEU+ revertants confirmed deviation in chromosome number. Most interestingly, 100% of haploid HIS+LEU+ revertants were hyperdiploid, while 90% of diploid HIS+LEU+ revertants were indeed aneuploid, indicating different mechanisms of chromosomal non-disjunction in the haploid and diploid strains. In addition, data on spontaneous levels of chromosome gain will be presented for yeast strains deficient in various components of DNA repair pathways. Thus the yeast DEL assay can be employed to detect chromosome gain, and will provide valuable information on the genetic and/or epigenetic mechanisms underlying chromosome gain.

962 DEVELOPMENT OF A WHOLE BODY PERFUSION FIXATION SYSTEM FOR CYNOMOLGUS MONKEYS.

A. C. Gibbs and J. Wilkins. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: J. Norris.

Whole body perfusion fixation avoids many of the artefactual changes observed microscopically in a variety of tissues following immersion fixation. Whole body perfusion is the preferred method of fixation for toxicology studies that require a detailed evaluation of, amongst other tissues, the central or peripheral nervous system. At Covance, we developed a system for whole body perfusion of primates in response to a Sponsor request for the technique to be performed on a chronic toxicity study where evidence of peripheral neuronal budding was required. The practical considerations involved in the perfusion of a large animal are numerous; the relatively large size of cynomolgus monkeys means that adequate perfusion of the extremities without causing tissue damage due to the perfusion pressure required is a difficult balancing act. The method used involves the introduction of an initial perfusate of heparinised saline containing sodium nitrite via the left ventricle; the right atrium is incised to allow the replacement of the blood in the circulatory system with the initial perfusate. Once the blood is replaced, the animal is perfused with 10% neutral buffered formalin until full fixation of all tissues is achieved. The system developed is very successful and excellent preservation of the cellular architecture was achieved in all tissues.

963 CONTINUOUS SUBCUTANEOUS INFUSION IN THE CYNOMOLGUS MONKEY.

S. N. Groom and G. Washer. *ClinTrials BioResearch Ltd., Senneville, Quebec, Canada.* Sponsor: G. W. Lulham.

The subcutaneous route is an important method of drug administration in humans. Continuous subcutaneous infusion was developed at CBTR to provide an alternative to subcutaneous injection. The feasibility of drug delivery by continuous subcutaneous infusion in the Cynomolgus monkey was investigated over a period of 28 days. Six Cynomolgus monkeys were surgically prepared with an indwelling subcutaneous lumbar catheter, fitted with CTBR's standard intravenous infusion jacket and tether assembly and infused subcutaneously with 0.9% Sodium Chloride, U.S.P via an in-line 0.22mm filter for 24 hours/day for 28 consecutive days. Infusion rates were selected to deliver daily dose volumes of 1 or 6 mL/kg/day (0.04 or 0.25 mL/kg/h). Dosimetry data for the 28-day period demonstrated all animals received within ± 10% of the nominal volume. Continuous 24 hour/day subcutaneous infusion of 0.9% Sodium Chloride, U.S.P. in the monkey at rates equivalent to 1 and 6 mL/kg/day for 28 days resulted no toxicologically significant effects upon clinical condition, body weight, hematology or clinical chemistry parameters. Treatment-related gross findings were confined to swelling at the infusion site (associated with mild edema and/or hemorrhage) in 2/3 high rate (6 mL/kg/day) animals. In light of these findings, 6 mL/kg/day was considered to represent the upper limit for subcutaneous infusion for 28-day studies.Subsequent to the conduct of the feasibility study a 13-Week continuous subcutaneous infusion toxicity study was successfully performed in Cynomolgus monkeys using infusion rates selected to deliver 3 mL/kg/day. The control data from this study confirmed the findings of the initial 28-day study and are presented.

964 CONNEX IN-LIFE DATA CAPTURE SYSTEM: A SYSTEM OVERVIEW.

T. J. Jameson and A. J. Little. *Covance Laboratories Europe, Harrogate, United Kingdom.* Sponsor: J. Norris.

The Connex data capture system has been developed at Covance Harrogate, England to record In-life Toxicology Data from a diverse range of species. The initial version of Connex was designed to record Food/9Water Consumption, bodyweights, clinical observations and tissue masses with the aim of replacing the Toxdata system or the manual data records used for more complex study designs. Subsequent developments have now extended this range to include test article administration. The existing Toxdata system relies upon data being recorded on to pre programmed magnetic discs. These discs are taken into animal rooms for data recording after which they are returned to a central "link room" where the information is uploaded on to a main frame computer and the data printed, this process is controlled using paper records. Connex excels over the existing systems in many ways, the first of which is the scheduling of data recording which is monitored by a dedicated program. This "Connex Console" program shows the current status of all studies being run on Connex and is available within the Animal Houses, Study Director group and Quality Assurance Department. Data generated using Connex is stored electronically and an interface has been designed permitting data to be reviewed in a safe environment. To ensure data is secure at all times extensive power failure, data transfer (network) failure and computer security/failure procedures have also been incorporated into the system. This poster provides an overview of both systems demonstrating how data collection is scheduled, stored, accessed and protected.

965 ASSESSMENT OF MURINE MICTURITION PATTERN AND UROTHELIAL PERMEABILITY AFTER CYCLOPHOSPHAMIDE ADMINISTRATION.

R. W. Wood, L. Eichel, E. M. Schwarz, E. M. Messing and J. S. Shojaie. *University of Rochester Medical Center, Rochester, NY.* Sponsor: R. K. Miller.

Toxicants can have adverse effects on the structure and function of the urinary bladder, resulting in elevated frequency of urination, changes in the functional capacity of the bladder, and in its ability to act as a barrier to intravesical resorption of xenobiotics. Noninvasive techniques have been developed that permit the evaluation of urinary frequency and void volume, as well as to describe the elimination of marker substances in urine that may undergo intravesical reuptake and hence prolongation of excretion with impairment of bladder mucosal barrier function. Cyclophosphamide (300 mg/kg IP) produces hematuria that persists for up to two weeks and is associated with

delayed clearance of fluorescein (1mg/kg IP), decreased urine production, reduced void volume, and increased voiding frequency. The resultant irritative voiding syndrome demonstrated the ability to measure syndromes characterized by overactive bladder, and may be useful in acute and chronic toxicity evaluations, medication development, and in the investigation of the molecular determinants of bladder structure and function. To produce elevated urine production, mice (female CRL CD-1) were offered a drinking solution of 0.125% saccharin and 3.0% glucose stabilized with parabens; this mixture results in at least a ten-fold elevation in urine production. Void volumes typically ranged from 150 to 300 µl, and occurred about 12 per hour. Thus measurable amounts of urine could be collected with a conventional fraction collector placed under a Nalgene mouse diuresis cage. For the measurement of void volume and frequency, the collection funnel was removed from the diuresis cage and replaced with a stainless screen. A urine pan sits on a balance configured to report changes of 15 mg and the resultant stable weight to a resolution of 1 mg. Character strings were sent via a terminal server to a remote networked computer that provided evaporation correction and event characterization. The system can measure many balances simultaneously, and provides descriptive statistics within- and across sessions. (Acknowledgments: DA05080, Departments of Urology, OB/GYN, Medicine.)

966 THE DESIGN OF A USER FRIENDLY DATA RECORDING SYSTEM.

A. J. Little and T. J. Jameson. *Covance Laboratories Europe, Harrogate, United Kingdom.* Sponsor: J. Norris.

End user involvement has been the key to the successful design and implementation of the Connex In-life data capture system both in terms of the programming and the computer workstations used in the laboratory environment. By consultation with end user departments the dedicated Covance Information Systems Department have been able to incorporate many excellent features into the Connex capture system. This poster will show the ease with which laboratory data can be recorded, demonstrating how external devices for electronic identification of animals and test substances are integrated in to the recording process. Many control measures have also been built in to the Connex system to prevent data entry errors and ensure data integrity. These measures include the comparison of every weight (animal, food or water) against background data at the time of recording. The system also forces balance calibration, audit trails any data changes and automatically records when animals have been electronically identified to perform procedures. To assist data entry purpose designed computer workstations have been developed for both the Rodent and Non-Rodent areas. The challenge when designing these workstations has been to provide a durable protection system for the integrated electronic components whilst maintaining the highest possible levels of ergonomics. The two very different solutions will be detailed together a new prototype "touch screen" workstation intended to further aid data capture.

967 ELECTRON PARAMAGNETIC RESONANCE (EPR) MEASUREMENTS OF MANGANESE OXIDATION STATE IN BIOLOGICAL SAMPLES: IMPLICATIONS OF THE ROLE OF OXIDATION STATE IN TOXICITY.

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Elevated exposures to manganese (Mn) and other metals have been associated with the increased incidence of Parkinsonism. This raises concern over the pending use of the antiknock gasoline additive methylcyclo-pentadienyl manganese tricarbonyl (MMT), which upon combustion may yield particles with Mn in a mixture of oxidation states. Mn oxidation state may play an important role in neurotoxicity, since studies have shown that Mn can impair dopaminergic systems via direct oxidation of dopamine, or through the generation of ROS via other redox processes. Here, we are developing an *in situ* method for the measurement of Mn oxidation state in biological tissues (blood and brain), in which divalent Mn (d^5) is quantified via electron paramagnetic resonance (EPR), total Mn is determined using magnetic-sector ICP-MS, and inferred trivalent Mn is determined by difference. We then conducted an *in vitro* study in fresh whole blood to determine the effects of Mn exposure of different oxidation states on the kinetic and steady state balance between Mn(II) and Mn(III). Since the complex matrix of biological samples and inherent Mn-binding can preclude the utility of simple aqueous EPR standards, we have investigated standards of aqueous Mn(II) (as "free" or hexa-aquo Mn(II)), Mn(II) + lysozyme (non-specific Mn binding), and Mn(II)-glutamine synthetase (specific Mn binding) to demonstrate quantifiable EPR spectra. Results to date have: (i) Established a detection limit of 30ppb for the measurement of Mn(II) by EPR; (ii) Suggested that the steady-state Mn(II):Mn(III) ratio of the redox reaction Mn(II)$\leftrightarrow$Mn(III) in whole blood differs, depending on whether the added (spiked) Mn was as Mn(II) or Mn(III), and; (iii) Suggested that the steady-state Mn(II):Mn(III) ratio is different in blood compared to brain tissues of $Mn(II)Cl_2$ exposed rats. These preliminary data suggest that the oxidation state of introduced Mn could influence the potency by which excessive Mn exposure exerts toxicity.

968 COMPARISON OF *AD LIBITUM* AND RESTRICTED FEEDING REGIMENS FOR CHRONIC STUDIES USING THE SPRAGUE-DAWLEY RAT.

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In recent years, there has been a trend towards restricting the amount of food provided to rats during chronic studies in an attempt to increase overall health of the animals and therefore, enhance survival. In the past few years CTBR has performed a number of studies with various types of diet restriction. Some of the long term carcinogenicity studies have been completed and the survival data at Week 104 was compared with survival data for some studies fed ad libitum. Other parameters, such as body weight and incidence and onset of masses were also compared. The data shows that the average survivability of in the males and the females with restricted feeding when compared to those fed ad libitum was higher. The amount of food given per day (ie.: 21g vs. 25g) also had an influence the survival in of the males. The overall health of the animals for which the diet was restricted was better (i.e.: reduced number of palpable masses and fewer age-related changes) and the body weights lower. In conclusion, the survival at completion of carcinogenicity studies can be improved by restricting the amount of food provided to the animals. The observed reduction in bodyweights can also be beneficial as it results in reducing the total amount of test article required to complete the studies.

969 A HIGH THROUGHPUT SCREENING ASSAY FOR HEPATOTOXICITY USING CRYOPRESERVED ANIMAL AND HUMAN HEPATOCYTES.

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Recently, we have developed a procedure for the successful cryopreservation of animal and human hepatocytes. We report here a high throughput screening procedure for hepatotoxicity using the cryopreserved cells. Cryopreserved hepatocytes were thawed and plated in 96-well plates preplated with test chemicals at the appropriate concentrations. After an incubation of 4 hrs, cytotoxicity was evaluated using two procedures: 1. Measurement of mitochondrial functions using the MTT assay. 2. Measurement of cellular ATP. The MTT assay was performed by adding the MTT reagent directly into the wells after the 4-hr incubation period, allowing an additional 3-hr incubation period for the metabolism of the reagent, addition of acidified isopropanol to extract the blue color metabolite, and quantification of absorbance at 650 nm. ATP quantification was performed by the addition of a cell lysis buffer after the incubation period followed by the quantification of ATP via chemoluminescence. The ability of the assays to distinguish hepatotoxic and nonhepatotoxic compounds was evaluated using the known hepatotoxicants tamoxifen, ethynyl estradiol, and the nontoxic analogue estradiol. Tamoxifen and ethynyl estradiol consistently yielded dose-dependent cytotoxicity using both MTT and ATP endpoints, while no significant cytotoxicity was observed with estradiol. The cytotoxicity measurement based on ATP quantification was found to be superior to the MTT assay because of the decreased cost and time required. We believe that this assay with cryopreserved hepatocytes and ATP cytotoxicity endpoint can be automated and used routinely for the screening of chemical libraries to allow rank-ordering of product candidates based on hepatotoxic potential.

970 EXTENDED DURATION POWDER DELIVERY SYSTEM USED IN PRE-CLINICAL STUDIES IN RODENTS.

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The Extended Duration Powder Delivery System (EDPDS) was developed at CTBR in order to fulfill a pharmaceutical industry demand for testing of dry powder formulations in an automated and reproducible manner, with minimal

intervention and at wide ranges of aerosol concentration.The EDPDS is sufficiently versatile to establish stable powder aerosol concentrations of different magnitudes (ie. 5 mg/L to 2000 mg/L) and for different lengths of time (ie. 10 minutes to 4 hours) without having to replenish the supply of test article and interrupt the exposure. Its modular design allows the system to be easily adapted by changing the size of the feed components and by controlling the feed rate with a variable speed motor which can be set to as low as 1 RPM to aerosolize minute quantities of test powder into the exposure chamber. The EDPDS has been used successfully on 5-day, 14-day and 28-day rodent inhalation toxicity studies. The system proved to be an efficient and highly reproducible means of delivering a range of powder concentrations on subacute studies that could be readily applied to subchronic, chronic and carcinogenicity inhalation studies.

971 SPECIES COMPARISON OF METHEMOGLOBIN REDUCTASE.

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Methemoglobin (MHb) formation is an effective strategy for treating cyanide (CN) poisoning. Methemoglobin reductase (MR) is the enzyme that reduces MHb back to hemoglobin (Hb). The endogenous, circulating, normal level of MR reflects the capacity to reduce MHb and is therefore an important factor in evaluating the anti-CN efficacy of MHb formers. To help determine which animal species would most accurately predict the human response to MHb formers, the activity of MR was measured (mean±SEM) in whole blood of several species following established analytical methods. The animals employed included rhesus monkeys (Macaca mulatta; samples collected at USAMRICD), African green monkeys (Chlorocebus aethiops; samples collected at USAMRICD), and beagle dogs (Canis familiaris; samples collected at WRAIR). The MR values in these animal species were compared with normal human values. The human range for MR (n = 30) was 12.6 - 24.2 IU/g Hb, with a mean of 16.6±0.44 IU/g Hb (Mayo Medical Laboratories, Rochester, MN). The mean MR level in rhesus monkeys (n = 15) was 20.2±0.74 IU/g Hb, falling within the normal human range. For African green monkeys (n = 19) the mean was 26.0±0.76 IU/g Hb, slightly higher than the normal human upper limit. For beagle dogs (n = 15) the mean MR level was 9.5±0.22 IU/g Hb, approximately 25% lower than the normal human lower limit. Although the beagle dog has been used extensively in the study of MHb formers, these data suggest that the rhesus monkey may also be an appropriate model.

972 COMPARISON OF RESULTS FROM CROSS-SITE VALIDATION OF CONTINUOUS INFUSION PROCEDURES, EVALUATING TWO ROUTES OF INTRAVENOUS ADMINISTRATION AND PRECANNULATED CRL:CD(SD)IGS BR AND FISHER CDF (F-344)CRLBR RATS.

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Identical validation studies were conducted at our Vienna, VA and Madison, WI laboratories, to determine the feasibility of using vendor-supplied precannulated rats, to compare the use and manageability of Fisher-344 and CD® rats, to evaluate the jugular and femoral routes of intravenous infusion, and to assess survival when a known vascular irritant is infused. The results of the two studies were compared to determine the similarities and differences between sites when a common protocol is followed and a harmonized technology is utilized. The rats were precannulated by the supplier (Charles River Laboratories) and eighty animals (10/sex/strain/route) were assigned to each study. Saline (0.9%) was administered to all rats at a rate of 15 mL/kg/1 hour daily, for Days 1-15; from Days 16-30 a 7.5% solution of Solutol HS was infused at the same rate to equal numbers of each sex/strain/route. A standard battery of ante- and post-mortem evaluations were performed, to include mortality, clinical signs, body weights, food consumption, clinical pathology, necropsy findings, and histopathology. Ten rats died, were sacrificed moribund due to sepsis and inflammation of the infusion site, or were removed from each study; the majority (70%) were male CD® rats exposed to Solutol with no predilection for route. Clinical observations included swelling at the cannulation site, primarily in the jugular CD® rats exposed to Solutol. Males of both strains exposed to Solutol had a slight decrease in body weights and food consumption with a less apparent effect in females during Weeks 3 and 4 of treatment. Clinical pathology changes were similar between studies and were primarily related to chronic cannulation (decreased red blood cell mass, increased neutrophil count, and increased globulin). In addition, lower cholesterol values were found in both strains of rats given Solutol. Microscopic evidence of catheter-site abcessation were more frequent in jugular CD® rats at the Vienna site, with no appreciable difference in lesion incidence among groups at the Madison site. These data indicate that similar results were obtained for both studies, precannulated rats are acceptable for use, femoral intravenous infusion is preferred for irritating formulations, and the Fisher rat is a superior model for chronic infusion.

973 ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) ANALYSIS OF URINARY METABOLITES OF 1,1,2,2-TETRACHLOROETHYLENE USING AN AUTOMATED SPE SAMPLE PREPARATION SYSTEM.

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1,1,2,2-Tetrachloroethylene [Perchloroethylene] (*PERC*, CAS 127-18-4), used extensively as a dry cleaning agent and in industrial degreasing processes, has been reported to increase the incidence of liver tumors in mice or of nephrotoxicity and renal tumors in male rats. Bioactivation of *PERC* is reported to occur by two pathways, oxidation by CYP2E1 and glutathione *S*-conjugation. Trichloroacetic acid (*TCA*, CAS 76-03-9) and dichloroacetic acid (*DCA*, CAS 79-43-6) are reported urinary metabolites of *PERC* oxidation whereas the glutathione conjugate, *S*-(1,2,2-trichlorovinyl) -L-glutathione (*TCVG*) is further cleaved to *S*-(1,2,2-trichlorovinyl)-L-cysteine (*TCVC*) and acetylated to N-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (N-ac-*TCVC*). A potential biomonitoring method was developed to measure urinary levels of *TCA*, *DCA*, *TCVC* and N-ac-*TCVC*. Stable isotope-labeled analogs of *TCVC* and N-ac-*TCVC* were prepared (Bartles and Miner, 1989) and deuterated *DCA* was purchased as internal standards. A BenchMate® II robotic workstation was used to automate sample preparation. *PERC* metabolites (>90%recovery) were analyzed using a ThermoQuest Finnegan LCQ tandem mass spectrometer. Chromatographic standards *TCVC* and N-ac-*TCVC* were synthesized as above. Compounds of interest were eluted within 12 min and detected using ESI-MS/MS in multiple segments, initially in the negative ion mode for detection of *TCA* and *DCA*, and subsequently in the positive ion mode for *TCVC* and N-ac-*TCVC*. Urine samples from rats exposed to 100, 500, or 1000 mg/kg *PERC* contained detectable amounts of *PERC* metabolites in urine over a 48-hr period. The LOD per injection was 30 fmol for *TCVC*, 800 fmol for N-ac-*TCVC*, 10 pmol for *DCA*, and 75 pmol for *TCA*. This procedure appears to offer significant advantages over typical extraction and derivatization methods for the same compounds by GC-MS. Thus, *PERC* internal dose for various exposures can be rapidly quantified using metabolites in a single assay using an automated sample preparation system.

974 INDUSTRIAL COMBUSTOR VALIDATION TEST MEASURING AIR EMISSIONS OF DIOXINS AND FURANS ON A TEQ BASIS USING EPA METHOD 23 AND HRGC/HRMS VERSUS THE AMBSTACK SAMPLER AND CALUX BIOASSAY.

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The analysis of polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinted dibenzofuran (PCDF) in gaseous samples by EPA method 23 and analysis by high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) is expensive and complex. We have designed a low cost unitized sampling system, the (AmbStack Sampler) and combined it with a reporter gene bioassay system, the Chemical Activated Luciferase Expression method (CALUX), to provide direct TEQ estimates of PCDD/PCDF contamination in air. The current study was designed to compare PCDD/PCDF determinations on a toxic equivalency (TEQ) basis by EPA method 23 and HRGC/HRMS and the AmbStack/CALUX system. In the current experiments simultaneous sampling by both methods were performed on a North American Package Boiler (NAPB) combusting a dopant (a mixture of 1,2 dichlorobenzene and copper naphthenate) at a flow rate adjusted to yield a HCl concentration at the stack of approximately 500 ppm at 7% O_2. The flue gas stream for this experiment was stable at a temperature of 140°C with a moisture level of about 11%. Samples were collected for three hours at a flow rate of 3.58 M^3 and analyzed by both HRGC/HRMS and CALUX analysis. HRGC/HRMS results were 2.75 ng TEQ/dscm (7% O2) versus 3.4 ng

TEQ/dscm (7% O2) by AmbStack/CALUX. EPA method 23 and HRGC/HRMS and AmbStack/CALUX provided similar estimates of TEQ contamination of combustion effluents. The AmbStack/CALUX system is useful as a low cost diagnostic tool to quickly measure PCDD/PCDF emissions from thermal combustion systems allowing optimization of conditions to minimize emissions of these toxic environmental contaminants.

975 DEVELOPMENT OF A HETEROPLASMIC MITOCHONDRIAL DNA STANDARD REFERENCE MATERIAL FOR DETECTION OF HETEROPLASMY AND LOW FREQUENCY MUTATIONS.

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A heteroplasmic human mitochondrial DNA (mtDNA) standard reference material (SRM) is being developed to provide quality control to medical, forensic and toxicological scientists who wish to determine their detection limits when examining low frequency mutations or heteroplasmic sites in DNA. While the detection of a mutation present in every mtDNA molecule is routine, it is extremely difficult to detect mutations present in only a low proportion of the molecules. In mtDNA diseases, the proportion with the mutation appears to increase with age (i.e., the mutation becomes more predominant in older individuals, who then exhibit the disease symptoms). To address these concerns, mtDNA mixtures containing a polymorphic/wild type site in different percentages (1, 2.5, 5, 10, 20, 30, 40 and 50%) have been constructed. With automated sequencing techniques (ABI 373 or 310), we were able to unambiguously detect the polymorphism present at the 30% level. With Denaturing Gradient Gel Electrophoresis (DGGE), resolution at the 5% level was feasible. With the addition of a Peptide Nucleic Acid (PNA) complementary to the wild type sequence, PCR of the wild type decreased, the mutant DNA selectively amplified, and detection became possible at the 5% level. This SRM can be employed to test and perfect more sensitive mutation detection techniques. Pre-symptomatic mtDNA diseases, once detectable, may become treatable, and perhaps even preventable. These more sensitive techniques may permit toxicologists to detect the effects of chemical and physical mutagens before they cause adverse health and environmental problems.

976 HISTORICAL CONTROL DATA FROM TOXICOLOGY STUDIES IN THE CAT.

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Reference data were compiled from laboratory cats comprising the control groups in experiments performed in our laboratories since 1995. In total, approximately 500 cats were used for various types of study of up to 71 weeks in duration, including: efficacy studies for vaccines or drugs, safety, pharmacokinetics and toxicology. The experimental protocol must take into account the particular environmental needs of the species. Group housing is advisable for long term studies, as are rest areas, scratching planks and toys. Single caging is sometimes necessary for toxicology investigations, but socialisation should be allowed for as long as possible each day. This species is well known for being difficult to handle. Technicians must be well trained, quiet and gentle; moreover, they must like cats! Some types of sophisticated examination routinely performed in other species, such as the dog, require an adaptation when applied to cats (e.g. Doppler-shift sphygmomanometry for blood pressure measurement and vascular access ports for repeated blood sampling in non-anaesthetised animals). The precautions necessary for the purchase, transportation and handling of SPF cats are similar to those used with other species. Physiological, biochemical (blood and urine), haematological and pathology data will be presented.

977 DETERMINATION OF TREMOROGENIC HARMANE AND HARMINE IN HUMAN BLOOD BY A SIMPLE AND RAPID LIQUID CHROMATOGRAPHY METHOD.

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A number of tremorogenic β-carboline alkaloids such as harmane, harmine, and harmaline have been found in common plant-derived foodstuffs, beverages, and inhaled substances. Because of their natural presence in the food chain, there is a growing concern regarding the potential risks of certain essential tremors associated with the long-term, low-level dietary exposure to these alkaloids. However, the lack of a sensitive quantitative method has rendered it impractical for the risk assessment. The purpose of this study was therefore to develop a simple and rapid analytical method to determine blood levels of two major β-carboline derivatives, harmane and harmine. Human blood was extracted with ethyl acetate and methyl-*t*-butyl ether (2:98) in an alkaline condition. After solvent evaporation, the samples were reconstructed in methanol. The samples were fractionated on a 250x4.6 mm C18 reversed-phase column with an isocratic mobile system consisting of 17.5 mM potassium phosphate buffer (pH 6.5) and methanol (30:70), followed by an on-line fluorescence detection. The method had the detection limit to determine 206 pg/ml and 81 pg/ml of harmane and harmine, respectively, in human blood. The intra-day precision (C.V.) at 25 ng/ml was less than 6.7% and 3.4% for harmane and harmine, respectively. The inter-day precision was 7.3% for harmane and 5.4% for harmine. The method is sensitive and reproducible and has proved useful for both laboratory and clinical studies of β-carboline toxicities. (Supported by NIEHS P30-ES09089.)

978 USING HISTORICAL CONTROL DATA TO QUANTIFY VARIABILITY INHERENT IN STANDARD NEUROTOXICITY MEASURES: FORE- AND HIND-LIMB GRIP STRENGTH AND HIND LIMB FOOT SPLAY.

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The EPA intends to require registrants of conventional "food use" pesticides, as well as neurotoxic registered pesticides, to submit data from acute, subchronic, and developmental neurotoxicity studies in rats. EPA will use this data in the implementation of the Food Quality Protection Act. To provide a basis for interpretation of neurotoxicity data by the scientific and regulatory communities, historical control data on fore and hind limb grip strength and hind limb foot splay from Huntingdon Life Sciences (HLS) were analyzed by Research Triangle Institute statisticians to identify and quantify all sources of variability inherent in these tests.The control data set was comprised of 31 studies conducted between 1993 and 1999, with 10 animals/sex/study. Evaluations were performed at Days 1,8 and 15 for acute studies, at Weeks 5, 9 and 13 for subchronic studies, and at Week 4 for 28-day studies. There were 2 replicates per evaluation at each time point. The quality assurance-reviewed data set was divided into one subset for each test. The continuous variables were: Body weight, testing interval, digital measure of grip strength or manual measure of landing foot splay. The categorical variables were: rat strain, gender, technician performing the test, equipment number, and study number. The reproducibility of the digital output for fore or hind limb grip strength and the manually measured hind limb landing foot splay was analyzed. Measurement error models were fit to the data to study possible sources of variability; such as, animals, trials, time points, technician, equipment, and studies. The relationships between fore and hind limb grip strength, hind limb grip strength and hind limb landing foot splay, and all three measures were also quantified.

979 ISOLATION OF HUMAN HEMOGLOBIN ADDUCTS AS BIOMARKERS OF EXPOSURE TO ACRYLAMIDE.

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Acrylamide is a commercially important chemical that is used in the drinking water purification process, production of grouting materials, and gel electrophoresis. The Environmental Protection Agency classifies acrylamide as a neurotoxin and a probable human carcinogen. Acrylamide readily and irreversibly forms hemoglobin adducts. Because hemoglobin adducts are not subject to physiological repair mechanisms, acrylamide adducts of hemoglobin may be efficient biomarkers for human exposure. Presently, the exact location of acrylamide adduct formation on the human hemoglobin molecule is unknown. The purpose of the present study is two-fold. First, the extent of acrylamide adduction occurring in oxygenated, deoxygenated, carbon monoxide bound, met, and β-93 cysteine residue blocked purified human hemoglobin (Hb A_0) samples shall be determined. Second, the specific site of adduction on the hemoglobin molecule will be identified. Fulfilling these two goals will provide insight into using human hemoglobin adducts as biomarkers for exposure to acrylamide. Reverse phase high-performance liquid chromatography (HPLC), mass spectrometry, and tryptic peptide maps will aid in the location of acrylamide adducts. Preliminary experiments using spectrophotometry and gel electrophoresis suggest that some aspect of the adduction

process is more available or reactive when Hb A_0 is deoxygenated. β-93 cysteine residues, which have been suggested as possible sites of acrylamide adduction, are much less accessible in deoxygenated Hb A_0. Since deoxygenated Hb A_0 appears to show the greatest amount of adduction, the β-93 cysteine residue site is probably not involved in acrylamide adduct formation.

980 NEITHER DIRECT CHEMICAL NOR CYANIDE ANTAGONISM CAN FULLY EXPLAIN THE ANTIDOTAL EFFECT OF N-ACETYL-L-CYSTEINE ON ACUTE ACRYLONITRILE INTOXICATION.

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Acrylonitrile (AN) is acutely toxic. Candidate mechanisms for toxicity include metabolism to cyanide (CN), covalent binding (CB) to proteins and depletion of glutathione (GSH). We have previously characterized the dose-dependent toxicokinetics of AN and the biomarkers CN, CB and GSH. We have now studied the effect of three mechanism-based antidotes on these parameters. Benzylimidazole (BI, 1mg/kg) blocks metabolism to CN. Thiosulfate (TS, 1g/kg) serves as a sulfur donor in CN detoxification. N-acetyl-L-cysteine (LNAC, 2mmol/kg) may 1.) decrease AN body burden by direct chemical antagonism, 2.) serve as a GSH precursor and 3.) serve as a sulfur donor like TS. Rats were given AN sc in the scruff of the neck and antidotes were administered ip simultaneously. Following AN-LD90 (2.2mmol/kg), all antidotes abolished the area under the curve (AUC) for CN in blood and brain but only LNAC decreased the AUC for AN in blood. However, this AUC for AN was much greater than that seen following AN-LD50 (1.5mmol/kg) alone. Tissue CB was not markedly changed by any antidote and only LNAC attenuated AN-induced GSH depletion. In rats treated with AN-LD90 + LNAC, blood levels of AN, LNAC and GSH were comparable (~1mM) and steady for the first 30 min. However, during this same time interval, liver GSH decreased over 90%. Mortality in rats given AN-LD90 + antidotes was 100% for saline, BI and TS but 10% for LNAC. We conclude that, although CN undoubtedly contributes to AN toxicity, abolishing the acutely toxic AUC for CN is not sufficient to preserve life. In addition, direct chemical antagonism of AN by LNAC in blood does not occur to any significant extent. (Supported by NIEHS ES 06141.)

981 IDENTIFICATION OF SITES OF HUMAN SERUM ALBUMIN MODIFICATION BY 2,4- AND 2,6-TOLUENE DIISOCYANATE.

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Toluene diisocyanate (TDI), a 4:1 mixture of 2,4- and 2,6- isomers used in the preparation of polyurethanes, is a recognized cause of occupational asthma. We have previously shown that the TDI isomers react readily with thiolated molecules. Clinical and experimental evidence indicates that serum albumin is adducted by TDI following inhalation of the chemical. We have in order to determine the site(s) of TDI binding to the protein and the significance of the adduct(s) to sensitization and asthma, we utilized radiolabelled TDI. Twenty fold molar excess human serum albumin was dissolved in PBS, pH 7.4 and reacted separately with custom prepared [14C]- 2,4 and [14C]- 2,6 TDI. The adducted protein was then subjected to four different proteolysis conditions without prior disulfide bond reduction: pronase E, trypsin, V8 and cyanogen bromide (CNBr) cleavage. Samples of both the digest and control where reduced with dithiothreitol (DTT) and analyzed by SDS-PAGE. The CNBr induced cleavage reaction was potentially the most informative regarding elucidation of site(s) of reaction by gel electrophoresis. CNBr cleavage bands, stained with Coomassie Blue, were excised from the gel, the proteins were eluted and evaluated for 14C content by liquid scintillation spectrometry. For 2,4-TDI, the majority of the 14C label, in non-reduced samples, was located in bands having apparent molecular weights of 49, 29, and 15 Kda. For non-reduced samples of 2,6-TDI, the label was located in bands of 29 and 15 Kda. Autoradiography confirmed the results for both isomers. Interestingly, fragments treated with DTT prior to SDS-PAGE retained little radiolabel suggesting that TDI was bound to thiol labile site(s). Two such sites of thiol lability on HSA are the free thiol (cysteine-34) and carboxylate residues. Further purification and analysis of the adducted peptides will provide information of the nature of the adducts and of the amino acids adducted. Such information will be invaluable for elucidation of the antigens involved in TDI hypersensitivity and asthma. (Supported by NIEHS 05651.)

982 EVIDENCE FOR THE PRESENCE OF A PUTATIVE AH RECEPTOR LIGAND IN CV-1 CELLS.

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The Ah receptor is a ligand activated transcription factor which has been shown to be largely responsible for mediating the cellular response to numerous environmental compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin. A role for the AhR in normal cellular biology has been previously postulated, but no endogenous ligand has yet been identified. The presence of a putative endogenous ligand has been determined by transient transfection of cultured CV-1 cells with various cytochrome P450 isoforms. CYP1A1, CYP1A2, CYP1B1, CYP2E1 and CYP3A4 expression vectors were transiently co-transfected into cultured CV-1 cells along with an AhR expression vector (pcDNA3/bmAhR) and the DRE-driven reporter (pGudLuc6.1). The subsequent effect of P450 expression on DRE-driven luciferase reporter activity was assayed. Expression of either CYP1A1, CYP1A2 or CYP1B1 in CV-1 cells reduced DRE-driven reporter activity in a concentration dependent fashion with the highest level of expression essentially abolishing reporter activity. Neither CYP2E1 nor CYP3A4 expression demonstrated any significant reduction in endogenous ligand levels as assayed through DRE-driven reporter activity. A stable CV-1 cell line was generated through stable co-transfection of CV-1 cells with CYP1B1 (pRc/CMV/1B1) along with a hygromycin resistance encoding vector (pRep). This resulted in a clone which displays an approximate 16-fold reduction in DRE-driven luciferase reporter activity when compared to control CV-1 cells co-transfected with pcDNA3/bmAhR and pGudLuc6.1. Cytosolic extracts generated from these two cell lines were fractionated on a C-18 microbore HPLC column using a 70% MeOH isocratic gradient. Fractions were collected, dried and then further analyzed for ligand activity in HepG2 40/6 cells which contain a stably integrated DRE-driven reporter construct. Many of the strong induction peaks identified in the CV-1 cytosolic extracts were absent in the CV-1/1B1 Clone-7 control cell line confirming the presence of putative AhR ligands which are capable of undergoing CYP1B1 metabolism to a less active product.

983 FUNCTIONALY IMPORTANT PHOSPHORYLATION SITES IN XAP2, A MEMBER OF THE UNLIGANDED ARYL HYDROCARBON RECEPTOR COMPLEX.

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Exposure to Xenobiotics such as 2,3,7,8 tetrachlorodibenzo(p)dioxin (TCDD) may result in a myriad of deleterious effects such as tumor promotion, wasting syndrome, and immunotoxicity all of which are mediated by the aryl hydrocarbon receptor (AhR) pathway. AhR is a ligand activated transcription factor that prior to exposure to dioxin, exists as a cytosolic 9S heterocomplex consisting of one molecule of AhR, a hsp90 dimer, and a immunophilin-like protein known as the Hepatitis B virus X-associated protein 2 (XAP2). The regulatory role of XAP2 in the AhR signal transduction pathway is being studied through examination of phosphorylation status. Two-dimensional phosphoamino acid analysis has demonstrated that this protein is primarily phosphorylated on serine residues. Identification of important phosphorylation sites was facilitated by the creation of serine to alanine mutations in XAP2-FLAG using site-directed mutagenesis. Loss of phosphorylation with these S to A mutations was identified through the following techniques: 32 P-orthophosphate labeling of COS-1 cells that were transiently transfected with XAP2-FLAG and S to A XAP2-FLAG expression vectors, anti-FLAG immunoprecipitations, tricine polyacrylamide gel electrophoresis, transfer to membrane, tryptic digestion of the radiolabeled protein, followed by peptide mapping techniques. Four S to A XAP2-FLAG mutants (43, 53, 131-2) exhibited loss of phosphorylation. Western blot analysis has determined that the S43A XAP2-FLAG mutant was unable to complex with hsp90 in COS-1 cells, while the S53A XAP2-FLAG mutant had decreased affinity for hsp90 COS-1 cells. The S43A XAP2-FLAG mutant would not radiolabel upon use of high levels of 32 P-orthophosphate, even though Western blot analysis determined that the protein was being expressed well. This data suggests that 43 could be a major phosphorylation site that may possibly influence phosphorylation of other serine residues in XAP2-FLAG. In conclusion, these results suggest that phosphorylation is essential for XAP2-FLAG to function properly in the cell.

984 PHOSPHORYLATION OF A PAS-REGION ARNT SERINE RESIDUE DOES NOT MODULATE ARNT:AHR OR ARNT:ARNT ACTIVITY.

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The ARNT protein belongs to the family of bHLH-PAS transcription factors and regulates a range of cellular processes by either homodimerizing or heterodimerizing with other bHLH-PAS proteins. Previously, the human ARNT protein was shown to be a phosphoprotein, but regulation of ARNT with respect to its phosphorylation status is poorly understood. Phosphoaminoacid analysis of [32P]-orthophosphate labeled human ARNT in COS-1 cells demonstrated that ARNT is predominantly phosphorylated on serine residues and several serine/threonine protein kinases phosphorylated bacterially expressed ARNT truncations *in vitro*. Solid-phase radio-sequencing of a tryptic phosphopeptide predicted one of the serine residues within the PAS-region as a probable phosphorylation site. Site-directed mutagenesis was used to change this serine residue to alanine and then two-dimensional phosphopeptide maps were generated to demonstrate the loss of the predicted PAS-region phosphorylation site. Both alanine and glutamic acid mutants were compared with the wild-type in an ARNT deficient cell line for their capacity to either (i) modulate activation of aryl hydrocarbon receptor (AhR) mediated reporter-vector expression in the presence and absence of tetrachlorodibenzo-p-dioxin or (ii) modulate an ARNT homodimer activated class B E-box reporter-vector expression. Changing this PAS-region serine residue to either an alanine or a glutamic acid did not influence AhR mediated or class B E-box-driven reporter activity. Although phosphorylation of this PAS-region serine does not modulate AhR:ARNT or ARNT:ARNT activity, this phosphorylation site may be important in other ARNT mediated expression systems.

985 NUCLEAR RECEPTOR COACTIVATOR, SRC-1 INTERACTS WITH Q-RICH SUB-DOMAIN OF THE AHR AND MODULATES ITS TRANSACTIVATION POTENTIAL.

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The Aryl hydrocarbon Receptor (AhR), a soluble cytosolic protein, mediates many of the toxic effects of TCDD and related chemicals. The toxic effects are largely cell-, tissue-, and promoter context dependent. Although many details of the overall dioxin signal transduction have been elucidated, the transcriptional regulation of dioxin induced genes like cyp1A1 is not completely understood. Previously, we have shown that the co-regulator RIP140 is a potential AhR coactivator. The role of coactivator, SRC-1 in AhR-mediated transcriptional regulation was examined. SRC-1 increased AhR-mediated, TCDD-dependent, reporter gene activity 3.2- and 3-fold in Hepa-1 and COS-1 cells, respectively. In *in vitro* interaction assays, SRC-1 was found to interact with AhR and not with ARNT. Although SRC-1 interacted with AhR even in the absence of TCDD, the addition of ligand further increased SRC-1 binding to AhR. Deletional mapping studies of the AhR domain that interacts with SRC-1 revealed that SRC-1 binds to the AhR transactivation domain. Finer mapping of the SRC-1-interacting sub-domains in the AhR transactivation domain suggested that the Q-rich sub-domain was necessary and sufficient for interaction, similar to that seen with RIP140 and the AhR. Using GFP-tagged constructs and co-immunoprecipitation assays, SRC-1 was shown to interact with AhR in cells. Unlike RIP140, LXXLL motifs in SRC-1 were necessary for interaction with AhR *in vitro* and for co-activation in Hepa-1 cells. This study demonstrates the potential role of SRC-1 in AhR transactivation, as well as possible differences in the mechanisms of RIP140 and SRC-1 recruitment to the AhR transactivation domain.

986 ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASES (ERKS) BY 2,2',4,4'-TETRACHLOROBIPHENYL IN RAT LIVER CELLS: ROLE OF CALCIUM.

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Polychlorinated biphenyls (PCBs) are widespread environmental contaminants. Much evidence indicates that exposure to PCBs can cause a number of toxic effects such as heptoxicity, reproductive and immunotoxicty etc. Although PCBs occur as mixtures in the environment, the various congeners present in the mixtures can be broadly grouped as coplanar, non-coplanar and mixed type congeners. Unlike the coplanar PCBs that exert their toxic effects by binding to the Ah-receptor, the non-coplanar PCBs do not bind with the Ah-receptor. The tumor promoting and neurotoxic effects of this group of PCBs suggest their interaction with other cellular components. In this study we investigated the effects of 2,2',4,4'-tetrachlorobiphenyl (TCB) and other PCB congeners on mitogen activated protein kinase (MAPK) signal transduction pathway involving ERK 1 and 2 in WB rat liver epithelial cells as a mechanism of their epigenetic effects. Activation of ERKs in PCB treated WB cells under various conditions was assessed by immunoblotting with phosphorylation (activated) state specific ERK antibodies. The results suggested that 2,2'4,4'-TCB activated ERKs 1 and 2 in a dose-dependent manner. Activation of ERKs sustained for up to at least 6 hrs. At 24 hrs after treatment there was a loss of activated ERKs. In contrast 3,3,',4,4'-TCB a coplanar PCB congener did not activate ERKs even at 20 μM. Activation of ERK by 2,2,4,4'-TCB was not inhibited by genistein (50 mM) a tyrosine kinase inhibitor, bisindoloylmaleimide (5 mM) a protein kinase C inhibitor, suggesting that two kinase families were not involved in ERK activation by 2,2',4,4'-TCB. ERK activation by TCB was not inhibited by manumycin a ras protein inhibitor, radicicol an inhibitor of raf-1 kinase or KN-93 an inhibitor of CaM kinase II. However, when the extracellular calcium was removed from the medium ERK activation by this agent was significantly decreased. Taken together, our results suggest that extracellular calcium-dependent cellular processes were involved in ERK activation by 2,2',4,4'-TCB. (Supported by NIEHS grant ES-04911.)

987 EXPRESSION OF TYPE XII COLLAGEN AND EMMPRIN IN NORMAL AND HYPOXIC PULMONARY ARTERIES.

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Pulmonary hypertension causes an increase in deposition of extracellular matrix material, particularly fibrillar collagen, in the adventitial tunic of the arteries. In a rat model, pulmonary vessels can be made hypertensive by hypoxia. If the animal is returned to ambient air within a couple of weeks, the condition is reversible, as long as the endothelium is intact. Type XII collagen is a molecule associated with collagen fibrils. Lying on the surface of the fibrils, it bridges, organizes and stabilizes fibrillar collagen arrangement. We have examined the expression of the mRNA for type XII collagen in normal, hypertensive and restored pulmonary vessels. We have also examined the expression of mRNA for EMMPRIN, an endothelial product that is a matrix metalloproteinase inducing molecule. As vessels produce excessive matrix and become hypertensive in response to anoxia, type XII collagen mRNA increases and EMMPRIN decreases. The MMP-inducing EMMPRIN mRNA increases in amount when the animal is returned to ambient oxygen, beginning the matrix degradation and the vessel structure remodelling that occurs in the return to a more normal phenotype. Once remodeling has occurred, the EMMPRIN mRNA amounts return to normal. These results give some insight into how the deposition of excessive amounts of fibrillar collagen may be stabilized in the hypertensive adventitia, and what may trigger the matrix metalloproteinases to remove it, to restore the vessel to a normal phenotype.

988 DIFFERENTIAL ACTIVATION OF C-HA-RAS ARE/EpRE BINDING PROTEINS BY BENZO[A]PYRENE AND THAPSIGARGIN IN VASCULAR SMOOTH MUSCLE CELLS.

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Efforts in this laboratory have focused on the characterization of vascular smooth muscle cell (vSMC) proteins that interact with the antioxidant/electrophile response element (ARE/EpRE) of *c-Ha-ras*. UV crosslinking studies have shown that multiple proteins of comparable molecular weights bind to *c-Ha-ras*, r*GSTYa* and *NQO$_1$* ARE/EpREs, though several unique proteins recognize the *c-Ha-ras* sequence. The present studies were conducted to gain further insight into the cellular localization of *c-Ha-ras* ARE/EpRE binding proteins, and their relative contribution to formation of specific DNA/protein complexes in response to benzo(a)pyrene (BaP) and thapsigargin. Our results showed that both nuclear and cytosolic vSMC proteins bind to the *c-Ha-ras* ARE/EpRE in BaP (3uM) -treated cells, yielding two complexes of comparable migration and apparent composition. Under more stringent nuclear protein extraction conditions, we also identified a third ARE/EpRE complex that was inducible by BaP (3uM) within 0.5 hours, and reached maximal induction by 2 hours. A fourth BaP-inducible ARE/EpRE complex was also identified, though it represented a minor component of total DNA binding activity.

Treatment of vSMCs with thapsigargin to release intracellular Ca^{2+}stores markedly induced the minor ARE/EpRE complex, along with two of the complexes observed in BaP-treated cells. Although the predominance of BaP- and thapsigargin- inducible complexes differed, the relative protein composition profiles in UV crosslinking gels was comparable for both treatments. Thus, inducible *c-Ha-ras* ARE/EpRE binding in vSMCs involves unique proteins that contribute to differences in complex formation in response to BaP and thapsigargin. (Supported by NIH Grants ES04849 and ES 09106.)

989 MECHANISMS OF C-HA-RAS ACTIVATION IN VASCULAR SMOOTH MUSCLE CELLS BY BENZO[A]PYRENE.

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Increased vascular smooth muscle cell (vSMC) proliferation and migration to the intima are early events in atherogenesis. Benzo[a]pyrene (BaP), a known carcinogen and atherogen, increases *c-Ha-ras* mRNA levels in vSMCs, and this response is believed to contribute to the appearance of proliferative (i.e. atherogenic) phenotypes. The purpose of these studies was to evaluate the mechanism of increased *ras* expression following BaP exposure in vSMCs. Multiple putative BaP-responsive regulatory DNA elements are discernable in the mouse ras promoter (i.e. electrophile and aryl hydrocarbon response elements), suggesting a transcriptional activation mechanism. Nuclear run-off experiments using vSMC nuclei demonstrated that BaP causes an increase in the rate of *c-Ha-ras* transcription. BaP-induced ras expression was not associated with mutations in the IDX alternative splice site- a mutation known to effect ras expression. Treatment of vSMCs with actinomycin D prior to BaP exposure resulted in decreased ras mRNA levels indicating that increased mRNA stability did not contribute to increased steady state mRNA levels. The mouse ras gene has been shown to utilize at least three different transcription start points located at -1045, -1030 and -1012 with respect to the first codon. Primer extension analysis was performed to determine if BaP alters transcription start point utilization during *ras* expression. Several novel transcription start sites were detected between bases -999 and -1284, however, the pattern of start sites was the same in vehicle- and BaP-treated vSMCs. Taken together, these results confirm that BaP increases expression of the *ras* gene in vSMCs through a transcriptional activation mechanism. (Supported by NIH Grants ES 04849 and ES 09106.)

990 GENERATION OF MUTANT GLUTATHIONE S-TRANSFERASE P1 CDNAS BY RANDOM MUTAGENESIS OF GSTP1C.

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Glutathione S-transferase P1, a major drug and electrophile metabolizing and detoxifying enzyme was shown recently to be encoded by a polymorphic gene locus, a result of nucleotide transitions in the region encoding the active site of the peptide. The resulting variant GSTP1 proteins exhibit significant functional differences. We propose that the active site of the GSTP1 peptide can be mutated to generate other GSTP1 cDNAs that encode GSTP1 peptides with more potent drug metabolizing activity. Such mutants can be used to confer resistance to otherwise toxic compounds including, mutagens, carcinogens, and anticancer agents. In this study, we describe the use of the technique of Degenerate Oligonucleotide Mediated Random Mutagenesis (DOMRM) to create mutant GSTP1 cDNAs. Since Try108 is a critical amino acid in the GSTP1 active site and AA104 is involved in the GSTP1 polymorphism, the degenerate primers were designed to facilitate amino acid substitutions at codons 104 and 108. The primers ranged between 20 to 100 nucleotides in length to improve template annealing. The degenerate primers spanned nucleotides of codons 103 to 110 with a change rate of 2 nucleotide per oligonucleotide, which should allow for the creation of mutant cDNAs, each coding a polypeptide whose primary structures differ by at least one amino acid. A nucleotide sequence (6 bases) with a mutation rate of zero was added to the 5' and 3' ends of the oligonucleotides to protect the integrity of the mutation rate throughout the primer. Using this strategy, we successfully generated random mutant GSTP1 cDNAs, which were subsequently ligated into the expression vector pBK-CMV. Structural and functional characterizations of the novel mutants are presently ongoing.

991 VIMENTIN GENE EXPRESSION IS ALTERED IN THE CENTRAL NERVOUS SYSTEM (CNS) OF HENS TREATED WITH DIISOPROPYLPHO-SPHOROFLUORIDATE (DFP).

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A single dose of DFP (1.7 mg/kg/s.c.) produces delayed neurotoxicity in hens in 7-14 days. DFP produces OPIDN by altering the levels and functions of serveral cytoskeletal proteins including enzymes. Vimentin plays an important role in the maintenance of brain homeostasis vital for neuronal survival after injury. Hence, we carried out a time course study in which hens were sacrificed at different time points; 1,2,5,10,20 days after intramuscular administration of DFP. Total RNA was extracted from brain regions, cerebrum, cerebellum, and brainstem as well as the spinal cord. Northern blots were prepared and hybridized to vimentin c-DNA probe. The results indicate that vimentin levels were down-regulated in spinal cord at 1 and 2 days to 47% and 50 % respectively. There was a moderate increase to 65% at 5 days and the levels remained at 58% and 61 % for 10 and 20 days, respectively. In brainstem the mRNA levels were down-regulated to 55% and 53% at 1 and 2 days, respectively and stayed at 62% and 65% during 5 and 10 days. At 20 days the levels decreased further to 53%. Hence the mRNA levels in susceptible tissues (brainstem and spinal cord) never recovered to control levels at 20 days. In contrast there was no decline in the transcript level in cerebrum(non-susceptible tissue) at 1 day (98%) and levels were up-regulated to 134%, 123% at 2 and 5 days respectively. On the other hand, the message levels returned to near control values at 10 and 20 days (105% and 97% respectively). In cerebellum (which is also a susceptible tissue) after an initial decline at 1 and 2 days (77% and 46% respectively), vimentin levels were re-induced rapidly (128%) at 5 days and stayed at the same levels (128%) until 10 days. The transcript levels came down to control vaules (101%) at 20 days. The differential and distinctive expression pattern of vimentin in DFP-induced OPIDN indicate its possible role in the astroglial pathology leading to CNS injury and axonal degeneration. (Sponsored by NIEHS grant ESO 5154.)

992 IMPACT OF CHEMICALLY-INDUCED OXIDATIVE STRESS ON CADHERIN/CATENIN COMPLEXES.

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Previous work has shown that chemically-induced oxidative stress disrupts the protein interactions of the E-cadherin/β-catenin/α-catenin complex in precision-cut mouse liver slices. Although these data suggest a role for oxidative stress in disruption of cell-cell adhesion mediated by cadherin/catenin complexes, our understanding of these effects are confounded by the fact that the composition of cadherin/catenin complexes is not homogenous in the liver. Both E- and N- cadherin are expressed in the liver, as well as β- and γ-catenin; thus four distinct complexes mediate cell-cell adhesion in the liver: E-cadherin/β-catenin/α-catenin; E-cadherin/γ-catenin/α-catenin: N-cadherin/β-catenin/α-catenin; N-cadherin/γ-catenin/α-catenin. Taking advantage of the retention of normal cellular heterogeneity offered by precision-cut mouse liver slices, the current study was designed to examine the impact of chemically-induced oxidative stress on cadherin/catenin complexes. Precision-cut mouse liver slices were challenged with diamide [25-250 μM; 6 hr] or tert-butylhydroperoxide [5-50 μM; 6 hr]. A polyclonal antibody against γ-catenin was used to immunoprecipitate proteins prior to Western blot analysis with monoclonal antibodies to E- or N-cadherin. Interestingly, no effect on protein interactions of γ-catenin with either cadherin was observed. Indirect immunofluorescence was used to co-localize catenins and cadherins following chemical challenge. A heterogeneous reduction in co-localization was seen in precision-cut liver slices. Taken together, these data suggest that oxidative stress selectively disrupts cadherin/catenin complexes in the liver. This response is dictated, in part, by the protein composition of the cell adhesion complex. (Supported by NIEHS center grant 09106.)

993 THIOL DISULFIDE REDOX IN GROWTH CONTROL OF HT29 CELLS.

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Redox sensitive proteins have been shown to effect changes in cell proliferation. To determine whether extracellular redox conditions affect cell proliferation in HT29 cells, we grew cells in cysteine-containing medium with additions of either cystine or GSSG to shift redox potential (Eh) to a more oxi-

dized condition. Cell proliferation rates were measured by both tritiated thymidine incorporation and BrdU incorporation. Results show that cell proliferation rate varied as a function of thiol-disulfide redox state. Over the range of Eh found for GSSG/GSH and cysteine/cystine pools in human plasma (-50mV to -130mV), the rate approximately doubled. As a model system to determine whether changes in the redox state of intracellular proteins occurred under these conditions, we examined the redox state of thioredoxin *in vivo*. Protein extracts were carboxymethylated, and modified proteins were separated by polyacrylamide gel electrophoresis under native conditions. Bands corresponding to varying redox states of thioredoxin were identified by western blot. Results show that thioredoxin is more oxidized in cells cultured in a more oxidized condition. These experiments demonstrate that changes in extracellular redox conditions induce changes in cell proliferation, and that corresponding changes in the state of an intracellular protein can be assessed by western blot of carboxymethylated cell lysates. (Supported by NIH Grant ES09047.)

994 TOXICANT- & PROSTAGLANDIN-MEDIATED INDUCTION OF ELONGATION FACTOR 1α.

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Prostaglandins (PGs) have many diverse, and often opposing, roles in cellular signaling pathways. In a model of toxicant-induced nephrotoxicity, PGE_2 and its stable synthetic analog, 11-deoxy-16,16-dimethyl PGE_2 (DDM-PGE_2), protect renal epithelial cells (LLC-PK_1) against 2,3,5-*tris*-(glutathion-*S*-yl)hydroquinone [TGHQ]-induced cytotoxicity via an unknown receptor-mediated mechanism(s). ^{35}S-methionine labeling of newly synthesized proteins following treatment of LLC-PK_1 cells with DDM-PGE_2 revealed the induction of a number of proteins. Electrospray mass spectrometric analysis identified in-gel tryptic digests of two ^{35}S-methionine labeled proteins to be elongation factor 1α (EF-1α) and actin. EF-1α is a critical actin-binding protein involved in peptide elongation during protein synthesis, which is also found in close association with the cytoskeleton. DDM-PGE_2 (1 μM, 24 h) induced EF-1α synthesis 12 fold as indicated by western blot analysis. The role of EF-1α, if any, in the cytoprotective mechanism(s) of DDM-PGE_2 remains to be determined. However, it is possible that elevated EF-1α levels may protect kidney cells from acute toxicity by facilitating the synthesis of additional cytoprotective proteins. Indeed, EK-3 cells (a TGHQ-transformed Eker rat-derived renal epithelial cell line) exhibit elevated constitutive EF-1α levels, which were further increased following additional exposure to TGHQ (300 μM; 1h). TGHQ also induced EF-1α synthesis in LLC-PK_1 cells (4 fold; 300 μM, 2 h). Moreover, TGHQ-induced tumors in Eker rats express elevated EF-1α levels. The ability of TGHQ to increase EF-1α synthesis, and elevated EF-1α expression in TGHQ-induced tumors, may indicate a role for EF-1α and PGs in TGHQ-mediated nephrocarcinogenicity. (ES 07247, GM 56321, ES 07784.)

995 CHANGES IN HISTONE PHOSPHORYLATION IN RESPONSE TO QUINOL THIOETHER INDUCED ONCOTIC AND APOPTOTIC CELL DEATH.

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Chromatin structure plays an important role in regulating gene expression. Histones are essential for maintaining chromatin structure and are therefore intimately involved in modulating gene expression. A role for the post-translational modification of histones in apoptosis has been proposed, but remains a subject for debate. In contrast, little information is available on histone modification during oncotic cell death. We have previously shown that 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ) induces growth arrest and cell death in human leukemia cells (HL-60) and porcine renal proximal tubular epithelial cells (LLC-PK1). We therefore compared histone phosphorylation patterns during TGHQ induced apoptosis of HL-60 cells and TGHQ induced oncosis of LLC-PK1 cells. TGHQ induced apoptosis in HL-60 cells is preceded by dephosphorylation of histone H2A. Because phosphorylation of histone H2A occurs throughout the cell cycle the results suggest that dephosphorylation of H2A may be due to growth arrest, which precedes apoptosis. In contrast, although TGHQ induced LLC-PK1 cell oncosis is also preceded by dephosphorylation of histone H2A, this is followed by the subsequent phosphorylation of histone H3. Phosphorylation of histone H3 at Ser-10 and Ser-28 plays an important role in chromatin condensation during mitosis and during premature chromosome condensation (PCC). Consistent with this, chromatin sensitivity to micrococcal nuclease, which preferentially cleaves active chromatin, decreases in response to TGHQ in LLC-PK1cells. Collectively, these results suggest that during oncosis LLC-PK1cells attempt to engage PCC, which leads to mitotic catastrophe and cell death (ES 07359, ES 07784).

996 POTENTIAL INVOLVEMENT OF PROTEIN PHOSPHATASE 2B AND ROTAMASE IN NORMALIZATION OF GAP JUNCTIONAL COMMUNICATION AND CONNEXIN43 PHOSPHORYLATION AFTER EXPOSURE OF V79 FIBROBLASTS TO A PHORBOL ESTER.

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Several kinases have been implicated in the regulation of gap junctional intercellular communication (GJIC), presumably through phosphorylation of the gap junction proteins. Connexin43 (Cx43) is one of the major gap junction proteins in the body. In many cell types, including V79 Chinese hamster lung fibroblasts, the Cx43 phosphorylation status may profoundly change concurrently with a strong inhibition of GJIC after activation of protein kinase C by 12-*O*-tetradecanoylphorbol 13-acetate (TPA). After removal of TPA, both parameters normalize within 3-4 hours, even in the presence of cycloheximide, indicating independence of protein synthesis. We have here studied the potential involvement of phosphatases in the normalization process. Protein phosphatase (PP) 1 and 2A inhibitors (okadaic acid, calyculin A) had no effect, while PP2B inhibitors (cyclosporin A, FK506) delayed the normalizations by about 1 hour. Cyclosporin A and FK506 are dependent on accessory proteins called cyclophilins and FK506-binding proteins (FKBPs) for the inhibition of PP2B. These proteins possess rotamase activity. The rotamase activity of FKBPs is also inhibited by rapamycin, an agent that does not inhibit PP2B. Rapamycin had no effect of the normalization of GJIC after TPA exposure, but surprisingly, it delayed the normalization of Cx43 phosphorylation status even more than cyclosporin A and FK506. Furthermore, TPA causes disappearance of gap junction plaques in immunofluorescence experiments. Cyclosporin A, FK506 and rapamycin delayed the normalization of reappearance of Cx43-containing gap junction plaques. These results may suggest that PP2B is involved in the normalization of GJIC after TPA exposure, while rotamase activity is involved in the normalization of Cx43 phosphorylation status.

997 ROLES OF MITOGEN-ACTIVATED PROTEIN KINASES IN OXIDATIVE STRESS MEDIATED CELL DEATH SIGNALING IN LLC-PK1 CELLS.

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The mitogen-activated protein (MAP) kinase family mediates important signal transduction pathways in response to diverse external signals. 2,3,5-*tris*-(glutathion-*S*-yl)hydroquinone (TGHQ) is a potent nephrotoxicant and nephrocarcinogen causing cell death in a reactive oxygen species (ROS) dependent manner. The extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNK/SAPK) are rapidly phosphorylated in renal proximal tubule epithelial cells (LLC-PK_1) following treatment with TGHQ, which also depends upon the generation of ROS. We now report that the activity of ERKs is elevated 4 fold 30 min after TGHQ treatment. Consistent with their phosphorylation pattern, activation of ERKs is transient and returns to constitutive levels by 5 hr. Moreover, the phosphorylation of p38 also increases in response to TGHQ treatment as evidenced by western blot analysis. We have previously shown that activated ERKs and p38 appear to contribute to cell death signaling, as inhibition of ERKs or p38 by PD098059 and SB202190 protected cells against TGHQ mediated cell death. In addition, TGHQ strongly activates the AP-1 and NFκB transcription factors. The DNA binding activity of AP-1 is abrogated by PD098059 and SB202190, presumably as a consequence of the decreased stress signaling. However, both kinase inhibitors potentiated TGHQ mediated NFκB activation. Furthermore, a NFκB inhibitory peptide partially reverses PD098059 mediated protection against cell death, suggesting that NFκB activation is an important element of the stress response. Taken together, the activation of MAP kinases, especially ERKs, plays a key role in cell death signaling in response to TGHQ induced oxidative stress in LLC-PK1 cells, and suppression of NFκB by MAP kinases may be involved in this cascade. (ES 07359, ES07784.)

998 THE EFFECTS OF MAP EXPRESSION ON METHYLMERCURY-INDUCED MICROTUBULE DISASSEMBLY.

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The sensitivity of microtubules (MTs) to methylmercury- (MeHg) induced disassembly was compared in undifferentiated, MAP1A- and MAP2C-transfected, and neuronally-differentiated P19 Embyronal Carcinoma (EC) cells. The extent of MT disassembly was examined qualitatively by mmunofluorescence microscopy and Western blotting and quantitatively by dot blotting of polymer and soluble proteins extracts. Immunofluorescence microscopy showed that MeHg disassembled MTs in a time- and dose-dependent manner and that MTs in both MAP2C-transfected and neuronally differentiated cells, but not those in MAP1A-transfected cells, were significantly more resistant to MeHg-induced MT depolymerization than those in undifferentiated cells. These results suggest that MAP2C has a greater ability to stabilize MTs against MeHg-induced disassembly than MAP1A. Surprisingly, however, when the extent of MT disassembly was assessed by Western blotting and by quantitative dot blotting no change was observed in the amounts of tubulin, MAP2, or MAP1A, in the polymer and soluble fractions in MeHg-treated samples, compared to the control cells that were not treated. These data indicate that although MeHg treatment results in the disassembly of Mts they were not depolymerized as detergent-soluble subunits. (Supported by NSERC Canada.)

999 PROTEIN-KINASE C (PKC) INHIBITS THE ACTIVITY OF ORGANIC ANION TRANSPORTING POLYPEPTIDE OATP1 AND OATP2 BY DIRECT PHOSPHORYLATION, BUT NOT BY INTERFERENCE WITH INTRACELLULAR VESICLE TRAFFICKING.

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Oatp1 and Oatp2 are expressed in hepatic sinusoidal membranes as well as apical membranes of kidney proximal tubules and choroid plexus in brain. Transport mediated by Oatp1 and Oatp2 is sodium-independent, ATP-independent and temperature-sensitive. They transport a wide spectrum of substrates with diverse structures. The substrate spectrum and tissue distribution indicate that Oatp1 and Oatp2 may play important roles in chemical disposition and drug-drug interactions. Several potential PKC phosphorylation sites in Oatp1 and Oatp2 have been revealed by amino acid sequence analysis. Therefore, the purpose of this study was to determine the effects of PKC on the transport activities of Oatp1 and Oatp2 by uptake assays in Oatp1- or Oatp2-cRNA injected Xenopus laevis oocytes. The present study demonstrated that the transport activities of Oatp1 ([3H]estrone-3-sulfate uptake) and Oatp2 ([3H]digoxin uptake) were inhibited by phorbol-12-myristate-13-acetate (PMA), a potent activator of PKC, in a dose- (1 nM to 10 mM) and time-dependent (5 to 60 min) manner. This inhibition is PKC specific in that an inactive PMA analogue, 4α-phorbol-12,13-didecanoate, had no effect on Oatp1- and Oatp2-mediated transport. In addition, the effect of PMA was blocked by a specific PKC inhibitor, bisindolylmaleimide (1 microM). PMA treatment increased phosphorylation of serine and threonine, but not tyrosine amino acid residues of the transporters. In contrast, pretreatment of oocytes with the microtubular inhibitor colchicine (1 mM for 8 hrs) did not prevent the decrease in transport elicited by PMA. Therefore, this study suggests that activation of PKC inhibits the function of Oatp1 and Oatp2, and that the mechanism by which PKC inhibits the transport of these transporters appears to be via direct phosphorylation, rather than interference with intracellular vesicle trafficking. (Supported by NIH grant ES-01142 and American Heart Grant KS-98-GS-11.)

1000 GENE EXPRESSION ARRAY ANALYSES OF SULFUR MUSTARD-INDUCED PROINFLAMMATORY MEDIATOR RESPONSE IN MOUSE EARS.

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Nucleic acid array technology is poised to become a principal technique in biomarker research. As part of the Chemical and Biological Weapons Defense Program, the United States Army Medical Research Institute of Chemical Defense is interested in identifying biomarkers predictive of exposure to vesicating agents such as sulfur mustard [bis(2-chloroethyl) sulfide; HD]. Studies were undertaken to profile gene expression patterns in RNA populations isolated from ear tissue of CD1 mice either treated or not treated with a candidate antivesicant compound prior to HD exposure. Adult, male mice were treated topically with either HD (0.16 mg/ear), indomethacin (IND), or IND followed by HD, on the medial ear surface. At approximately 6 hr or 24 hr following exposure, biopsies were taken, snap frozen in liquid nitrogen, and stored ≤ -70C. Messenger ribonucleic acid (mRNA) was isolated and a complementary deoxyribonucleic acid (cDNA) probe was radioactively labeled using gene-specific primers. After hybridizing the labeled cDNA probe to a 588-gene expression array (Atlas Mouse cDNA Expression Array, Clontech, Palo Alto, CA), data were analyzed using AtlasImage™ 1.0 (Clontech). Normalization was performed with two housekeeping genes, ubiquitin and ribosomal protein S29, by comparing expression on arrays probed with IND-, HD-, and HD/IND-treated samples to an array probed with a naïve sample. Genes indicative of tissue damage, stress, and inflammation have been identified from HD-exposed tissue, while HD/IND-treated tissue shows increased expression of genes involved in repair of damaged tissue. These data contribute to the understanding of the early events associated with HD-induced skin injury. (Supported by Contract No. DAMD17-89-C-9050 and by Battelle Memorial Institute.)

1001 CHARACTERIZATION OF SULFUR MUSTARD-INDUCED PROINFLAMMATORY MEDIATOR RESPONSE IN MOUSE EARS.

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Sulfur mustard, [bis(2-chloroethyl) sulfide; HD] is an alkylating agent that produces incapacitating blisters in exposed tissue. Recent reports suggest that proinflammatory genes may be useful biomarkers of vesicant-induced injury. Studies were undertaken to characterize levels of gene expression in animals exposed to HD using the mouse ear vesicant model (MEVM). Adult, male CD1 mice, housed under AAALAC guidelines, were exposed topically to HD (0.16 mg/ear), or a pre-treatment of either hydrocortisone (HC), indomethacin (IND), olvanil (OLV), or dexamethasone (DEX) and HD, on the inner surface of the right ear using established procedures. The left ear served as a vehicle (dichloromethane) control. At approximately 2 hr, 6 hr, and 24 hr following exposure, skin biopsies were taken, snap frozen in liquid nitrogen, and stored ≤ -70 C. Skin samples were analyzed for levels of mRNA using a Ribonuclease Protection Assay (RPA). There was an elevation of mRNA coding for Macrophage Inflammatory Protein-2 (MIP-2) at HD-exposed sites relative to control tissue at each timepoint, with peak-levels occuring at 24 hr. Without pre-treatments, Monocyte Chemoattractant Protein-1 (MCP-1) gene expression increased two-fold at 2 hr, peaked at 6 hr, and returned to a two-fold increase at 24 hr, and Interleukin-1β (IL-1β) message levels increased dramatically at 6 hr then decreased at 24 hr. Significant reduction in mRNA coding for MIP-2 was evident with DEX and IND pre-treatments at 24 hr compared to samples that had no pre-treatment. Pre-treatment with DEX decreased MCP-1 gene expression at 6 hr. Production of mRNA coding for IL-1β was significantly decreased at 6 hr with HC and DEX pre-treatments. These data contribute to the understanding of the early events associated with HD-induced skin injury. (Supported by Contract No. DAMD17-89-C-9050.)

1002 EFFECT OF PENTOXIPHYLLINE ON COLLAGEN SECRETION BY RAT HEPATIC STELLATE CELLS EXPOSED TO ACETALDEHYDE.

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Physiopathology of liver fibrosis is far from being completely understood, and the efficacy of the avaliable therapeutic strategies is disappointing. Increased evidence has so far implicated TNF-α as a pivotal molecule involved in liver damage. Pentoxiphylline (PTX) has been reported to inhibit TNF-α production as well as to decrease collagen deposition. Activated stellate cells are responsible for increased collagen deposition in liver fibrosis and cytokines are recognized as important mediators in this process. The aim of this study was to determine the effect of PTX on oxidative stress, collagen and TNF-α secretion by rat hepatic stellate cells (CFSC-2G) pretreated with acetaldehyde. Media containing 175μg/ml of acetaldehyde were added to rat hepatic stellate cells 24 hours after seed and replaced 48 hours later with media containing 200μM/ml of PTX which was collected after 24 hours. Cells

were counted and used to determine lipid peroxidation by malondialdehyde production. Collagen secretion was measured by the hydroxiproline micromethod in culture media. TNF-α secretion was quantified by ELISA. Neutral red and MTT assays were performed in presence of different concentrations of PTX to select the one that did not alter cellular function. A concentration above 250μM/ml decreased substantially cell function. PTX did not alter collagen secretion (0.3731 ± 0.035μmol/10^6 cells) in rat hepatic stellate cells. Acetaldehyde treated cells increased 2.6 times collagen secretion (1.264±0.22μmol/10^6 cells) while lipid peroxidation values enhanced by four times compared with control cells. In presence of PTX, acetaldehyde treated cells diminished by 3.1 times collagen secretion (0.3951±0.049∝mol/10^6 cells) compared to those not exposed to this drug, the concentration of collagen in the media was similar to that of control cells (0.4847±0.058∝mol/10^6cells). Similar results were obtained when anti-TNF-α was added. A slight increment in TNF-α was observed after acetaldehyde treatment which decreased with PTX treatment. The present study demonstrates that rat hepatic stellate cells increase collagen secretion as a result of acetaldehyde treatment. PTX brings collagen secretion levels to normal values probably by suppressing TNF-α secretion.

1003 THE ATM PROTEIN IS INVOLVED IN REPAIRING DNA DOUBLE STRAND BREAKS WITH SPECIFIC ENDS.

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Ataxia telangiectasia (A-T) is a human genetic disease characterized by progressive cerebellar degeneration, hypersensitivity to ionizing radiation (IR), and high cancer risk. The hallmarks of A-T cells are IR sensitivity and abnormally high rates of spontaneous and IR-induced chromosomal breakage and rearrangement. Compared to normal cells, A-T cells have slower kinetics and lower fidelity of repairing IR-induced DNA double strand breaks (DSB), indicating that there is a primary defect in DSB repair in A-T cells. The ATM (Ataxia telangiectasia mutated) gene encodes a 350 KD protein kinase that belongs to a PI-3 kinase family that includes proteins involved in DNA repair and cell cycle regulation, such as DNA-PK. The recent discovery that the ATM protein is able to bind to DSB, independent of DNA-PK, further supports a distinctive role of the ATM in DSB repair. We are investigating the role of ATM in DSB repair by measuring the capacity of nuclear extracts prepared from ataxia telangiectasia (A-T) fibroblasts to rejoin restriction endonuclease cleaved plasmid DNA. A reversion vector, SupF22 (a generous gift of M. Seidman), which was constructed to detect small deletions, was used in our experiments. SupF22 was linearized with 7 different endonucleases that cut between the two microhomologies. SupF22 reverts to an active SupF gene through precise deletion of 53 base pairs of DNA between two copies of the microhomology sequence GGGT and can be scored as blue colonies in the indicator bacterial strain. We found that DSB repair efficiency in both normal and A-T extracts decreased in the order: 4 bp 5' overhang > 2 bp 5' overhang > 3 bp 3' overhang or blunt ends. DSB repair fidelity in normal extracts ranged from 0 to 0.26%, regardless of the nature of DSB ends. Meanwhile, the repair fidelity of DSB with 5' overhang in A-T extracts was close to that in normal extracts. However, the repair fidelity was dramatically decreased in A-T extracts when SupF22 carrying DSB with blunt or 3' overhang ends was incubated in A-T extracts, a difference as much as 10 folds was observed in our experiments. These results indicated that the ATM protein might be involved in repairing DSB with blunt or 3' overhang ends.

1004 INDUCTION OF XPA BY CISPLATIN IN CISPLATIN-RESISTANT OVARIAN CARCINOMA CELLS.

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Purpose: Recent evidence suggests that DNA repair gene expression is regulated. Increased DNA repair gene expression has been implicated as a mechanism for resistance to cytotoxic chemotherapy. The role that regulation of DNA repair plays in cell survival is of great significance to our understanding molecular events associated with the response of metastatic cancer cells to cytotoxic chemotherapy. XPA is an essential part of the nucleotide excision repair pathway which removes the majority of DNA damage induced by the cytotoxic chemotherapeutic agent cisplatin (CP). XPA mediates the assembly of the excision complex at the site of DNA damage. We have investigated XPA regulation in response to CP treatment in CP-resistant and CP-sensitive ovarian carcinoma cells to test the hypothesis that CP-resistance is associated with DNA repair gene inducibility. **Methods:** Paired CP-sensitive (A2780) and CP-resistant (A2780/CP70) cells were treated with CP at their respective LC50 (3 μM and 40 μM) for 1 h. XPA expression was analyzed by Western blot of whole cell lysates. XPA promoter regulation was analyzed in reporter gene assays using XPA promoter/reporter gene constructs containing XPA promoter fragments of different lengths. Transcription factor binding to XPA promoter fragments was determined by electrophoretic mobility shift and super-shift analyses. **Results:** Preliminary data indicate that CP treatment induces XPA expression and XPA promoter activity in the CP-resistant cells but not in the CP-sensitive cells. XPA promoter induction was dependent on the presence of an upstream region of the XPA promoter. Electrophoretic mobility shift analyses indicated that multiple factors in both CP-sensitive and CP-resistant cells bind this upstream promoter fragment essential for CP induction. However, CP-sensitive cells contain an additional factor not found in CP-resistant cells. **Conclusions:** These data support the hypothesis that XPA expression is induced in response to CP treatment in CP-resistant tumor cells but not in CP-sensitive tumor cells. The results suggest the hypothesis that induction is inhibited by a negative acting XPA promoter binding factor in CP-sensitive cells. Current efforts are aimed at determining whether increased XPA expression is also associated with increased DNA repair capacity in CP-resistant cells and at identifying the putative negative acting factor present in CP-sensitive cells. (Supported by NIEHS grant R01 ES06460 and a Pilot Project award from NIEHS Center grant P30 ES06639.)

1005 EVALUATION OF LESION BYPASS IN *XERODERMA PIGMENTOSUM* VARIANTS CELL EXTRACTS.

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Sponsor: A. Puga.

The conversion of unrepaired DNA damage in the genome to mutations during DNA replication is a critical step in the initiation of carcinogenesis. A number of specialized DNA polymerases that are involved in replication and mutagenesis at DNA damage site in mammalian cells have recently been identified. These polymerases are products of a gene superfamily with homology to the bacterial SOS response gene *umuC*, which includes the *RAD30*, *RAD30B*, *REV3* and *DinB* genes. The human *RAD30* gene, which is mutated in the skin cancer prone disease Xeroderma Pigmentosum Variant (XPV), encodes a novel DNA polymerase, eta [η], required for error-free bypass of UV-induced pyrimidine dimers in DNA. To further investigate the DNA polymerase requirements for lesion bypass in normal and XPV lymphoblasts, we have developed an *in vitro* primer extension assay utilizing cell-free extracts. Primed oligonucleotides, 55-56 bases in length, containing known mutagenic lesions including an abasic (tetrahydrafuran) residue, pyrimidine dimer, or a (6-4) photoproduct at nucleotide 39 from the 5'terminus, were used as templates for DNA synthesis in these cell extracts. Replicated products were separated and analyzed by 15% PAGE. Our results show that replication is arrested at an abasic site following DNA synthesis in all extracts tested, as shown by the formation of synthesis products which are 38nt and 39nt in length. However some bypass of the abasic lesion was also observed, demonstrating that these cell extracts contain the polymerase activities required for lesion bypass. We have also performed experiments using aphidicolin, an inhibitor of the replicative DNA polymerases, α, δ and ε. Consistent with a requirement for additional DNA polymerase activities for replication at an abasic site, replication on damaged and undamaged DNA is differentially inhibited by aphidicolin. This *in vitro* assay can be used to investigate the biochemical requirements for bypass of a variety of lesions, including single benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide [BPDE] adducts, in human cell extracts. (Supported by NIH ES08079 [MPC] and ES07250-11 [BRJ/P.Stambrook, PI].)

1006 HIGH FREQUENCY OF LOSS OF HETEROZYGOSITY AT CHROMOSOME 11 IN BENZENE-INDUCED THYMIC LYMPHOMAS IN P53+/- TRANSGENIC MICE.

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In an ongoing study, benzene inhalation exposure has induced a high frequency (>80%) of thymic lymphomas in p53+/- mice beginning at six months of exposure. To characterize the presence and extent of loss of heterozygosity (LOH) on chromosome 11, we have examined the status of seven microsatellite markers in 27 benzene-induced and six spontaneous thymic lymphomas. LOH at microsatellite markers is an indication of large genetic alterations such as gross deletions, recombination or chromosomal loss. Benzene-induced thymic lymphomas exhibited six patterns of LOH and 59% (16/27) of induced lymphomas exhibited the same pattern. Each of the six spontaneous thymic lymphomas analyzed showed a unique pattern of LOH,

only one of which was exhibited by the benzene-induced thymic lymphomas. Over 90% (25/27) of the benzene-induced thymic lymphomas exhibited loss of the functional p53 allele, and the loss of p53 was accompanied by LOH at other microsatellite markers suggesting the LOH was a result of gross alteration of chromosome 11. The results indicate that benzene treatment is inducing a high frequency of LOH on chromosome 11 in p53+/- mice, and that loss of the functional p53 allele or some other gene on chromosome 11 is a key event in the induction of thymic lymphomas in these mice.

1007 FURTHER EVIDENCE FOR A PALINDROMIC ORIENTATION OF TRANSGENE PROMOTER SEQUENCE CRITICAL FOR TUMORIGENIC RESPONSIVENESS IN TG.AC MICE.

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Tg.AC mice have been shown to develop skin papillomas when exposed to carcinogens and this transgenic mouse model is being used in a short-term carcinogenesis assay for safety testing of pharmaceuticals. A subset of hemizygous Tg.AC mice were found to be refractory to carcinogens. We have previously shown that hemizygous Tg.AC mice (both responders and nonresponders) contain about 40 copies of transgene but the nonresponders had lost a 2 kb BamHI fragment containing z-globin promoter sequence (Thompson et al., 1998). Restriction enzyme and S1 nuclease digestion experiments strongly suggested that the 2 kb BamHI fragment results from a palindromic orientation of the transgene, though alternative interpretations could not be ruled out. Ongoing attempts to PCR and/or clone the "head-to-head" juncture have been unsuccessful, probably due to the symmetrical structure of the palindrome. Restriction enzyme and S1 nuclease digestion experiments indicated two subsets of nonresponder Tg.AC mice, one with a large, slightly asymmetric deletion across the "head-to-head" juncture of the palindrome and another with only a small asymmetric deletion in the same 2 kb BamHI fragment. Prompted by evidence for asymmetric deletions in the palindromic structure, we attempted to PCR across the "head-to-head" junction of transgene in nonresponder DNA using a strategy where one of the PCR primers was designed based on sequence thought to be deleted on only one side of the palindrome. The resulting PCR products were cloned and sequencing results confirmed the suspected palindromic orientation of transgene in Tg.AC mice. Furthermore, the deletions seen in nonresponder mice resulted in the loss of z-globin symmetry in the "head-to-head' region of the palindrome. Although a large number of full-length copies of transgene are present in Tg.AC mice, loss of "head-to-head" symmetry in palindromic structure, even by deletions as small as 75 bp, result in complete loss of ability of carcinogen to activate transgene expression.

1008 SELECTION OF DRUGS TO TEST THE SPECIFICITY OF THE TG.AC ASSAY BY SCREENING FOR INDUCTION OF THE *GADD153* PROMOTER *IN VITRO*.

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Increased experience with short-term carcinogenicity assays that use genetically altered mouse models, such as the Tg.AC assay, is required for understanding the strengths and limitations of these assays. To help select pharmaceuticals to test the limits of specificity of the Tg.AC assay, a rapid throughput *in vitro* assay was developed that is predictive of *in vivo* activity in the Tg.AC assay. This assay measures activation of a hamster *gadd153* promoter construct that is stably expressed in HepG2 cells. The *gadd153* promoter is responsive to DNA damage, oxidative stress, and endoplasmic reticulum impairment. 100 noncarcinogenic pharmaceuticals were screened in the *gadd*153 assay, along with two other gene induction assays less predictive of Tg.AC activity (zeta-globin promoter induction in K562 cells and fos promoter induction in HepG2 cells). About 20% of the drugs we screened induced the *gadd153* promoter. Several criteria were used to select the three best candidates for subsequent *in vivo* testing in a Tg.AC assay: whether drug solubility in acetone or ethanol was sufficient to elicit systemic toxicity, the level of *gadd153*, zeta-globin, and fos promoter inductions by the drug, the potency of the drug in the *in vitro* assays, and the cost of the drug required for a 6-month study. Testing whether noncarcinogenic pharmaceuticals that activate select stress response pathways *in vitro* can elicit papillomas in the Tg.AC skin paint assay will accelerate the evaluation of the specificity of the Tg.AC assay and assist in defining its appropriate usage in the safety testing of pharmaceuticals.

1009 CLONING AND DEXAMETHASONE REGULATION OF THE RAT ARYL SULFOTRANSFERASE (SULT1A1) GENE.

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Aryl sulfotransferase (SULT1A1) catalyzes the sulfation of phenols, acetaminophen, minoxidil and other substrates. Previous reports demonstrated that rat hepatic SULT1A1 mRNA levels are induced by glucocorticoid treatment. In the present study, a 1921 bp fragment of the 5' flanking region of the SULT1A1 gene was isolated from a rat genomic library using the GenomeWalker kit (Clontech Laboratories, Inc.). A computerized analysis of the sequence for potential transcription factor binding sites indicated that the SULT1A1 gene does not contain a consensus glucocorticoid response element (GRE). In order to examine the regulation of SULT1A1 gene transcription by glucocorticoids, luciferase reporter constructs containing either the intact 1921 bp SULT1A1-5'flanking region or SULT1A1-5' deletion fragments were prepared and transiently transfected into primary cultured rat hepatocytes. Transfectants containing the intact SULT1A1-5':luceriferase construct displayed substantial increases in luceriferase activity (of~30-fold) in response to 24h treatment with dexamethasone (DEX, 10^{-7}M) relative to DMSO-treated controls. In hepatocytes transfected with either of two deletion constructs prepared from the SULT1A1-5' flanking region (beginning -768 bp and -477 bp relative to the transcription start site), DEX (10^{-7}M) produced ~17- and ~14-fold increases in luceriferase activity, respectively. The results suggest that a functional *cis*-acting DEX-responsive element is located within ~477 bp of the SULT1A1 core promoter. (Supported by NIH Grant ES05823 and NIEHS Center Grant ES06639.)

1010 TISSUE DISTRIBUTION OF ALCOHOL DEHYDROGENASE, ALDEHYDE DEHYDROGENASE AND CYTOCHROME P450-2E1 IN CHRONIC ETHANOL-TREATED SPRAGUE-DAWLEY RATS.

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The class I alcohol dehydrogenase (ADH) is the major mammalian enzyme involved in ethanol oxidation. The ethanol-inducible cytochrome P450-2E1 (CYP2E1) and the catalase-H2O2 system also metabolize ethanol to acetaldehyde. Acetaldehyde is further metabolized to acetate principally by the mitochondrial aldehyde dehydrogenase (ALDH2), as well as the cytosolic aldehyde dehydrogenase (ALDH1). Many of the toxic and pathologic effects associated with chronic alcohol consumption are attributed to increased formation of acetaldehyde and reactive oxygen species. In order to characterize the effect of chronic ethanol treatment on protein levels of ADH, ALDH and CYP2E1, the tissue distribution of these enzymes was examined by Western blot analysis. Male Sprague-Dawley rats were fed a low-carbohydrate liquid diet containing 35% ethanol-derived calories for 100 days. Pair-fed control rats received the liquid diet containing dextrose isocaloric to the ethanol calories consumed by the ethanol-fed rats. Tissue homogenates were prepared from 12 tissues including liver, kidney, heart, lung, stomach, intestine, pancreas, spleen, bladder, testis, muscle and brain. The CYP2E1 protein levels were increased in liver and kidney of ethanol-treated rats compared to control animals. In contrast, chronic ethanol treatment did not alter the class I ADH protein levels in any of the tissues where the protein was detected. Similarly, ALDH2 protein levels were not altered by the ethanol treatment when compared to controls. These results suggest that chronic ethanol treatment increases CYP2E1 protein in rat liver and kidney, but does not influence class I ADH or ALDH2 protein levels in rat tissues. The induction of CYP2E1 by ethanol, and the consequent increase in acetaldehyde formation and oxidative stress, may partially explain the fatty accumulation in the liver and kidney that is associated with chronic alcohol abuse. (Supported by R01 AA11885 [VV] and R01 AA09300 [DRP].)

1011 METABOLISM OF THE NICOTINE BY-PRODUCT NNAL BY HUMAN UDP-GLUCURONOSYLTRANSFERASES (UGTS).

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The tobacco-specific and carcinogenic nitrosomine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is the major metabolite formed from the carbonyl reduction of NNK, a nitrosation product of nicotine. The major detoxification step of NNAL is felt to be the formation of NNAL-beta-O-D-glu-

cosiduronic acid (NNAL-Gluc) catalyzed by the superfamily of UGTs. In humans, we have demonstrated that the UGT1 and UGT2B genes are differentially expressed in different tissues of the gastrointestinal tract. This work has led to the cloning of twelve of the fifteen known human UGT cDNAs, each being characterized for substrate specificity following expression in V79, HK293 or insect SF-9 cells. Highly purified NNAL was incubated with total cellular extracts expressing the different UGTs to examine the ability of the expressed enzymes to form NNAL-Gluc. Two forms of the glucuronide are generated with human liver microsomes as detected by TLC, NNAL-Gluc-a (rf=0.68) and NNAL-Gluc-b (rf=0.74). Expression of UGT2B4, 2B7, 2B10 and 2B15 does not support glucuronidation of NNAL. UGT1A7, expressed in biliary and gastric epithelium as well esophageal tissue, formed approximately ten fold more NNAL-Gluc-a than NNAL-Gluc-b. On a comparable level, UGT1A9, which is expressed in liver, intestine and esophagus, formed similar ratios of NNAL-Gluc as found with UGT1A7. UGT1A10, found in all extrahepatic tissues, catalyzed only NNAL-Gluc-a. UGT1A6, not found in the esophagus but in most other tissues, also generated only NNAL-Gluc-a. There was no detectable activity for UGT1A1, 1A3, 1A4 or 1A8. It is worth noting that three of the active proteins, UGT1A7, 1A9 and 1A10 are expressed in esophagus, a tissue that is susceptible to the carcinogenic actions of tobacco smoke adducts. (Supported by USPHS grants CA79834 [RHT], and DFG STR493/3-1 [CPS].)

1012 ETHANOL METABOLISM AND SENSITIVITY IN MICE WHICH LACK CYP2E1 EXPRESSION.

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Ethanol is metabolized to acetaldehyde mainly by the alcohol dehydrogenase pathway (ADHs) and to a lesser extent by microsomal oxidization (CYP2E1) and the catalase-H2O2 system. Acetaldehyde, which is responsible for some of the ethanol-induced effects, is further oxidized to acetate by aldehyde dehydrogenases (ALDHs). In this study, a transgenic Cyp2e1(-/-) mouse line was used to elucidate the role of this enzyme in ethanol metabolism and sensitivity. Ethanol was administered to both male and female Cyp2e1(-/-) and 129/sv wt mouse lines and comparisons of the pharmacokinetic parameters and sleep times were evaluated. Differences were evaluated by quantitating the concentrations of ethanol and acetaldehyde in whole blood at 15, 30, 60, 120, and 180 minutes following a single i.p. dose of ethanol (2.0, 3.0, 4.0, or 5.0 g/kg) by gas chromatography-headspace analysis. Differences in sleep time were determined for each genetic stock and sex following a single i.p. dose at 3.0, 4.0, or 5.0 g/kg of ethanol. Following i.p. administration, the rates of ethanol elimination from whole blood were similar regardless of dose or genetic stock. However, the blood acetaldehyde levels were higher in wt mice than in Cyp2e1(-/-) mice. Interestingly, female mice achieved higher ethanol blood levels than the males following equivalent dosing. In contrast, acetaldehyde was found to reach higher levels in male animals yet not in females. Male mice exhibited a longer sleep time than females. The Cyp2e1(-/-) mice yielded longer sleep times than the control animals in both sexes. These data suggest that although the contribution of CYP2E1 in ethanol oxidation may be limited, this enzyme may play a role in ethanol sensitivity in the brain. In vitro metabolic studies will be performed on microsomal enzymes isolated from the brain and liver of both genetic mouse lines. (Supported by R01 AA11885 [VV] and NIAAA Training Grant 5T32AA07464 [TLZ].)

1013 ISOLATION AND SEQUENCING OF CYP1A CDNAS FROM FIVE SPECIES OF BUTTERFLYFISH: A CASE FOR DIET-DRIVEN EVOLUTION IN A CRITICAL BIOTRANSFORMATION ENZYME.

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Complementary DNAs of cytochrome P450 1A (CYP1A) have been cloned and sequenced from the livers of five butterflyfish species (*Chaetodon auriga, C. citrinellus, C. melannotus, C. plebius,* and *C. vagabundus*) collected at Lizard Island, Australia. The cDNAs were obtained by reverse-transcriptase polymerase chain reaction using primers derived from conserved codons in the amino acid coding region of teleost CYP1A. The 1186-1188 base pair products are within the open reading frame encoding 395 amino acid proteins containing the I-helix and heme binding regions typical of CYP proteins. The nucleotide and derived amino acid sequences are most closely related to the butterflyfish, *C. capistratus*. A rooted genetic distance tree was obtained using these sequences and sequences from ten teleost sequences. Based on parsimony analysis, *C. vagabundus* and *C. auriga* group together, *C. plebius* and *C. melannotus* group separately, and these form separate branches from *C. capistratus*. *C. citrinellus*, a phenotypically distinct member of the genus, does not group with the other butterflyfish. Interestingly, *C. vagabundus*, *C. auriga*, and *C. citrinellus* maintain generalist feeding patterns, while *C. plebius* and *C. melannotus* display highly specialized feeding preferences for chemically defended hard and soft corals, respectively. This evidence suggests that diet-related factors may have driven the molecular changes exhibited in CYP1A among butterflyfishes and provides a basis for the differential expression of this enzyme among the species.

1014 SODIUM AZIDE-INDUCED THERMOTOLERANCE AND STRESS PROTEIN INDUCTION IN WILD-TYPE AND MUTANT STRAINS OF *C. ELEGANS*.

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Sodium azide, a mitochondrial toxicant, has long been used to anesthetize the nematode *C. elegans*, but the mechanism by which this organism can survive this exposure is not known. We hypothesized that survival may involve cellular stress response mechanisms, and as such may produce cross-tolerance to thermal stress. Worms pre-exposed to 10 mM azide showed significantly increased thermotolerance to 37°C, with a 0.68 survival probability (SP) compared with 0.07 SP for control worms taken directly to 37°C without prior azide exposure ($p<0.05$). This is, however, less than the 0.92 SP for worms pre-exposed to 33°C (the classic heat shock phenomenon). Using SDS-PAGE, Coomassie Brilliant Blue staining, and Western Blot analysis, we observed induction of hsp70 and hsp16 following azide exposure, although levels of induction were low compared to the worms pre-exposed to 33°C. Under unfavorable conditions, *C. elegans* enters a non-feeding, non-aging life cycle stage known as the dauer stage. We tested *daf-21*, a constitutive dauer-forming hsp90 mutant, and were able to demonstrate azide-induced thermotolerance, indicating that hsp90 may not be critical to the worm's response to stress. Azide-induced thermotolerance was also displayed by several other constitutive dauer-forming mutants (*daf-2*, *daf-7*, and *daf-14*). Also, by selecting for individual worms that survived 37°C without prior exposure to azide or elevated temperatures we have identified and characterized several intrinsically thermotolerant substrains of the N2 wild type *C. elegans* strain.

1015 IDENTIFICATION AND CHARACTERIZATION OF AN E3 UBIQUITIN- PROTEIN LIGASE IN THE SOFT SHELL CLAM (MYA ARENARIA).

M. L. Kelley and R. J. Van Beneden. *University of Maine, Orono, ME.* Sponsor: M. E. Hahn.

Certain softshell clam (Mya arenaria) populations in eastern Maine have a high prevalence of gonadal tumors; the cause of these tumors is unknown. A significant contaminant found in these environments is dioxin, a known carcinogen. A cDNA fragment with sequence similarity to the E3 family of ubiquitin-protein ligases was first identified from gill mRNA of dioxin-exposed clams. E3 enzymes are responsible for ligating ubiquitin molecule(s) onto a protein, targeting it for degradation. E6-AP is the best studied member of the E3 ubiquitin-protein ligase family defined by a conserved region of approximately 350 amino acids termed the "homologous to the E6-AP carboxyl terminus" (hect) domain. The hect domain includes several highly conserved residues. This includes an absolutely conserved cysteine residue 30-40 amino acids from the carboxyl terminus which is the site of ubiquitin thioester bond formation. E6-AP targets the tumor suppressor p53 for degradation; a decrease in p53 protein is known to affect cell cycling, thus promoting cell proliferation. This pathway has been observed in human cervical cancer. The cDNA sequence of clam E3 has been determined and is structurally similar to the vertebrate E6-AP at the predicted amino acid level (47-48% and 49% identical to mouse and human E6-AP, respectively). We are exploring whether the clam E3 protein is functionally similar to other E3 proteins by determining if it covalently binds ubiquitin, a hallmark of this protein-degradation pathway. We will also identify proteins targeted for degradation by clam E3. Results of these studies may contribute to an understanding of gonadal tumor progression in this invertebrate model system.

1016 ANALYSIS OF GENE EXPRESSION IN F-344 RATS FOLLOWING DERMAL EXPOSURE TO FUELS AND SOLVENTS.

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In an effort to establish safe exposure scenarios for military personnel coming into contact with fuels and solvents, we proposed to develop a predictive mathematical model of early cellular responses of the skin. Several components of jet fuels are considered to be dermal irritants. Cellular responses to irritants are complex and not completely understood. Cellular damage releases cytokines and chemokines, which are involved in the initial inflammatory response. One such chemokine, nitric oxide (NO), causes dilation of the vasculature. NO is produced by inducible nitric oxide synthase (iNOS). The iNOS gene is upregulated during the inflammatory response. Therefore, the analysis of iNOS mRNA may be useful for studying the process of dermal irritation. Isolation of high quality, undegraded RNA is crucial to the analysis of gene expression patterns. However, the isolation process becomes particularly challenging in the skin. Skin is tough, difficult to homogenize, and is abundant in RNAses. We developed a technique of harvesting rodent skin which minimizes the degradative effects of RNAse, while providing a sample that is easily homogenized in the isolation reagent. Our technique involves immediately submersing the freshly harvested skin sample in a solution of 10mM vanadium chloride to inhibit RNAse, then mincing the tissue and flash freezing with liquid nitrogen. Frozen, minced tissue is then pulverized with a stainless steel mortar and pestle and homogenized in a commercial isolation reagent containing phenol and guanidine thiocyanate. We were able to obtain RNA with an average yield of 1.0 - 5.0 μg RNA/mg skin and a OD_{260}:OD_{280} ratio of 1.6 to 1.8. Gel electrophoresis and northern analysis with 28S and actin cDNA probes confirm the quality of the RNA that we obtained by this method. (Supported by CDC/NIOSH RO1 OH03654-01 and AFOSR 92HE05COR.)

1017 EFFECTS OF ADENOVIRAL VECTORS ON MOUSE LIVER PROTEIN PATTERNS.

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Adenoviral vectors, when administered intravenously, are associated with acute hepatotoxicity and transient vector expression. It has been suggested that continued viral gene expression may limit the duration of vector expression due to cellular immune responses and may also contribute to the hepatotoxicity observed. To further decrease viral gene expression, two vectors were constructed to eliminate E4 viral gene expression. One vector, Av4dlP, contained the E4 coding region but was deleted in the E4 promoter; another vector Av4orf3, was deleted in the E4 coding region except for orf3. The parental vector Av3, contained the complete E4 gene and its promoter. Each of these three vectors also contained the nuclear localized β-galactosidase gene (nBg) as a reporter. A one week comparison study was performed in C57BL/6 mice. Mice were treated with a single intravenous administration of one adenoviral vector at a dose of 5 x 10^9 particles/mouse and were sacrificed 1 week after vector administration. Liver function tests (LFTs) were performed the day after vector administration and every other day until termination. Liver samples were collected at necropsy for immunohistochemistry to evaluate gene transfer and vector expression, histopathology and proteome analysis. There was a clear ranking of the adenoviral vectors with regard to the severity of the effects on liver protein patterns. The Av4dlP vector was the least hepatotoxic when all parameters, including liver protein pattern, liver function tests and histopathological investigation were taken into consideration. A large set of liver proteins was altered by the vectors of which 12 have been identified. The proteins thus far identified indicate impairment of mitochodrial function and effects on the cytoskeleton. While the mechanism of adenoviral vector hepatotoxicity remains unclear, proteome analysis is a sensitive tool for comparisons of cellular effects of vector modifications and may aid in the selection of less toxic vectors.

1018 PROTEOMIC ANALYSIS IN RENAL TOXICITY.

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Proteomic analysis involves the systematic separation, identification and characterisation of proteins present in a tissue or other biological sample. By comparing the proteins present in samples from animals given a test material with those present in samples from control animals, it is possible to identify directly those proteins that are potentially related to target organ toxicity. In molecular investigations, proteomic analysis is often regarded as equivalent to genomic analysis. In toxicity studies, although the pattern of gene activity will be abnormal in a tissue with a pathological lesion, there can be a poor correlation between the level of activity of different genes and the relative abundance within the tissue of the corresponding proteins. This is especially true where the mode of action of the test material interferes with protein synthesis and/or post-translational modification. Consequently, the information about a pathological process that can be derived from analysis at the level of gene activity is incomplete. Unlike genes, until very recently it was not technically possible to subject proteins to high throughput, automated analysis. Proteomics technology has now bridged that gap, making it possible to analyse proteins using high throughput, automated techniques. This paper describes the results obtained when proteomes were analysed from a rat repeat-dose toxicity study using the known nephrotoxin, Gentamicin. A treatment related response was obtained for the kidney cortex proteomes that was evident at dose levels lower than those which had elicited changes in conventional toxicopathological endpoints. 40mg/kg/day gentamicin produced only minor changes by histopathological investigation and no changes in clinical pathology parameters. Preliminary proteomic analysis showed changes in >100 expressed proteins in the kidney cortex at doses of 0.1, 1, 10 and 40mg/kg/day. Ongoing work to be presented includes identification of affected proteins and proteomic analysis of urine and plasma from the treated animals.

1019 ARYL HYDROCARBON RECEPTOR-MEDIATED INHIBITION OF MAMMARY TUMOR GROWTH IN AN ATHYMIC NUDE MOUSE MODEL BEARING MCF-7 CELL XENOGRAFTS.

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6-Methyl-1,3,8-trichlorodibenzofuran (6-MCDF) binds to the aryl hydrocarbon receptor (AhR) and exhibits partial AhR antagonist activity for several receptor-mediated toxic responses. In contrast, 6-MCDF induces AhR-mediated antiestrogenic activity in the rodent uterus and human breast cancer cell lines. The role of the AhR in mediating the antiestrogenic/antitumorigenic activity of 6-MCDF was further investigated in athymic nude mice implanted with an estrogen pellet and bearing MCF-7 human breast cell xenografts. MCF-7 cells express a functional AhR and estrogen receptor α (ERα), and 10^7 cells in a Matrigel suspension were transplanted on both sides of the mammary fat pad. After allowing 1 week for tumor formation, one group received corn oil (vehicle control) and a second group was treated with 25 mg/kg/day 6-MCDF by oral gavage. A 7-fold increase in tumor volume was noted in the control over a period of 5 weeks; however, in animals treated with 6-MCDF, no significant induction of tumor volume was observed over the same time period. Postmortem analysis showed no significant differences in body and organ weights between the two treatment groups, and CYP1A1-dependent hepatic microsomal ethoxyresorufin *O*-deethylase was not significantly induced by the dose of 6-MCDF used in this study. Results of preliminary studies using other breast cancer cell lines including Ah-nonresponsive benzo[a]pyrene-resistant MCF-7 cells confirm the role of the AhR in mediating the antitumorigenic response of 6-MCDF. (CA64801 and ES09106)

1020 METHYLENE-SUBSTITUTED 1,1'-DIMETHYLDIINDOLYLMETHANE ANALOGS AS INHIBITORS OF CARCINOGEN-INDUCED MAMMARY TUMOR GROWTH IN RODENTS.

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Diindolylmethane (DIM) is an aryl hydrocarbon receptor (AhR) agonist and inhibits estrogen-induced responses in the rodent uterus and carcinogen-induced mammary tumor growth in female Sprague-Dawley rats. A number of new methylene-substituted analogs of DIM have been synthesized and investigated for their antiestrogenic and antitumorigenic activities in rodent models. Initial studies examined 1,1'-dimethyldiindolylmethane analogs substituted at the CH_2 bridge with 4-hydroxyphenyl, 4-methoxyphenyl and 1-naphthyl substituents. All of these compounds were only weakly active in AhR binding or transformation assays, and at doses as high as 100 mg/kg/day (X3), no induction of hepatic microsomal CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity was observed in C6B3F1 mice. Additionally, treatments do not induce estrogenic responses in the mouse

uterus. Despite the apparent lack of AhR agonist activity, the C-substituted DIM analogs inhibited growth of 7,12-dimethylbenz[a]anthracene-induced mammary tumors at doses as low as 1.0 mg/kg/every second day. At this dose, there was no significant tumor growth throughout 21 days of treatment, whereas in vehicle control animals, tumor volumes increased >3.5-fold. Thus, these analogs are more potent inhibitors of mammary tumor growth than DIM, and preliminary results suggest that their mechanisms of antitumorigenic action are at least, in part, through AhR-independent pathways. (CA64081 and ES09106)

1021 ABERRANT METHYLATION OF THE *p16INK4a* PROMOTER AND ALTERED PROTEIN EXPRESSION IN AFLATOXIN B_1 (AFB_1)-INDUCED MOUSE LUNG TUMORS.

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The *p16INK4a* tumor suppressor gene is a cyclin-dependent kinase inhibitor, and inactivation of the gene by an accumulation of methylated cytosine-guanine (CpG) sites is a common occurrence in human and animal primary tumors. Carcinogen exposure can induce the activity of DNA methyltransferases, the enzymes responsible for methylation of CpG sites; thus, *de novo* methylation of the *p16INK4a* promoter might be induced by aflatoxin B_1 (AFB_1). In this study, female AC3F1 (A/J x C3H/HeJ) mice were treated with AFB_1. Six to fourteen months after dosing, mice were killed and tumors collected. Analysis of DNA samples from 61 AFB_1-induced whole AC3F1 mouse lung tumors was performed to assess methylation status. Direct sequencing of sodium bisulfite modified DNA revealed that 66% (40/61) of the samples were partially methylated at 30% or more of the CpG sites analyzed. Twenty AFB_1-induced tumor sections were examined for altered expression of the *p16INK4a* protein by immunohistochemical staining. In comparison to normal lung tissue, where greater than 90% of nuclei stained positively, decreased expression was observed in 39% (7/20) of tumor samples with 0-60%, of the cells staining positive for p16. In the 7 samples with decreased expression, 5 showed methylation of greater than 30% of the CpG sites examined. The correlation between p16 immunohistochemical and methylation of at least 30% of the CpG sites was 56%. This suggests that methylation of the *p16INK4a* promoter plays a role in, but is not absolutely required for AFB_1-induced mouse lung tumorigenesis. (Supported by Medical Research Council of Canada Grant # MT-10382.)

1022 POTENTIAL MECHANISMS OF TUMORIGENIC ACTION OF DIETHANOLAMINE IN MICE.

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Diethanolamine (DEA), a secondary amine found in a number of consumer products, has been reported to induce liver tumors in mice. The lack of genotoxicity in short-term assays and a Tg.AC bioassay, suggests a nongenotoxic mechanism of action. In an attempt to define the latter, the potential nitrosation of DEA to form N-nitrosodiethanolamine (NDELA) *in vivo* and the consequences of the known metabolic incorporation of DEA into phospholipids were examined. A tumorigenic dosage of DEA (160 mg/kg/day) was administered with or without supplemental sodium nitrite (170 ppm in drinking water) to B6C3F1 mice via dermal application (with or without access to the application site) or oral gavage for 2-4 weeks. Blood levels of DEA reflected the dosing method used; oral>dermal with access>dermal without access. No NDELA was observed in the urine, blood or gastric contents of any group of treated mice at levels of ≥1.5 ppb. Liver choline and phosphocholine were decreased 62-84% in an inverse relation to blood DEA levels. In a separate group of orally dosed mice, elevations in diacylglycerol and protein kinase C isoforms (PKC) and decreased S-adenosylmethionine (SAM) levels occurred. It was concluded that DEA induced choline deficiency, a known carcinogenic nutritional state. Subsequent chronic induction of PKC activity and decreased SAM levels may underlie DEA tumorigenesis in mice.

1023 CHOLINE INHIBITS DIETHANOLAMINE (DEA)-INDUCED MORPHOLOGICAL TRANSFORMATION IN SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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DEA and its fatty acid condensates are widely used in commerce. DEA is hepatocarcinogenic in mice, and although negative in a standard battery of genetic toxicity tests, it was positive in the SHE cell morphological transformation assay. The goal of this work was to determine whether the carcinogenic effects of DEA may be caused by altered choline homeostasis. SHE cells were cultured in medium containing DEA (0, 10, 50, 100, 250, and 500 μg/ml) and [33P]-phosphorus was used to label phospholipid pools. After 7 days, cells were harvested, lipids extracted and [33P]-labeled phospholipids were quantified by autoradiography after thin layer chromatographic separation. In control cells, phosphatidylcholine (PC) accounted for 43±1% of total phospholipid synthesis. DEA treatment decreased PC synthesis at concentrations >50 μg/ml, with PC reduced to 14±2% of cell phospholipids at 500 μg/ml. DEA inhibited choline uptake into SHE cells at concentrations >50 μg DEA/ml, reaching a maximum 80% inhibition at 250-500 μg/ml. Additionally, [14C]-DEA was detected in phospholipid head groups. The concentration-dependent reduction in PC synthesis and incorporation of [14C]-DEA into phospholipids was inhibited when DEA-treated cells were cultured with excess choline (30 mM). Similarly, DEA (10-500 μg/ml) transformed the SHE cells in a concentration-dependent manner, whereas choline supplementation inhibited DEA-induced morphological transformation. Thus, DEA disrupts intracellular choline homeostasis by inhibiting choline uptake and altering phospholipid synthesis. Since excess choline blocks these effects and inhibits cell transformation, the results suggest that the cell transformation and carcinogenicity of DEA are secondary to biochemical changes consistent with intracellular choline deficiency.

1024 MODULATION OF MITOMYCIN C CYTOTOXICITY BY OLTIPRAZ.

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The phase II detoxification enzyme, NAD(P)H: quinone oxidoreductase 1 (NQO1), has been shown to be induced by the dithiolthione oltipraz. Oltipraz may be able to modulate greater intracellular cytotoxicity of mitomycin C through the induction of NQO1. A single base pair polymorphism in exon 6 of the NQO1 protein has been shown to significantly reduce NQO1 enzymatic activity. To determine whether an unfavorable NQO1 genotype may be phenotypically altered by pharmacologic modulation to increase mitomycin C efficacy, two human cancer cell lines (MCF-7 and Lovo) were studied using an optimal oltipraz dose of 100uM. Effects on NQO1 enzyme activity after treatment with oltipraz were assayed using a spectrophotometric method and compared to baseline enzymatic activities in non-treated cells. NQO1 activity in oltipraz-treated cells increased two- to three-fold. In additional studies where pre-treatment of the cells with 100μM oltipraz over an optimal induction time course of 48 hours was followed by a 24-hour treatment with 0.5μg/mL mitomycin C, the number of colonies counted in flasks containing MCF-7 cells was reduced by 50% when compared to cells treated with mitomycin C alone. Lovo cells undergoing the combined oltipraz/mitomycin C treatment had colony numbers reduced by nearly 100% compared to treatment with mitomycin C alone. The human cancer cell lines, MCF-7 and Lovo, appear to exhibit an increased mitomycin C cytotoxicity when pre-treated with oltipraz. This may translate for cancer patients with an at-risk NQO1 genotype as an opportunity to increase mitomycin C sensitivity and produce therapeutic improvement in mitomycin C cytotoxicity.

1025 POTASSIUM BROMATE-INDUCED RAT CLEAR CELL RENAL TUMOR IS INDEPENDENT OF CODING REGION MUTATIONS IN THE VON HIPPEL-LINDAU GENE.

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Potassium bromate (KBrO3) is a rat renal carcinogen and a major drinking water disinfection by-product from ozonization. While KBrO3 is a human nephro- and neuro-toxicant, its carcinogenicity in humans is unknown. Clear cell renal tumors, the common form of human renal carcinomas, are rare in animals but can be induced by KBrO3 in Fischer 344 rats. Alteration of the

von Hippel-Lindau (VHL) gene is frequently observed in clear cell renal carcinomas in humans and has been associated with the same tumor type induced by N-nitrosodimethylamine in Wistar rats. Detection of VHL mutations in KBrO3-induced rat renal tumors would allow direct comparison of mutation spectra with those in humans, providing an indication of relevance for carcinogenicity of KBrO3 in human kidney. Formalin-fixed paraffin-embedded clear cell renal tumors from male Fischer 344 rats treated with KBrO3 for 2 years in the drinking water and kidney samples from untreated control were microdissected for DNA extraction. The coding region and exon-intron boundaries of the VHL gene were examined by polymerase chain reaction, which was optimized to amplify fragmented DNA from archived tissues, and direct DNA sequencing. More than 90% of the DNA sequencing has been completed. No mutations were detected in any of 9 selected kidney tumors and 1 control. This suggests that induction of clear cell renal tumor by KBrO3 in Fischer 344 rats is not associated with mutations in the coding region of the VHL gene. Other VHL alterations, such as allele loss and methylation in the promoter region, have been reported in human clear cell renal carcinomas and are under investigation in these rat tumors in order to clarify the role of VHL in KBrO3-induced rat renal tumorigenesis. (This abstract does not reflect EPA policy.)

1026 IMPROVED METHODOLOGY FOR THE SIMULTANEOUS DETERMINATION OF ENDOGENOUS ESTROGENS AND THEIR METABOLITES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL AND ULTRAVIOLET DETECTION (HPLC/EC/UV).

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A major risk factor in the etiology of breast cancer is the length of women's exposure to endogenous estrogens. Early menarche, early first pregnancy and late menopause have all been shown to increase the risk of breast cancer development. Several metabolites of endogenous estrogens including 4-hydroxyestradiol and 16-alphahydroxyestrone have been shown to be genotoxic in various experimental systems. However, the relative importance of these metabolites in breast cancer etiology remains unclear. In order to prove any hypothesis regarding a relationship between these metabolites (and others) and breast cancer development assays that are rugged, rapid, sensitive, and reproducible are needed for the analysis of biological samples collected during epidemiological studies. Taking as a point of departure methodology described in personal communications with P Gamache and J Lavigne, we have developed a HPLC/EC/UV method that separates 12 estrogens/metabolites (estriol, 16-alphahydroxyestrone, 4-hydroxyestradiol, 2-hydroxyestradiol, 2-hydroxyestrone, 4-hydroxyestrone, estradiol, 4-methoxyestradiol, 2-methoxyestradiol, estrone, 4-methoxyestrone and 2-methoxyestrone) in less than half the time of previous methods. After solid phase extraction of the analytes from human samples, a binary gradient reverse-phase (C18) HPLC separation with a mobile phase system of ammonium acetate / citric acid buffer (pH 3) and acetonitrile was used to baseline resolve these components in 20 minutes. Detection at the low pmol level was obtained using 8 coulometric sensors. This method, because of its speed and sensitivity, will facilitate the routine analysis of estrogen metabolites. (Supported by grants from Arkansas Breast Cancer Research program and Office of Women's Health, FDA.)

1027 THE TEMPORAL LOSS OF DNA REPIAR CAPACITY BY ESTROGEN EXPOSURE.

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In this study we report the effects of both natural and stilbene estrogen exposures on the DNA repair capacity of the normalized mouse Leydig cell line, TM3, using the host cell reactivation assay. We have used 17β-estradiol, 17α-estradiol, diethylstilbestrol (DES) and Bisphenol A as test compounds. Cells were exposed to ether a sub-chronic 12 day treatment (100-ng DES only) or an acute 72 hour treatment. A significant ($p < 0.05$) dose dependent decrease in the DNA repair of the Leydig cells was observed when exposed to DES (1 pg, 1 ng, and 100 ng/ml). A non-significant decrease in DNA repair was observed with 1 pg/ml Bisphenol A. The DES concentration of 100 ng/ml resulted in the maximum decrease of DNA repair (53.57%), followed by DES, 1 ng/ml (53.27%), Bisphenol A, 1 pg/ml (43.51%), and DES, 1 pg/ml (24.28%). 17β-estradiol and 17α-estradiol treatment resulted in a non-significant increase of DNA repair, 32.10% and 54.37% respectively. Sub-chronic treatment with 100 ng/ml DES resulted in a non-significant decrease in DNA repair activity (26.15%). The data presented here is novel and intriguing as it provides direct evidence for a mechanism for the induction of genomic instability by DES and Bisphenol A. However, whether the observed attenuation in DNA repair capacity by estrogen exposure leads to accumulation of DNA damage remains to be ascertained.

1028 AN INDUCIBLE DNA REPAIR PROCESS IS INDEPENDENT OF NON-HOMOLOGOUS END-JOINING (NHEJ).

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DNA repair is vital for maintaining genomic stability. The role of DNA repair in assuring normal development and preventing carcinogenesis is demonstrated in individuals with DNA repair disorders such as Xeroderma Pigmentosum and Cockayne syndrome. We have discovered a novel, inducible DNA repair response in cells treated with ionizing radiation (IR) at the G1/S border of the cell cycle. This process differs from constitutive repair processes by producing very long repair patches (VLRP) of at least 150 nucleotides in addition to constitutive repair patches of 3-5 nucleotides. One signal for the induction of VLRP is double-strand breaks produced by ionizing radiation and bleomycin. In the current study, we examined whether several components of the non-homologous end joining pathway that rejoins double-strand breaks are involved in this inducible repair process. Cells with mutations in the XRCC4 gene, as well as the p86 subunit of Ku and the catalytic subunit of the DNA-dependent protein kinase (DNA-PK) were irradiated at the G1/S border of the cell cycle and repair patch size was analyzed by alkaline cesium chloride density gradients. XR-1 cells which lack the XRCC4 gene were able to produce VLRP of 150 bases, and the VLRP were produced at a higher level than seen in normal human fibroblasts. Two cell lines with mutant Ku86, xrs6 and MEF Ku86 -/-, were also able to produce long patches of 200 and 150 bases. These cell lines also produced a higher level of VLRP than is seen in normal fibroblasts. Finally, we examined two cell lines with a deficiency in the catalytic subunit of the DNA-PK. Hamster V3 cells and MO59J human glioblastoma cells both produced long patches of 175 bases, while the V3 cells produced VLRP at normal levels and the MO59J cells overproduced the long patches. These results indicate that neither the XRCC4 gene product nor the components of the DNA-PK are required for the production of long patches. The overproduction of long patches in several of the cell lines appears to be the result of a mutation in the p53 gene in these cells because the overproduction is also seen in the corresponding control cell lines and in fibroblasts that are p53 deficient. Further investigation will focus on whether components of the double-strand break repair process of homologous recombination are responsible for producing VLRP. (Supported by NCI CA62059.)

1029 DNA-DAMAGE BY HYDROXYLAMINE O-SULFATE AND METHYL ETHYL KETOXIME O-SULFATE.

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Long term inhalation of methyl ethyl ketoxime (MEKO) causes a dose-dependent induction of liver tumors in male rats and male mice. The mechanistic basis for the sex-specific tumor induction is not known. Oxime O-sulfates have been implicated as toxic metabolites involved in the induction of DNA-damage by oximes and sec. nitroalkanes. We compared the ability of synthetic MEKO O-sulfate (purity > 98%) and hydroxylamine O-sulfate to induce DNA-damage (8-oxo-deoxyguanosine and 8-amino-deoxyguanosine) in calf thymus DNA and mutagenicity in Salmonella typhimurium TA100. MEKO O-sulfate was stable in aqueous solution at pH 7.4; less than 5% decomposition was noted after 48 h at 37°C. To study DNA-damage, MEKO O-sulfate and hydroxylamine O-sulfate were incubated with DNA in buffer at pH 7.5 for 4 h at 37°C, DNA was isolated, hydrolysed and 8-oxo-deoxyguanosine and 8-amino-deoxyguanosine were quantified by HPLC with electrochemical detection. At concentrations of 0.72 mM, MEKO O-sulfate did not induce detectable levels of 8-amino-deoxyguanosine and caused only a minor increase in the concentration of 8-oxo-deoxyguanosine. At 7.2 mM MEKO O-sulfate, 8-oxo-deoxyguanosine was increased from 63±28 pml/mg DNA to 280±52 pmol/mg DNA and 8-amino-deoxyguanosine was formed (57±30 pg/mg DNA). Hydroxylamine O-sulfate in identical concentrations induced large increases in 8-oxo-deoxyguanosine (up to 13 732±4 306 pg/mg DNA) and induced formation of 8-amino-deoxyguanosine in concentrations of 30 012±1745 pmol/mg DNA. In agreement with the DNA damage studies, only hydroxylamine O-sulfate caused mutations in Salmonella typhimurium. The results suggest that oxime O-sulfates are stable in aqueous solution and

that reactive intermediates from oxime O-sulfates *in vivo* should only be formed after further enzymatic activation to yield hydroxylamine O-sulfate.

1030 MOLECULAR DOSIMETRY AND REPAIR STUDIES ON N^2,3-ETHENOGUANINE IN HEPATOCYTES AND NONPARENCHYMAL CELLS FROM RATS EXPOSED TO VINYL CHLORIDE.

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Exposure to vinyl chloride (VC) monomer causes hepatic angiosarcomas, tumors that originate from endothelial cells lining the hepatic sinusoids, in rats and humans. This study investigated the molecular dosimetry of the VC-induced, promutagenic DNA adduct N^2,3-ethenoguanine (N^2,3-εGua) in hepatocytes and nonparenchymal cells (NPC). Based on evidence that NPC (a population enriched in endothelial cells) were deficient in base excision repair relative to hepatocytes, it was hypothesized that higher concentrations of N^2,3-εGua would be present in NPC DNA following VC exposure. 12 wk-old male Sprague-Dawley rats were exposed to 0, 10, 100, or 1100 ppm VC for 1 or 4 wk (6 hr/d, 5 d/wk). A recovery group was similarly exposed for 4 wk and maintained 1 wk before being killed. The liver of each animal (n=3-5) was perfused with collagenase *in situ*, and hepatocytes were separated from NPC by differential centrifugation. DNA from each population was hydrolyzed in 0.1*N* HCl, and N^2,3-εGua was isolated with immunoaffinity chromatography. The adduct was quantitated with gas chromatography-high resolution mass spectrometry, and the guanine content of each sample was determined by HPLC. The exposure-response curve with respect to N^2,3-εGua was supralinear with a plateau between 100-1100 ppm, and there was a 2-4 fold accumulation of the adduct between 1 and 4 wk in both hepatocytes and NPC. However, no significant cell type-specific differences in adduct concentrations were observed in controls or rats exposed to any VC concentration. Surprisingly, there were no significant adduct concentration differences between the 4 wk and recovery groups with either cell type, suggesting that N^2,3-εGua is poorly repaired *in vivo*. The persistence of this adduct may increase its mutagenic potential, although it appears that factors in addition to relative adduct concentrations are important determinants of the susceptibility of NPC to VC-induced carcinogenesis. (This work was supported in part by NIH grants ES07126 and ES05948.)

1031 DEOXYADENOSINE (dAdo) ADDUCTS FORMED BY THE SYN- AND ANTI-DIOL-EPOXIDES OF 7,12-DIMETHYLBENZ[A] ANTHRACENE (DMBA) ACCOUNT FOR ITS TUMOR INITIATION ACTIVITY IN MOUSE SKIN.

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The current study was designed to explore the possibility that the dAdo adducts formed by the bay-region diol-epoxides of DMBA (syn- and anti-DMBADE) account for the tumor initiation activity of DMBA. Groups of SENCAR mice (4/group) were topically treated with 10 nmol of DMBA, 50 and 100 nmol of both syn- and anti-DMBADE and were sacrificed at 3, 6 and 24 h after the treatment. DNA adduct formation in epidermis was analyzed by nuclease P1-enhanced 32P-postlabeling assay. Tumor initiation activity was assessed by a two-stage initiation-promotion protocol and formation of specific mutations in codons 12, 13 and 61 of the Ha-ras gene in skin tumors was assessed by a PCR-RFLP protocol. Results from these experiments showed that both syn- and anti-DMBADE formed stable dAdo adducts in mouse epidermis, most of which co-migrated with the stable dAdo adducts formed by the parent compound. In addition, both the syn- and anti-DMBADEs possessed tumor initiation activity. Essentially all (100, 96 and 94%, respectively) of the papillomas produced by syn- and anti-DMBADE contained a -CAA- to -CTA- mutation in codon 61 of Ha-ras gene similar to the parent compound DMBA. Collectively, these results indicate that the dAdo adducts induced by both bay-region diol-epoxides of DMBA lead to the mutations in codon 61 of Ha-ras gene and consequently initiate tumorigenesis in mouse skin (Supported by NIEHS ES 03124, ES 08389, and from The Council for Tobacco Research 3995 [M.-s.T.]; NIEHS Center grant ES 07784 and CA 79442 [J.D.].)

1032 EXPOSURE-DEPENDENT ACCUMULATION OF N7-(2-HYDROXYPROPYL)GUANINE (7-HPG) IN DNA OF TISSUES OF F-344 RATS AFTER INHALATION EXPOSURE TO PROPYLENE OXIDE (PO).

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PO is an industrial chemical shown to induce tumor formation (2-3% incidence) in nasal passages of rodents in the high exposure groups (≥ 300 ppm) in carcinogenicity bioassays. Nasal tumors were accompanied by nonneoplastic changes suggesting that cytotoxicity and compensatory cell proliferation may be involved in PO carcinogenesis. In the present study, exposure-dependent accumulation of 7-HPG in nasal respiratory epithelium (NRE), lung and liver was determined in male F344 rats exposed to PO (0, 5, 25, 50, 300 or 500 ppm) by the inhalation route for 3 days (6 hr/day) or 20 days (6 hr/day; 5 days/week). The exposures studied ranged from low concentrations, such as those potentially occurring in the workplace, to high concentrations that were carcinogenic in rodents, thus providing valuable information for biologically based risk assessment for PO. Preliminary results (n=3) showed a linear response in 7-HPG accumulation for all three tissues and both lengths of exposure. The nose had the highest concentration of adducts followed by lung and liver, with linear regression slopes 1/6 and 1/15 that of the nose, respectively. These differences are thought to be due to greater amounts of PO reacting with nasal tissue and a combination of direct and systemic exposure in the lung. We have previously shown an increase in cell proliferation in the NRE only at carcinogenic exposures. While 7-HPG is not promutagenic, loss of the adduct by chemical depurination and base excision repair can produce abasic sites. Abasic sites were measured in these three tissues and testis using the ARP method and showed no increase in rats exposed to 500 ppm PO for 20 days, suggesting that this is not a mechanism of mutagenesis for 7-HPG. Thus, the modest increase in nasal tumors may be related more to increased cell proliferation than to the very high number of 7-HPG adducts. (Supported in part by CMA and CEFIC.)

1033 ASSOCIATION BETWEEN THE XRCC1 399 GLN POLYMORPHISM AND ADENOCARCINOMA OF THE LUNG.

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Genetic predisposition to cancer has been associated with hereditary genetic defects in DNA repair. Maintenance of genomic integrity is critical in the protection against mutations that lead to cancer. XRCC1 is a DNA repair enzyme involved in the base excision pathway. Three polymorphisms have been identified in the XRCC1 gene that code for nonconservative amino acid changes at codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln). Significant associations have been reported between the 399 Gln allele and higher levels of both AFB_1-adducts and GPA somatic mutations. We conducted a case control study to test the hypothesis that the 399 Gln allele is positively associated with adenocarcinoma of the lung. In this study, 74 lung adenocarcinoma samples and 68 control samples were genotyped at position 399. Polymorphic frequencies in the adenocarcinoma samples were 43% for Arg/Arg, 37% for Arg/Gln and 20% for Gln/Gln. In the control samples, XRCC1 polymorphic frequencies were 46% for Arg/Arg, 47% for Arg/Gln and 7% for Gln/Gln. A marginal significance (p=0.07) was noted between lung adenocarcinoma and presence of the 399 Gln/Gln allele. Additional samples are being analyzed to determine if this association is significant ($p \leq 0.05$). (Supported by NIH Grants 1 F32 ES05836-01 and CA70190 from NIEHS under the U.S. DOE cooperative agreement No. DE-FC04-96AL-76406.)

1034 ANALYSIS OF MUTATION IN LUNG OF BIG BLUE RAT INDUCED BY EXPOSURE TO DIESEL EXHAUST.

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Exposure to diesel exhaust (DE) is known to cause lung tumors in rats. To clarify the mutagenicity of DE, we estimated mutant frequency (MF) and determined the mutation spectra in rat lung after exposure to DE using lambda/lacI transgenic rats (Big Blue system). Male Big Blue rats were exposed for 4 weeks to 1 or 6 mg/m3 of DE, which contains suspended particulate mat-

ter. Control rats were maintained in filtered air. After exposure to 6 mg/m3 DE, MF in lung was 4.8-fold higher than in control rats ($p<0.01$), Sixty-nine mutants were identified after exposure to 6 mg/m3 DE. The major mutations were A:T to G:C and G:C to A:T transitions. Remarkably, G to T transversion of the lacI gene at site 221 was a hot spot induced by exposure to DE. DNA adducts formed by DE were analyzed using a 32P-postlabel-TLC method and the amount of 8-hydroxydeoxyguanosine (8-OHdG) was measured using HPLC. Relative adduct level and amount of 8-OHdG were significantly increased in the rats exposed to DE. The level of cytochrome P450 1A1 mRNA was shown by northern blot analysis to be significantly increased in the rats exposed to 6 mg/m3 DE. These results indicate that DE causes lesions in genomic DNA and acts as a mutagen in rat lung.

1035 INFREQUENT P53 MUTATIONS IN LIVER TUMOURS INDUCED BY 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2[5H]-FURANONE (MX) IN RATS.

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MX, a disinfection by-product in chlorinated drinking water, is mutagenic in bacteria and in mammalian cells. In a recent carcinogenicity study with Wistar rats [Komulainen H. et al., J. Natl. Cancer Inst. 89:848-856, 1997], MX caused tumours at multiple sites, including the liver. In order to evaluate the molecular mechanisms of the MX-induced carcinogenesis, we analyzed mutations in p53 gene (exons 4-7) and expression of p53 gene in formalin-fixed, paraffin-embedded liver tumours of the rats (altogether 16 adenomas, 8 carcinomas, 23 cholangiomas, 3 cholangiocarcinomas). Mutations were investigated by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by direct sequencing. Four mutations (3 G:C->A:T transitions, one A:T->T:A transversion) were detected in 2/16 adenomas and 1/3 cholangiocarcinomas, all in high dose females of the study. An immunohistochemical overexpression of the p53 gene was detected in all cholangiomas and cholangiocarcinomas, but in none of the adenomas or carcinomas. The results suggest, that p53 mutations are not involved in MX-induced liver carcinogenesis in Wistar rats. (Supported by Academy of Finland, Finnish Research Programme on Environmental Health.)

1036 CHRONIC TOXICITY OF DIETHYLNITROSAMINE, MANNITOL AND CAPROLACTAM IN HETEROZYGOUS *p53*-DEFICIENT (+/-) AND WILD (+/+) TYPE MICE.

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This study compares the carcinogenicity of diethylnitrosamine (DEN) in conventional mice to that in *p53*-deficient mice, chiefly to assess the potential of DEN to induce DNA adducts in the liver of transgenic mice. Mannitol (MAN) and caprolactam (CAP) were also tested. Sixty male wild type mice (+/+), 8 weeks old, and 60 heterozygous (+/-) C57BL/6 (*p53*-deficient) mice, 8 weeks old, were used. Of the 6 experimental groups, groups 1(wild type, (+/+) and 2 (*p53*-deficient heterozygous; (+/-) served as room controls. Groups 3 (+/+) and 4(+/-) were exposed orally (gavage) to 50 μmoles/kg bw DEN weekly for a total of 10 doses. Group 5 (+/-) was exposed to 15,000 ppm of CAP in the diet for up to 26 weeks. Group 6 (+/-) was exposed to 50,000 ppm of MAN in the diet for up to 26 weeks. At 10 weeks, part of the liver was removed for DNA adduct analysis by the immunoslot blot method which measures O^4-EtT (O^4-ethylthymidine) adducts. Absolute and relative liver weights were measured, and liver microscopy (H&E) was performed. The liver was also evaluated immuno-histochemically by staining for proliferating cell nuclear antigen (PCNA) and by measuring the replicating fraction (RF). No significant or consistent body or liver weight changes were present in any of the groups. No consistent and pertinent changes in RF values were present in any of the groups and no other pertinent and consistent morphologic changes were evident in the livers. The O^4-EtT modified bases of liver DNA indicated a significant increase in both groups 3 and 4 mice, when compared to their respective controls, although not significantly different from each other. None of the tested substances produced neoplasms in p53 (+/-) mice. This was unexpected for DEN, especially since it produced DNA adducts in the liver.

1037 TOXICITY OF INHALED BROMODICHLOROMETHANE IN p53+/- TRANSGENIC MICE.

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The purpose of this study was to determine the dose response curves and target organ toxicity for the water disinfection by-product bromodichloromethane (BDCM) as the basis for selecting doses for long term cancer studies in the p53 +/- mouse model. Wild type and p53+/- mice on the FVB/N and C57BL/6 backgrounds were exposed to BDCM vapor daily for 6 hr/day at concentrations of 0, 1, 10, 30, 100, and 150 ppm for 1 week and at 0, 0.3, 1, 3, 10, and 30 ppm for 3 weeks. In the 1-week exposure study dose-dependent mortality and morbidity were observed at concentrations of 30 ppm and above and were as high as 100% at 150 ppm. In the 3-week exposure study mortality and morbidity was only found in the 30 ppm exposure groups and was 0, 17, 50, and 33% for the wild type C57BL/6, p53+/-C57BL/6, wild type FVB/N, and the p53+/- FVB/N mice, respectively. Dose-dependent tubular degeneration, necrosis, and associated regenerative cell proliferation (greater than 10 fold over controls) were seen at concentrations as low as 10 ppm in the kidneys of all strains at 1 week. Pathological changes were more severe in the FVB/N compared to the C57BL/6 and were more severe in the heterozygotes compared to the wild type. However, recovery and return of the percentage of cells in S-phase to control levels was seen at 3 weeks. Similar dose-dependent increases in hepatic necrosis and regenerative cell proliferation were observed, but were induced only at concentrations of 30 ppm and higher. The maximum tolerated dose for longer-term studies with these mice is on the order of 10 ppm. Differences in toxicity indicate that the exact strain and genotype should be used when doing range finding studies to select doses for p53+/- cancer studies.

1038 TUMORIGENIC POTENTIAL OF CARBARYL IN THE P53 KNOCKOUT MOUSE MODEL.

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Using the genotoxic compound urethane, we have previously shown the heterozygous p53 knockout mice to be sensitive to the development of vascular tumors within a short period of time. The essentially zero level of vascular tumors in control p53 knockout mice made this system particularly suitable for studying low potency compounds suspected of being vascular carcinogens. Therefore, this model was used to assess the significance of vascular tumors noted in a long term study in CD-1 mice with carbaryl. Although increased incidences of vascular tumors were observed in male mice at 100, 1000 and 8000 ppm, no clear dose-effect relationship was obtained. In previous studies, carbaryl had shown clastogenic activity in an *in vitro* CHO assay with S9 activation but was negative in an *in vivo* chromosomal aberration test (rat bone marrow). Metabolism data obtained in the rat had suggested the possible formation of epoxide metabolites. In order to investigate whether Carbaryl might induce vascular tumors by a genotoxic mechanism it was administered continuously via the diet to groups of 20 male heterozygous p53 knockout mice at concentrations of 0, 10, 30, 100, 300, 1000 and 4000 ppm for 180 days (8000 ppm exceeding the MTD in CD-1 mice). Carbaryl induced no neoplastic or preneoplastic changes in the vascular tissue of any of the organs examined (nor in any other tissue). Neoplasms, recognized as those which typically occur spontaneously in untreated mice of this strain, were sporadically observed in a few animals from the intermediate dose groups with no evidence of a dose or treatment relationship. Under the conditions of this study Carbaryl did not show any carcinogenic potential in the p53 heterozygous mouse. In particular there was no evidence that the apparent increase in vascular tumors in the previous lifetime mouse study reflect a genotoxic mechanism.

1039 SUBACUTE EXPOSURE OF P53-HEMIZYGOUS (+/-) MICE TO 4-(N-METYHL-N-NITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK).

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We investigated the sensitivity of the p53-hemizygous (+/-) mouse to exposure to 4-(N-Methyl-N-nitrosamino)-1-(3-pyridyl)-1-Butanone (NNK). A single exposure of A/J or (A/J x TSG-p53)F1 mice to NNK results in 100% or 97% of multiple lung adenomas within 16/17 weeks. In our study NNK was

administered at a dose, that is cumulative comparable to the exposure levels occuring in life-long smokers. NNK was administered to 26 male and 26 female p53-hemizygous (p53 +/-) C57BL mice intraperitoneally 5 times once a week during 5 consecutive weeks in a dose of 1 mg/kg body weight. Half of these animals were then kept for a subsequent 9 week and the other half for a 22 week treatment-free period. The animals were regularly observed, body (weekly) and organ weights as well as feed intake (weekly) were determined. Organs and tissues were subjected to gross and histopathological investigations. Treatment with NNK did not induce any effects on general condition, species-specific behavior, mortality, body weight development and food consumption. Liver weights were significantly reduced in females after 27 study weeks. Histopathologically no indications of neoplasia or pre-neoplasia were seen in liver and lungs. It is concluded, that within a period of 27 weeks the p53-hemizygous (p53 +/-) C57BL mice is insensitive to the carcinogenic effect of NNK seen in A/J mice and Syrian golden hamsters at a cumulative dose comparable to the lifetime-exposure of smokers.

1040 TUMORIGENIC POTENTIAL OF COMPLEX MIXTURES IN p53 DEF. MICE.

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Previous studies have demonstrated that manufactured gas plant tar (MGP tar) is highly tumorigenic in mouse lung following ingestion. The present study evaluates the potential tumorigenic activity of manufactured gas plant tar, pharmaceutical grade coal tar (PG tar) and benzo[a]pyrene B[a]P in $p53^{def}$ mice. Diets containing MGP tar (0.3%), PG tar (0.3%) or B[a]P (100 ppm) were fed to $p53^{def}$ mice for 26 weeks. A negative control group was maintained on a non-adulterated basal gel diet. Mice dosed with a single ip injection of 2.1 mg of B[a]P in a tricaprylin vehicle and maintained on a non-adulterated basal gel diet were positive controls. Animal body weights and diet consumption were monitored throughout the study. Ingestion of a 100 ppm B[a]P diet resulted in 55% of the mice developing forestomach tumors with a multiplicity of 2.9 tumors/mouse. Mice injected with 2.1 mg B[a]P had a 29% forestomach tumor incidence with a multiplicity of 1 tumor/mouse. In contrast, a much lower tumorigenic response was observed in mice ingesting MGP tar and PG tar. In these animals, 5 and 18% of the mice developed forestomach tumors with a multiplicity of 0.36 and 0.15 tumors/mouse, respectively. Interestingly, no lung or liver tumors were observed in any of the treated groups. These results are in contrast to the results of previous studies that demonstrated MGP tar to be highly tumorigenic in mouse lung. The lack of lung tumorigenicity may be due to the dose of each mixture administered and/or the exposure time given to animals. These results also suggest that the lung and liver tumorigenicity previously observed with other strains of mice may not involve p53 gene mutations. (EPRI Grant WO2963-06)

1041 CANCER BIOASSAY AND GENOTOXICITY OF INHALED BENZENE IN P53+/- AND C57BL/6 MICE.

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P53+/- and wildtype C57B1/6 mice have been exposed to benzene by chronic inhalation to assess tumorigenic and genotoxic responses. To determine the time to maximum tumor induction, the length of exposure was for 36 weeks or until the survival in the benzene exposed groups reached 20% of unexposed controls. Mice were exposed to either 0 ppm or 3000 ppm x hr/week of benzene using three exposure regimens: 100 ppm (6 hrs/day 5 days/week); 100 ppm (10 hrs/day 3 days/week; MWF) or 200 ppm (5 hrs/day 3 days/week; MWF). The frequency of micronuclei in peripheral blood was determined at 1, 5, 13 and at 33-38 weeks of exposure. At 26 weeks of exposure to benzene the incidence of thymic lymphomas was: 22% in the 100 ppm exposure groups, 2% in the 200 ppm exposure group and 0% in the air controls. In the 100 ppm exposure groups, 36 weeks of benzene exposure induced a high frequency of thymic lymphomas in p53+/- mice, >80% incidence compared to 2% in unexposed controls. In the 200 ppm exposure group, the incidence of thymic lymphomas in p53+/- mice at 36 weeks was 17%. The incidence of thymic lymphomas in C57B1/6 mice at 36 weeks at all levels of benzene exposure was less than 5%. The frequency of micronuclei was elevated in all exposure groups at all time points examined. The increased frequency of micronuclei in peripheral blood was equivalent or greater in the C57B1/6 mice than in the p53+/- mice. These data indicate that benzene inhalation exposure is carcinogenic in p53+/- mice, but at these levels of benzene exposure p53+/- mice are not more sensitive to the genotoxic effects of benzene than wild type mice.

1042 SPONTANEOUS TUMOR PROFILE IN THE P53 DEFICIENT TG.AC (V-HA-RAS) BIGENIC MOUSE MODEL.

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The p53+/- and Tg.AC transgenic mouse models are under consideration as adjuncts or alternatives for carcinogen identification. A p53+/- mouse with a Tg.AC hemizygous alteration was generated as another possible short-term bioassay model and to provide a mechanistic understanding of carcinogen induced neoplasia. Male (92) and female (100) bigenic mice were observed under normal husbandry conditions. Mean survival time in humane- and moribund-euthanized mice was greater in male than female mice with 50% mortality occurring at 37.3 and 30.6 weeks of age respectively. Seventy-one percent of female mice died prior to when 50% mortality was attained in male mice and only 5 female mice survived to the terminal sacrifice at 49 weeks of age (after 43 weeks on study). Jaw masses (presumably odontogenic tumors) and enlarged spleens were the most frequent necropsy findings (70% of animals). At 20, 25, 30, and 49 weeks of age, jaw masses were found in 2, 7, 9, and 19% of male mice, respectively, and 3, 11, 14, and 29% of female mice, respectively. Surprisingly, uterine masses (presumably leiomyosarcomas) were first found as early as 12 weeks. At 15, 20, and 25 weeks, uterine masses were found in 13, 15, and 19%, respectively, of female mice. The bigenic model permits comparison to sporadic tumor incidence in p53+/- or Tg.AC mice. Inherent genetic susceptibilities for tumor formation were suggest that there are tissue specific interactions between p53 and v-Ha -ras. Since both ras and p53 are detected in many human cancers, a mechanistic understanding of critical gene events and interactions occurring in neoplasia may help in defining carcinogen specific mechanisms of cancer using this model. (This work was sponsored by NIEHS [N01-ES-65399].)

1043 ANALYSIS OF RESPONDER STATUS ACROSS MULTIPLE GENERATIONS OF TG.AC TRANSGENIC MICE.

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The v-Ha-ras transgenic Tg.AC mouse model has been proposed as an alternative short term *in vivo* model to the conventional two-year bioassay for the identification of carcinogens. When exposed to 12-0-tetradecanoyl-phorbol-13-acetate (TPA), Tg.AC mice respond with skin papillomas as a reporter phenotype. During the last several years, an increased incidence in TPA-exposed non-responder Tg.AC mice has been observed. The present study monitored the response of male and female homozygous Tg.AC mice from six different generations to 2.5 µg TPA applied topically either two or three times per week for 12-21 weeks. The incidence and multiplicity of papillomas from three studies initiated in late 1997 through mid 1998 was decreased as compared to three studies using mice from a colony founded in late 1998. Between these two sets of experiments, the incidence of mice exhibiting at least 20 papillomas increased from 30% to 90%. Genomic DNA blots have conclusively linked an altered genotype to the non-responder phenotype. The latter studies exhibited remarkable consistency in regard to tumor incidence, multiplicity, and latency following TPA exposure, with no difference noted between sexes. These studies demonstrate the robustness of the papilloma response in recent multiple generations of Tg.AC mice and the utility of TPA as an appropriate positive control. (This work was sponsored by NIEHS [N01-ES-95442].)

1044 CARCINOGENIC ENDPOINT EVALUATION IN TG. AC TRANSGENIC MICE GIVEN CYCLOSPORIN A DERMALLY FOR 26 WEEKS.

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The purpose of this study was to assess the potential carcinogenicity of cyclosporin A when applied topically to homozygous TG. AC mice for 26 weeks. Groups of 15 male and 15 female mice approximately 10 weeks old were given 200 µl/mouse topical doses daily of the following for 26 weeks:

acetone vehicle, 2.5 µg of 12-0-tetradecanoly phorbol-13-acetate (positive control), 0.05, 0.4 or 0.8 µg of cyclosporin A dissolved in acetone. Visual observation for papillomas began after 4 weeks and tumor palpation began after 12 weeks. Clinical observations were done daily. Each mouse was necropsied and a complete set of tissues was evaluated histologically. No treatment-related effect was noted on weekly body weight or feed consumption. Papillomas were noted grossly and microscopically at the application site in all but two of the positive control mice. There was no significant difference in the survival rates of the three cyclosporin A treated groups when compared to the control group. The number of squamous papillomas at the application site was statistically ($p < 0.05$) increased in the females given 0.8 µg cyclosporin A compared to the controls (3/14 vs 0/14, respectively) but was not statistically increased in the males (2/15 vs. 0/15, respectively). The incidence of all other tumors among cyclosporin A treated animals was comparable to control animals.

1045 POINT MUTATIONS OF THE TRANSGENE IN FORESTOMACH AND SKIN TUMORS OF RASH2 TRANSGENIC MICE INDUCED BY N-METHYL-N NITROSOUREA (MNU) OR N-ETHYL-N-NITROSOUREA (ENU).

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It has been reported that forestomach and skin squamous cell tumors are induced by a single injection MNU in transgenic mice carrying the human proto-type c-Ha-ras gene (rasH2 mice) with an extremely high incidence of point mutations of the transgene. On the other hand, our previous studies in which rasH2 mice received a single injection of vinyl carbamate revealed that the transgene mutations were extremely low in the lung tumors induced. To examine the frequency of the transgene mutation, rasH2 mice received an intraperitoneal injection of 37.5 mg/kg MNU or 120 mg/kg ENU, and were reared until 26 weeks. In the MNU group, squamous cell tumors of the forestomach were found in 16/16 males and 20/20 females, and skin papillomas in 70% of these mice. In the ENU group, the forestomach tumors were found in 14/20 males and 7/20 females, and skin papillomas in 15%. The incidences of the transgene mutation of the forestomach tumors and skin tumors in the MNU group were 87% and 82%, respectively, those in the ENU group being 15 and 50%, respectively. Real-time RT-PCR analysis showed that the human c-Ha-ras gene expression level elevated in both MNU- and ENU-induced tumors. Our results suggest that the transgene mutation is not always related to the inductions of the forestomach and skin tumors.

1046 A 26-WEEK STUDY IN TRANSGENIC RASH2 MICE WITH ETHYLENE THIOUREA.

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Male and female transgenic rasH2 (TgrasH2) mice (15/sex), which are being evaluated by ILSI as a more rapid alternative to lifetime mouse bioassays, were given ETU in their diets at 0.6% (6000 ppm) for 26 weeks. ETU is a nonmutagenic carcinogen which was positive for thyroid and liver neoplasm's in a chronic mouse (B6C3F1) bioassay. After 26 weeks, male and female mice had lower body weights compared to controls: 76% and 85%, respectively ($p<0.01$). There were no moratalities. There were no significant clinical signs of toxicity during the study. 14/15 males and 15/15 females had enlarged thyroids at final sacrifice and liver weights were significantly increased over controls: males and females 134% and 120%, respectively ($p<0.01$). Histopathology results in treated mice included thyroid tumors in males (8/15) and females (11/15). No thyroid tumors were seen in controls. Additionally 6 male mice and 2 female mice had liver adenomas, versus none in the respective control groups; 1 treated male and 2 treated females had lung adenomas. In conclusion, ethylene thiourea, a nongenotoxic carcinogen, administered in the diet of TgrasH2 male and female mice at 0.6% for 26 weeks, induced thyroid and liver tumors. These results provide support for the TgrasH2 model as a rapid alternative carcinogenicity test system for nongenotoxic agents.

1047 A 26-WEEK STUDY IN TRANSGENIC RASH2 MICE WITH SULFISOXAZOLE.

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Male and female transgenic rasH2 (TgrasH2) mice (15/sex), which are being evaluated by ILSI as a more rapid alternative to lifetime mouse bioassays, were given the antimicrobial sulfisoxazole (non-carcinogenic in a chronic study in B6C3F1 mice) in their diets at levels of 0.5%, 2% and 3% for 26 weeks. A 4-week range-finding study in wild-type mice (CB6F1) at 5% caused 50% mortality in males. At 26 weeks male mice at 2 and 3% had lower mean body weights compared to controls: 89%, and 85%, respectively ($p<0.01$) . Male body weights at 0.5% were comparable to controls and females at 0.5% were greater (109%, $p<0.01$) than controls. Females at 2 and 3% were comparable to controls. Male/female mortality was: 1/1, 0/1 and 5/0 in the 0.5, 2 and 3% groups, respectively. There were no significant clinical signs of toxicity. Gross and histopathology findings were unremarkable. In conclusion, sulfisoxazole, a non-carcinogen, administered in the diet of TgrasH2 male and female mice for 26 weeks, induced no false positive neoplastic or pre-neoplastic lesions. These results provide support for theTgrasH2 model as an alternative rapid carcinogenicity test system for nongenotoxic test agents.

1048 DOSE-DEPENDENT SUPRA-ADDITIVITY FOR THE INDUCTION OF MOUSE SKIN PAPILLOMA BY CHRONIC TREATMENT WITH VARIOUS DOSE COMBINATIONS OF 7,12-DIMETHYLBENZ[A]ANTHRACENE (DMBA) AND 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA).

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Toxicity testing is usually performed on single chemicals and includes high dose levels. In contrast, humans are exposed to a multitude of substances at low dose. One question therefore is whether supra-additive effects of mixtures can be observed also with doses around the no-observed-adverse-effect levels (NOAEL) of the single chemicals. Using the wellknown example of a synergistic tumorigenic effect on mouse skin of a combination of the DNA-reactive carcinogen DMBA with the tumor-promoting agent TPA, deviation from additivity was investigated as a function of dose. Twentyfive groups of 16 female NMRI mice were treated on the back twice weekly for up to two years with 100 µl acetone containing various dose combinations of DMBA and TPA (0, 0.1, 0.3, 1, 3 nmol per application; full 5x5 dose matrix). Papilloma formation was recorded weekly. Kaplan-Meier estimators for censored observations of time-to-the-first-papilloma were derived for all groups. DMBA and TPA alone increased the papilloma incidence at and above dose levels of 0.3 and 1 nmol, respectively. For the statistical analysis of combination effects, logrank tests for interaction were performed in a 2x2 design. Combinations of equipotent doses of the single chemicals (DMBA+TPA at 0.3+1 and 1+3 nmol), resulted in significant supra-additivity ($p=0.014$ and 0.045, respectively). In contrast, synergism was not observed when the combined treatment was carried out with the two components at their NOAEL (0.1+0.3 nmol ; $p=0.98$). The observed dose dependence for supra-additivity may have an impact on the risk assessment of environmental mixtures.

1049 PAH AND METAL MIXTURES IN METROPOLITAN SOILS OF NEW ORLEANS.

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Evaluating the quantities and associations of mixtures of metals and organic compounds is an important step in toxicological assessment. This study compares mixtures of polycyclic aromatic hydrocarbons (PAHs) and selected metals (Pb, Zn, Cd, Mn, Ni, Cu, Cr, V) of New Orleans (n=28) soil samples and Mississippi River alluvium (n=6), parent material of the lower delta. Surface (2.5 cm deep) soil samples were air-dried and sieved (2 mm screen). Accelerated solvent extraction (ASE) was applied to soil extraction for analysis with gas chromatography-mass spectrometry (GC-MS). Metals were extracted using a 5:1 ratio of 1 mol L^{-1} nitric acid (room temperature) to soil, shaken for 2 hours, centrifuged (1000 x g - 15 min.), and filtered. Metal ions in the extracts were determined using inductively coupled plasma-atomic

emission spectrometry (ICP-AES). Mann-Whitney tests show the differences ($p < 0.001$) between urban soils and alluvium. Pearson product moment correlation reveals that urban soils collected from inner to suburban New Orleans generally exhibit a strong association (correlation coefficient = 0.78, $p = 10^{-6}$) between PAHs and metals. Specific compounds, Acenaphthylene, Benzo(a)pyrene, and Benzo[g,h,I]perylene exhibit a correlation coefficient of > 0.88 and $p < 10^{-9}$. The results suggest that the distribution of mixtures of PAHs and metals are similar and that they are predicted by the soil metal maps of metropolitan New Orleans. (Acknowledgements: Research on soil metals funded by the ATSDR/MHPF cooperative agreement # U50/ATU398948, and on soil PAHs by DOD Grant # DSWA01-97-1-0028.)

1050 METAL MIXTURES OF MODERN ALLUVIUM: BASELINE DATA FOR MISSISSIPPI RIVER DELTA SOILS.

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Establishing baseline data is essential for comparative research on the concentrations and associations of metals of various sample collections. This study evaluates the mixtures of metals (Pb, Zn, Cd, Mn, Ni, Cu, Co, Cr and V) in alluvium samples of the lower Mississippi River delta that originate from the entire watershed. Surface (2.5 cm deep) soil samples were air-dried and sieved (2 mm screen). Metals were extracted using a 5:1 ratio of 1 mol L^{-1} nitric acid (room temperature) to soil, shaken for 2 hours, centrifuged (1000 x g - 15 min.), and filtered. Metals were determined with inductively coupled plasma-atomic emission spectrometry (ICP-AES). Samples were stratified into groups according to location a) along the riverbank (n=42), b) distant to roads and railroad tracks (n=58), and c) next to roadways and railroad tracks (n=41). Mann Whitney rank sum tests show that metal concentrations in group b < group a < group c ($p < 0.001$). The respective medians for groups b, a, and c are: Pb 4.0, 4.0, 8.8; Zn, 9.5, 10.3, 22.5; Cd 0.8, 0.8, 1.0; Mn 81, 155, 324; Ni 3.4, 3.8, 6; Cu 1.8, 2.6, 9; Co 2.5, 2.6, 3.7; Cr 0.6, 0.8, 1.1; and V 1.8, 2.3, 7.0. Pearson product moment correlation shows closest associations for metals within groups a and b (correlation coefficients >0.80 and $p < 10^{-15}$). In group c, Pb, Mn, Ni, Cu, Co and V, exhibit strongest associations (correlation coefficient >0.80, $p < 10^{-12}$). These baseline data apply to modern alluvium of the Mississippi River delta. (Acknowledgements: ATSDR/MHPF cooperative agreement #U50/ATU398948.)

1051 PAH AND METAL MIXTURES IN BAYOU ST. JOHN SEDIMENTS.

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Bayou St John (BSJ) is an historic waterway and location of the first settlement (~1700) of New Orleans. This study evaluates the quantities and associations of mixtures of polycyclic aromatic hydrocarbons (PAHs) and selected metals (Pb, Zn, Cd, Mn, Ni, Cu, Cr, & V) of the bayou (n=10) and compares them with Mississippi River delta (MRD) alluvium (n=6) and New Orleans (n=28) soil samples. Sediments were collected with a Wildco-Ekman bottom dredge. The samples were air dried and ground to a fine powder. Accelerated solvent extraction (ASE) was applied to sediment extraction for analysis with gas chromatography-mass spectrometry (GC-MS). Metals were extracted using a 5:1 ratio of 1 mol L^{-1} nitric acid (room temperature) to soil, shaken for 2 hours, centrifuged (1000 x g - 15 min.), and filtered. Metal ions in the extracts were determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Mann-Whitney tests show differences ($p < 0.001$), with PAH in BSJ sediments > urban soils > MRD alluvium (PAH index ratio of 11k:3.6k:0.2k). No difference appears between metal content of sediments and urban soils. Pearson product moment correlation reveals that metals and PAH are not significantly associated. Several metals show significant associations, Pb-Cr, Mn; Zn-Cd, Ni; Cd -Ni, V; and Mn-Cr. Most PAHs are significantly associated. PAHs accumulate in Bayou Saint John sediments and, in contrast to urban soils, are mixed with, but not associated with metals. (Acknowledgements: Research on soil metals funded by the ATSDR/MHPF cooperative agreement # U50/ATU398948, and on soil PAHs by DOD Grant # DSWA01-97-1-0028.)

1052 ADDRESSING CONCERNS ABOUT TOXIC INTERACTIONS AT HAZARDOUS WASTE SITES.

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Traditionally, potential for harm from exposure to chemicals and radiation at hazardous waste sites is assessed one substance at a time. People concerned about "synergism" among pollutants find traditional assessment disconcerting. "Hanford Downwinders", concerned about releases from Hanford (south-central Washington), asked the Agency for Toxic Substances and Disease Registry (ATSDR) to discuss toxic interactions in general, and interactions between radioactive and nonradioactive substances in particular. In response, we reviewed literature on interactions among noncarcinogens and among carcinogens, mechanisms of interactions, and doses of interactions. Doses are critical. People often equate thresholds for toxic effects with conservative screening levels typically hundreds to tens of thousands of times below their lowest toxic doses (or doses that might result in measurable increases in non-threshold effects). For interactions between chemical and radioactive substances, we reviewed literature on combinations of tobacco smoke with (1) occupational and environmental exposures to radon progeny and (2) occupational exposures to plutonium oxide. The databases for these combinations were, respectively, the most plentiful and the most relevant to Hanford. Because non-additivity commonly results from saturation of shared elements in overlapping mechanisms, neither harmful nor protective interactions are likely from environmental exposures to mixtures of substances at levels orders of magnitude below their thresholds. However, ATSDR continues to evaluate the possibility interactions among chemicals with overlapping mechanisms of action might, in the event of massive releases, produce synergistic or antagonistic effects of public health significance. (The opinions expressed are those of the authors and may not necessarily represent ATSDR policy.)

1053 ANALYSIS OF A MIXTURE OF ESTROGEN AGONISTS IN AN ER-α REPORTER GENE ASSAY.

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Response surface methodology has been successfully applied to the analysis of a wide range of chemical mixtures in order to describe interactions among the chemicals while accounting for biological variability. One objective of our work is to apply similar techniques to the analysis of mixtures of endocrine-active compounds. A full 4x4x4 factorial design (64 groups) was used where each chemical (17β-estradiol at 0, 0.01, 0.1, 1.0 nM; ethinylestradiol at 0, 0.001, 0.01, 0.1 nM; diethylstilbestrol at 0, 0.01, 0.1, 1.0 nM) was assessed individually and in all possible combinations in an MCF-7 cell ER-α Environ Sci Technol 29:2140-6, 1995). A response surface was estimated using a non-linear mixed (population-averaged) model, as a sigmoid-shaped relationship was expected. The model was parameterized to allow for within-replicate correlated responses while assuming data across replicates were independent. The definition of additivity used was that given by Berenbaum (J Theoretical Biology 114:413-31, 1985). The overall test for additivity was rejected indicating a complex interaction among the three chemicals. At the highest concentrations, an antagonistic interaction was apparent, presumably due to competition for a common receptor. However, a similar analysis was performed using a 3x3x3 design in which the highest concentration of each chemical was omitted. In this region of the dose response, the overall test for additivity was not rejected, indicating an additive relationship among the three chemicals. This finding demonstrates the successful application of response surface approaches to ER-α agonists, and illustrates that interactions can differ greatly between different regions of the dose response. Funded by The Chemical Manufacturers Assoc. (Arlington, VA).

1054 REINVENTING MIXTOX: PRIORITY CHEMICAL MIXTURES AND THE INTERACTIONS-ADJUSTED HAZARD INDEX.

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Feedback from Regional Superfund risk assessors indicated that EPA's original Mixtox interactions data base was only marginally useful; it provided no indication of the numerical change in the Hazard Index due to interactions, and thus no quantitative alterations were being made in the site assessments.

This pilot project initiates the transformation of EPA's Mixtox data base into a tool directly useful for Superfund baseline risk assessment. Binary chemical mixtures (chemical pairs) were prioritized by screening analytical results from final National Priority List (NPL) sites using RfD-based screening concentrations, followed by application of an algorithm to rank the frequency of toxicologically significant binary mixtures at NPL sites. Priority inorganic and organic chemical pairs were selected from the top-ranking pairs. Trichloroethylene and lead were the predominant chemicals in the organic and inorganic priority pairs, respectively. Binary weight-of-evidence (WOE) determinations for interactions were then developed for 12 priority pairs using the current EPA mixture risk methodology. Of the 24 WOE classifications of joint toxic action for these 12 pairs, three were "greater than additive," four were "less than additive," one was "adequate evidence of additivity," and the rest were default classifications of additivity (due to conflicting or inadequate information). The WOE classifications, converted to numerical scores, are used to estimate an interactions-adjusted hazard index, as demonstrated in a software mock-up. (This abstract does not necessarily reflect EPA policy.)

1055 A MECHANISTIC ANIMAL-REPLACEMENT APPROACH FOR PREDICTING THE MEDIAN LETHAL CONCENTRATION (LC_{50}) OF MIXTURES OF NARCOTIC CHEMICALS IN THE RAT.

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The acute median lethal concentration (LC_{50}) in several aquatic species is associated with a relatively constant lipoid chemical concentration (C_{lipid}) of 40-160 mM. Recent studies suggest that these lipoid concentrations could be associated with the occurrence of non-specific chemical narcosis in mammals as well. In the case of chemical mixtures, if the exposure concentration of each component contributing towards the lipoid concentration can be calculated, then they can be added together to determine the set of ambient concentrations of chemicals in a mixture corresponding to LC_{50}. Further, the contribution of each chemical to the lipoid concentration can be predicted on the basis of its lipid:air partition coefficient (P_{la}). These observations provide a unique opportunity for exploring the development of animal-replacement algorithms for predicting LC_{50} of mixtures of narcotic chemicals in mammalian species, more specifically the rat. The objective of the present study was to develop an algorithm for predicting the LC_{50} of narcotic chemical mixtures based on knowledge of the P_{la} and the molar fraction (F_i) of each mixture component. An algorithm that considers C_{lipid} (40 - 160 mM), fraction of blood flowing through the metabolizing tissue, hepatic extraction ratio, and oil:air partition coefficient was developed. Results show that the above algorithm adequately predicted the rat LC_{50} of over 20 non-specific narcosis-inducing organic chemical mixtures containing alcohols, ketones, esters, alkanes, haloalkanes, and aromatics. The average ratio of the predicted mixture LC_{50} values to the experimental values was 1.1 (sd=0.45, n=22). The algorithm developed and validated in this study represents the first mechanistic animal-replacement tool ever developed for predicting the rat LC_{50} of narcotic chemical mixtures.

1056 PHYSIOLOGICAL MODEL-BASED ESTIMATION OF AN UNCERTAINTY FACTOR (UF) ACCOUNTING FOR PHARMACOKINETIC INTERACTIONS IN MIXTURES.

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While it may be difficult to obtain the necessary information to model the pharmacokinetics of chemicals in mixtures, it should be feasible to predict the maximal effect of interactions on the kinetics of each mixture component on the basis of theoretical and mechanistic considerations. The objectives of the present study were to: (i) predict and validate the theoretically-possible, maximal impact of interacting chemicals on the blood concentration profile of each component in mixtures of 10 volatile organic chemicals in the rat, and (ii) derive an UF by comparing the kinetics of chemicals obtained during single exposure to those obtained during mixture exposure. With the hepatic extraction ratio (E) set equal to zero or one, the physiologically-based pharmacokinetic (PBPK) model yielded an envelope demarked by the maximal and minimal blood concentration vs time profiles for each component of the chemical mixtures. The experimental data on blood concentrations of parent chemicals collected during exposure to increasingly complex mixtures (2, 4, 5, 8 or 10 components) approached but did not exceed the simulated envelope. The ratio of the area under the concentration of parent chemical vs time curve between the maximal interaction effect (i.e., while E=0 or E=1) and single exposures provides a factor that quantifies the theoretically-possible change in kinetics of components during mixed exposures. Mechanistically-based algebraic expressions, based on PBPK model equations, were developed to quantify UF which ranges between 1.2 and 10.1 in rats and 1.3 and 11.9 in humans at steady-state, for chemicals investigated in this study. This UF, in fact, is conceptually similar to the inter-individual UF for pharmacokinetic differences currently used in health risk assessment.

1057 TARGET ORGAN VARIABILITY IN THE TOXICITY OF CHEMICAL MIXTURES.

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To estimate the toxic action of chemical mixtures it is often assumed that the interactive toxicity of a chemical mixture is consistent across target organs, such as brain, liver, and kidney. To test this assumption a series of parallel experiments were performed to compare the phenotype-dependent interactive toxicity of simple mixtures of toxic metals in primary cultures of rat hepatocytes, rat osteoblastic bone cells (ROS17/2.8), and in Rhesus renal cortical cells (LLC-MK2). To minimize the differences between culture conditions and maximize the ability to make distinctions based on cell type alone, Ham's F12 medium supplemented with 5% FBS, glutamine, and gentamicin was used for all cultures. Cells were treated for 24 hr with 0-30 μM CdCl2, 0-60 μM HgCl2, or a 1:2 molar ratio of the mixture. Cytotoxicity was determined at 24 hr by lactate dehydrogenase release. The data were analyzed statistically and graphically by the linear model of null interaction of Kodell and Pounds. The hepatocytes were most sensitive, and the osteoblastic bone cells were least sensitive, to both metals. The Cd LC50s were ~5, 15 and 18 μM for the hepatocytes, LLCMK2 and ROS cells respectively. The cell type-dependent response to Hg was similar with LC50s of 19, 38, and 42 μM respectively. In contrast, the cytotoxicity of mixture was response additive in all three cell types tested. However, cell and tissue dose is an important determinant of toxicity. Variability in multiple target organ toxicity must be considered and a systems approach must be pursued when integrating and estimating the joint toxic action from exposures to multiple chemicals. (Supported by ATSDR U61/ATU581481.)

1058 EFFECT OF ETHYLBENZENE CO-EXPOSURE ON STYRENE'S NASAL TOXICITY IN RATS.

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Styrene is widely used in the production of plastics and resins. It is manufactured from ethylbenzene and thus co-exposure to both compounds is likely. While both compounds are respiratory irritants, styrene exposure results in damage to tissues of the nasal passages. Studies in this laboratory (Sidel and Schatz, 1998,1999) have demonstrated the toxic and proliferative effects of exposure to 50 ppm styrene. In this study, we characterized the effects of co-exposure with 300 ppm ethylbenzene. The co-exposure prevented the nasal toxicity (indicated by elevated levels of LDH and GGT in nasal lavage fluid) seen with styrene exposure alone. Acid phosphatase activity was increased by both styrene and ethylbenzene exposures alone and the response to the co-exposure was additive. It is possible that an inflamatory response (vs. toxicity) is involved with these solvents. The co-exposure also prevented styrene's proliferative effects in both olfactory and respiratory epithelia of the nose. Styrene is metabolized by CYP P450s to the reactive epoxide styrene 7,8-oxide, which is thought to be responsible for its toxicity. The co-exposure caused greater reductions in the activity of CYP P450s involved in styrene metabolism (CYP 2B1, CYP 4B1 and CYP 2E1) in nasal microsomes than caused by styrene alone. Activity of microsomal epoxide hydrolase was unchanged while GST activity was decreased with the co-exposure compared to styrene exposure alone. These data indicate that the reduction in activity of CYP P450s is a major factor in the alteration in toxicity.

1059 *IN VITRO* EVALUATION OF THE WEIGHT OF EVIDENCE METHOD FOR TOXICOLOGIC INTERACTIONS OF CHEMICAL MIXTURES.

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ATSDR, in collaboration with TNO, is conducting studies to evaluate the weight of evidence (WOE) method to estimate joint toxic action of chemical mixtures [Environ. Health. Perspect. 106: 1353-1360. (1998)]. An *in vitro* study, which measured the formation of MTT-formazan, was undertaken to evaluate the predictive capability of the WOE method. Rat kidney slices were used in two series of experiments, one with a quaternary mixture of similarly acting chemicals and the other with a binary mixture of dissimilarly acting chemicals. The quaternary mixture consisted of the cysteine conjugates of trichloroethylene (TCE), tetrachloroethylene (PCE), hexachlorobutadiene (HCBD), and 1,1,2-trichloro-3,3,3-trifluoropropene (TCTFP). The binary mixture consisted of $HgCl_2$ and the cysteine conjugate of HCBD. In each series, 120 kidney slices were assayed concurrently. For the binary mixture, the experimental design consisted of a complete factorial design, whereas for the quaternary mixture it consisted of 20 different combinations of varying doses. The results of the experimental studies agreed with the WOE assessment-based predictions which showed that, in the dose range tested, the quaternary mixture evidenced dose-additive toxicity. Additional agreements with the WOE assessment indicated that the HCBD conjugate had no impact on the toxicity of $HgCl_2$, but $HgCl_2$ antagonized the toxicity of the HCBD conjugate. The results of this *in vitro* study suggest that (1) the WOE method can be used for assessing joint toxic action across a range of doses and, (2) it can be used to screen/prioritize mixtures for experimental and follow up studies for multiple toxicity end points.

1060 STUDY DESIGNS FOR ASSESSING INTERACTIONS IN CHEMICAL MIXTURES.

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A study design for assessing interactions between chemicals in mixtures should have well-defined objectives and should answer three general questions. How many different dose combinations should be tested? Which mixture combinations should be tested? How many replicates of each selected mixture combination should be tested? We have developed a sequential approach for identifying appropriate chemicals combinations to test interactions. Our approach maximizes efficiency by focusing on local rather than global characterization of chemical interactions. To demonstrate our approach, we characterized concentration-response relationships for six compounds (Aminog, Atrazine, Flavone, MCanthrene, Naphthoflavone, and Quercetin) on 11-ketotestosterone and estradiol production in gonadal tissue cultures. Gonadal tissue was harvested from female bass (2-3 years of age) during the peak reproductive season (February - April) to maximize steroidogenic activity and available gonadal mass. Tissue was minced and incubated in culture medium with or without test agents for 48 hours in the absence or presence of exogenous testosterone to control for substrate limitation of steroidal aromatase activity. Duplicates of each treatment were performed in two experiments using tissue from a different female. Estradiol and 11-ketotestosterone in culture medium were measured by validated RIA procedures. Concentration-response curves were used to evaluate the magnitude of response necessary to detect departure from additivity in combination experiments and to identify important chemical combinations for testing. The results indicate advantages over full factorial and block designs with respect to efficiency and for conformity with criteria for reliable interaction analysis.

1061 EVIDENCE FOR DOSE ADDITIVITY OF CHLOROFORM (CHC13) AND BROMODICHLOROMETHANE (BDCM) IN MICE.

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Chemical disinfection of drinking water, while one of the public health triumphs of the 20th century, results in the production of a variety of disinfection byproducts (DBPs), including CHc13 and BDCM. The objective of the present study was evaluation of the nature (additive, nonadditive) of the hepatotoxic interaction between CHC13 and BDCM. Female CD-1 mice, ~65-70 days old, were gavaged daily for 14 days witheither CHC13, BDCM or binary combinations of CHC13 and BDCM in an aqueous vehicle (10% Alkamuls®) at a constant volume of 10 ml/kg. Dosing solutions were made fresh daily. On day 15, hepatotoxicity was assessed by serum sorbitol dehydrogenase (SDH). The experiment consisted of 12 treatment groups: one vehicle control group; three of CHC13 alone, 0.1, 1.0 and 3.0 mmol CHC13/kg/day; three of BDCM alone, 0.1, 1.0 and 3.0 BDCM/kg/day; three mixture groups at a 1:1 mixing ratio of CHC13:BDCM and total mixture dosages of 0.1, 1.0 and 3.0 mmol/kg/day: and, two mixture groups at a 2.7:1 mixing ratio of CHC13:BDCM and total mixture dosages of 1.0 and 3.0 mmol/kg/day. The 2.7:1 mixing ratio of CHC13:BDCM was based on their average seasonal proportions at 35 water treatment facilities (Krasner et al., 1989). The data were analyzed with a threshold additivity model (Gennings et at., 1997); the null hypothesis is dose additivity. An overall test for additivity of these 5 mixtures was not rejected (p=0.798). For the 1:1 mixing ratio, the mean experimental SDH values (IU/l) vs the means (95% prediction intervals) predicted by the threshold additivity model were: at 0.1 mmol/kg-14.5 vs 13.4 (6.6, 20.3); at 1.0 mmol/kg-24.1 vs 27.7 (13.5, 41.8); and, at 3.0 mmol/kg-155.7 vs 118.5 (17.5, 219.6). For the 2.7:1 CHC13:BDCM mixing ratio, the mean experimental vs predicted (95% prediction interval) SDH levels were:at 1.0 mmol/kg-26.4 vs 26.4 (12.1, 40.8); and, at 3.0 mmol/kg- 141.9 vs 111.0 (41.7, 180.2). The closeness of the predicted and observed mixture responses indicates that CHC13 and BDCM were dose additive for hepato

toxicity at these mixture doses and combinations. (This abstract may not reflect EPA policy.)

1062 TOXICOLOGICAL INTERACTIONS AMONG ARSENIC, CADMIUM, CHROMIUM, AND LEAD IN HUMAN KERATINOCYTES.

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The objective of this study was to characterize potential interactions among arsenic (As), chromium (Cr; 1:1 mixture of Cr^{3+} and Cr^{6+}), cadmium (Cd), and lead (Pb) with respect to cytotoxicity at a mixing ratios of interest in human keratinocytes. Three immortal keratinocyte cell lines (RHEK-1, HaCaT, and NM1) and normal human epidermal keratinocytes (NHEK) were exposed to increasing concentrations of the individual metals or to a mixture of the four metals. Cell viability was determined by MTT (thiazolyl blue) assay after 24 hr treatment. The overall range of concentrations studied for the individual metals was from 0.3 to 300μM. A 4-metal mixture (1X) was prepared at the concentrations of LD_{50}s of As, Cr, and Cd; but 100μM of Pb was utilized, as we were unable to get substantial cell killing at any dose used. Thorough statistical analyses of the cytotoxicity data were carried out to test for deviations from additivity and discern the nature of interactions among the four metals. An approach using an "additivity response surface", permits testing the hypothesis that metals in a mixture act in an additive fashion. In RHEK-1 and HaCaT, the responses were antagonistic at high concentration (0.3X). However, at the middle concentration (0.1X and 0.03X), there was a significant synergistic interaction. A synergistic response predominated at the middle concentration groups (0.3X and 0.1X) in NHEK and NM1. These findings suggest that the metals studied might exhibit different mechanism of toxicity in NHEK and NM1, which has very similar growth characteristics to normal cells, as compared to the other immortal keratinocyte cell lines. (This study was supported by ATSDR [Cooperative Agreement U61/ATU881475], and NIEHS Superfund Basic Research Program [P42 ES05949].)

1063 BIOCHEMICAL EFFECTS OF MANUFACTURED GAS PLANT TAR IN RATS.

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Previous studies have demonstrated that mouse lung is a target organ for the genotoxic effects of manufactured gas plant tar (MGP tar) following ingestion. In addition, the chemicals primarily responsible for these effects appear to be benzo[b]flouranthene, benzo[a]pyrene (B[a]P) and an unidentified component of MGP tar. The genotoxic effect of MGP tar in species other than mice has not been fully evaluated. Therefore, the present study evaluated the genotoxic effects of MGP tar in rats following ingestion. Powder diets containing neat MGP tar, soil contaminated with MGP tar, and organic extracts of these soils were fed to CD rats for 13 days. In addition, diets containing B[a]P ranging from 0.5 to 100 ppm were also evaluated. Metabolites of polycyclic aromatic hydrocarbons in urine and chemical: DNA adduct formation in lung, liver, forestomach and mammary tissues were evaluated. Ingestion of soil adulterated with MGP tar resulted in two major DNA adducts in lung tissue. One of the these adducts is derived from B[a]P while the second is formed from the unidentified component of MGP tar. Ingestion of diets containing neat MGP tar and an extract of contaminated soil also resulted in the unidentified DNA adduct being formed in lung and forestomach tissue. However, no DNA adducts were detected in liver and mammary tissues. In the case of rats ingesting 0.5 to 100 ppm B[a]P a single major adduct was only detected in tissues of rats receiving 50 and 100 ppm of B[a]P. These results indicate that the PAH component of MGP tar is readily bioavailable following ingestion similar to the results observed with mice. In addition, the unknown compound within the mixture may also play a key role in the formation of chemical:DNA adduct in rat tissue. (EPRI Grant WO2963-06.)

1064 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF AIDS COMBINATION THERAPIES IN SWISS (CD-1®) MICE.

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Combination therapy with anti-HIV and opportunistic infection drugs is a common practice in treatment of AIDS patients including pregnant women. Reproductive and developmental toxicity of most individual drugs is known but not the effects of combination therapies. To understand the reproductive and developmental consequences of combination therapies, commonly used anti-HIV drug AZT and opportunistic infection therapies pyrazinamide, rifabutin and clarithromycin were evaluated by 10 to 30 day gavage studies in Swiss (CD-1®) mice either alone or AZT with one of the opportunistic infection drghugs. The doses include AZT 200 and 400; pyrazinamide 300, 1500 and 3000, rifabutin 80, 320 and 640 and clarithromycin 250, 500 and 1000 mg/kg/day. AZT alone caused an increase in resorptions and decrease in litter size. Pyrazinamide alone caused slight declines in fetal weights and slight increases in the duration of gestation and resorptions. Rifabutin alone decreased the pregnancy rate and fetal weights. Clarithromycin alone decreased the pregnancy rate and reduced the litter size. Pyrazinamide, rifabutin or clarithromycin when given in combination with AZT increased the reproductive and developmental toxicity far greater than any one drug. Combination therapies caused decreases in pregnancy rates and litter sizes and increased the number of resorptions, stillborn pups and pup deaths. Significant numbers of gross external alterations in pups were not observed following administration of any drug alone or in combination. Clarithromycin appears to be the most toxic and pyrazinamide the least toxic of the three opportunistic infection therapies with regard to enhancement of reproductive and developmental toxicity of AZT. Results of these studies indicate that the reproductive and developmental toxicity of combination therapies could be more severe and possibly different from the individual drugs.

1065 BACKGROUND DNA MODIFICATIONS (I-COMPOUNDS) IN LIVER AND COLON OF FEMALE F-344 RATS FED WITH DIETS SUPPLEMENTED WITH SUNFLOWER, RAPESEED, OLIVE, OR COCONUT OIL.

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I-compounds are bulky covalent DNA modifications formed in tissues of untreated laboratory animals detectable by the ^{32}P-postlabeling method. They appear to be derived mainly from DNA-reactive intermediates of nutrient and oxygen metabolism. In this study, the postlabeling method was adapted for the analysis of DNA of rat colon, a target organ for fat-associated carcinogenesis, and the effect of various types of fat supplements was investigated. Groups of 5 female F344 rats were fed for 4 weeks diets containing 17 percent sunflower, rapeseed, olive, or coconut oil. DNA adducts were analyzed by the nuclease P1-enhanced version of the ^{32}P-postlabeling assay. Chromatography on 'home-made' tlc sheets was in 2 directions/2 solvents for the liver samples and 2 directions/3 solvents for the colon. Concentrations of sodium phosphate, urea, and ammonia (colon only) were optimized for the resolution of the respective adduct spots. In the liver, which is not a target organ, 5 spots could be clearly distinguished. All were present in all animals. Mean adduct levels of the individual spots ranged from 5 to 58 per 10E^9 nucleotides (RAL-values). Between the groups, there was a significant difference in only one spot, with RAL-values 2.5 times higher in the rapeseed group as compared with all others. In the colon, 7 spots were seen consistently. Mean adduct levels ranged from 3 to 89 per 10E^9 nucleotides. No difference between the groups was significant, primarily because of large variabilities within the groups. Epidemiological evidence suggests that various types of fats modulate the process of colorectal carcinogenesis differently. In a human biomonitoring study, DNA adducts associated with lipid peroxidation were higher in white blood cells of humans set on a sunflower oil-based diet compared to rapeseed oil. The present data derived from a target organ of an animal model do not confirm such differences for other DNA modifications of so far unknown structure. (Supported by the Swiss Federal Office of Public Health No.316.98.0677.)

1066 EVALUATION OF THE ORAL SUBACUTE TOXICITY AND GENOTOXIC POTENTIAL OF TRICHLOROMETHYL PHENYL CARBINYL ACETATE (TPCA).

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To evaluate the acute systemic toxicity of trichloromethyl phenyl carbinyl acetate (TPCA), 60 rats (10/sex/group) were dosed with 100, 300 or 1000 mg/kg/day, via gavage, for 14 days. Clinical signs included decreased food consumption and fecal volume, labored breathing, moribundity, and yellow anogenital staining. Hypertrophy of centrilobular hepatocytes was observed in males and females at 300 and 1000 mg/kg, and eosinophilic cytoplasmic inclusions were observed in male kidneys at all dose levels. The presence of eosinophilic cytoplasmic inclusions is unique to male rats and is compatible with the diagnosis of alpha 2-i-globulin nephropathy. According to an EPA Policy Statement, the male rat kidney response to chemicals that induce alpha 2-micro-globulin accumulation is probably not relevant to human risk assessment. Based on the effects seen in the liver of rats treated with 300 and 1000 mg/kg per day, the no-observed-effect-level (NOEL) was 100 mg/kg per day. TPCA was also tested in the bacterial reverse mutation assay using Escherichia coli tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9 and in Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537. TPCA did not cause a positive response in any of the tester strains in the presence or absence of S9. The clastogenic potential of TPCA was also evaluated using Chinese hamster ovary (CHO) cells, both in the presence and absence of an Aroclor-induced S9-activated test system. In the S9-activated test system, CHO cells were exposed to TPCA for 4 hours; in the non-activated test system, treatment was for 4 and 20 hours. No evidence of a clastogenic potential of TPCA was observed. TPCA did not show genotoxic potential based on negative results in both Ames and cytogenetic assays.

1067 DNA ADDUCTS IN LIVER, LUNG AND ISOLATED LUNG CELLS OF RATS AND MICE EXPOSED TO 160 PPM [RING-U-^{14}C]-STYRENE BY NOSE-ONLY INHALATION FOR 6 HOURS.

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Clara cells are predominant in mouse but almost absent in rat and human lung and contain various CYPs, which may oxidize styrene to the rodent carcinogen styrene-7,8-oxide (SO). SO or other reactive metabolites might form DNA adducts and cause the bronchioalveolar tumors seen in mice exposed to 150 ppm styrene. In rats no tumors were seen at 1000 ppm. 12 Rats and 60 mice were exposed to 160 ppm ^{14}C-styrene (52 mCi/mmol) for 6 h by nose-only inhalation in a recirculating exposure system. Liver and lungs were isolated 0 and 42 h after exposure. Type II cells and Clara cells were isolated from rat and mouse lungs, respectively. DNA adduct profiles in mice and rats differed quantitatively and qualitatively in liver, total lung and lung cells. At

0 and 42 h after exposure, the 2 isomeric N7-guanine adducts of SO (GS) were present in liver at 3.0±0.2 and 1.9±0.3 (rat) and 1.2±0.2 and 3.2±0.5 (mouse) per 10^8 bases. Several unidentified adducts were present at 2-3 times higher concentrations than GS in mouse, but not rat liver. In both rat and mouse lung GS was the major adduct at ~1 per 10^8 bases at 0 h and these levels halved at 42 h. In both rat Type II and non-Type II cells, GS was the major adduct and was ~3 times higher in Type II cells than in total lung. DNA adduct levels in Clara cells and non-Clara cells were similar to total lung. The hepatic covalent binding index (CBI) at 0 and 42 h was 0.19±0.06 and 0.14±0.03 (rat) and 0.25±0.11 and 0.44±0.23 (mouse), respectively. The pulmonary CBIs were 0.17±0.04 (rat) and 0.24±0.04 (mouse). Compared to CBIs for other genotoxicants, these values indicate that styrene has only very weak genotoxic potency. The overall results of this study suggest that a non-genotoxic or epigenetic mechanism, possibly caused by a cytotoxic metabolite, is involved in the tumorigenicity in chronically exposed mice. (This study was supported by the Styrene Information and Research Center and Shell International Chemicals.)

1068 FURFURAL DOES NOT INDUCE UNSCHEDULED DNA SYNTHESIS (UDS) IN THE *IN VIVO* B6C3F1 MALE AND FEMALE MOUSE HEPATOCYTE DNA REPAIR ASSAY.

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In this study, the ability of furfural to induce UDS in hepatocytes after *in vivo* administration to male and female B6C3F1 mice was examined. From a preliminary toxicity study, the oral maximum tolerated dose (MTD) of furfural was determined to be 320mg/kg body weight. In the UDS study rats were treated with single oral 50, 175 and 320mg/kg doses of furfural. Negative controls were treated with distilled water and positive controls with either 20mg/kg dimethylnitrosamine (DMN, for 2 to 4 hr expression of UDS) or 200mg/kg *o*-aminoazotoluene (OAT, for 12-16 hr expression of UDS). Hepatocytes were isolated by liver perfusion either 2-4 hr (3 mice per furfural treatment level, 3 negative and 3 positive controls) or 12-16 hr (3 rats per furfural treatment level) after treatment. Hepatocytes were cultured in medium containing [^{3}H]thymidine for 4 hr and UDS assessed by grain counting of autoradiographs. Furfural, at doses of 50, 175 and 320mg/kg, did not produce any statistically significant increase in the net nuclear grain count after either 2-4 or 12-16 hr of treatment. In contrast, significant increases in net nuclear grain counts were observed in hepatocytes from male and female mice treated with either DMN for 2-4 hr or OAT for 12-16 hr. Net grain counts were significantly increased from -1.26 to +38.8 ($p<0.01$, pooled t-test) for male mice treated with DMN; from -2.41 to 30.78 ($p<0.001$, pooled t-test) for female mice treated with DMN; -2.79 to 17.23 ($p<0.001$, pooled t-test) for male mice treated with OAT; and -2.57 to 28.78 ($p<0.001$, pooled t-test) for female mice treated with OAT. The percentage of hepatocytes with net grain counts >5 was significantly increased for both positive controls while those for furfural-treated groups and controls were similar. These results demonstrate that furfural does not produce DNA damage in male or female mice hepatocytes *in vivo* at doses up to the MTD of 320 mg/kg. (Supported by FEMA.)

1069 CARCINOGEN INDUCED DNA DELETIONS IN MICE.

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Genetic instability and deletions are involved in carcinogenesis. We have used the mouse pink-eyed unstable (pun) reversion assay to detect DNA deletion events *in vivo* in the mouse. The pun phenotype is a dilute fur coat and pink eyes resultant from a lack of pigmentation. Spontaneous reversion of the mutation produces mice pigmented with spots in either tissue. Such reversions are the result of homologous recombination deleting one copy of a tandem duplication that interrupts the p gene. Deletion during embryogenesis results in a visible fur spot or an eyespot in the offspring.Induced pun reversion can be detected by the furspot assay following exposure to various carcinogens such as X-rays, cis-platinum(II)diamine dichloride (cisplatin), EMS, MMS, ENU, benzo(a)pyrene (B(a)P), trichloroethylene, benzene, sodium arsenate, polychlorinated biphenyls, dioxin and cigarette smoke. Eyespots are induced in this study by X-ray, cisplatin and B(a)P. As the position of an eyespot indicates the time during embryo development at which the deletion event occurred, examining the eyespots within the region of the retinal pigment epithelium (rpe) that correlates to the time of induction allows an increased sensitivity. Pregnant dams are induced 10 days post coitum and the pups examined for furspots 10 days post partum. Eyes are taken 30 days post partum and the rpe is recovered and examined. The eyespot assay requires less than 15 mice per chemical, substantial less than the furspot assay or many other phenotypic *in vivo* assays. These results further substantiate carcinogen induced genomic instability due to an increased level of homologous recombination. Since 25% of our genome consists of repeated elements, deletion between such repeats may be a significant step in carcinogenesis.

1070 EFFECT OF DIETARY FOLATE DEFICIENCY ON ARSENIC GENOTOXICITY IN MICE.

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Arsenic, a human carcinogen found in drinking water supplies throughout the world, is clastogenic in human and rodent cells. An estimated ten percent of Americans are deficient in folate, a methyl donor necessary for normal nucleotide metabolism, DNA synthesis, and DNA methylation. Folate deficiency has been shown to enhance the activity of several mutagens, and may also increase arsenic genotoxicity. We are evaluating induction of micronuclei (MN) in peripheral blood erythrocytes of male C57Bl/6J mice fed folate-deficient (FD; 0 mg/kg diet) or folate-replete (FR; 5 mg/kg) diets for 7 weeks. During week 7, mice on each diet (n=4 per group) were given 4 consecutive daily doses of sodium arsenite (0, 2.5, 5, or 10 mg/kg in water) or acrylamide (positive control; 50 mg/kg in water) via oral gavage. Kinetochore immunostaining was employed to help distinguish between MN resulting from chromosome breakage and MN resulting from chromosome loss. Statistically significant increases in MN were observed in both FD and FR mice at 5 and 10 mg/kg arsenite in polychromatic erythrocytes (PCEs), but not normochromatic erythrocytes (NCEs). At 10 mg/kg, MN-PCE levels in FD mice were 4-fold higher than control levels (16.1±3.9/1000 vs. 4.1±0.8/1000), while increases in FR mice were 1.6-fold higher (5.6±1.3/1000 vs. 3.4±0.7/1000). For all dose groups, MN-NCE levels were higher in FD mice than in FR mice. The observed MN were of the negative kinetochore staining type, suggesting that they derived from chromosome breakage rather than whole chromosome loss. Our results indicate that dietary folate deficiency increases arsenic-induced clastogenesis in mice at high doses. Since both arsenic and folate deficiency interfere with DNA repair, dysregulation of this process may contribute to the high MN levels observed. (This abstract does not necessarily represent US EPA policy.)

1071 GENOTOXICITY OF METHYLPHENIDATE HYDROCHLORIDE IN THE *IN VIVO* MOUSE MICRONUCLEUS ASSAY.

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The potential of methylphenidate HCl (MPH) to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) of mice was evaluated *in vivo*. Male and female Crl:CD-1®(ICR)BR mice were gavaged with single doses of MPH; the positive control, cyclophosphamide (CP); or the MPH vehicle control (phosphate buffer). Based on preliminary toxicity assessments, MPH doses used were 87.5, 175, and 350 mg/kg for males and 75, 150, and 300 mg/kg for females. MPH-treated animals were individually housed. Animals (n=5/sex) in the MPH and vehicle treatment groups were euthanized and bone marrow harvested at 24 and 48 hours post-treatment; positive control animals were evaluated only at 24 hours. Coded bone marrow preparations were evaluated for cytotoxicity by calculating the PCE to red blood cell ratio for at least 200 erythrocytes. At least 2000 PCEs per animal were scored for micronuclei, and the frequency of micronuclei was expressed as the percent micronucleated cells. One male in the high-dose group died within the first hour after treatment; no other animals died. Dose-related transient clinical signs (eg, hyperactivity, licking, and/or chewing), consistent with the known pharmacological activity of MPH, were observed at all dose levels in both sexes, primarily on the day of dosing. Plasma data from MPH-treated satellite animals confirmed exposure to methylphenidate and the primary human metabolite. At the dose levels used, MPH was not cytotoxic to the bone marrow. MPH did not induce increases in the frequency of micronucleated PCEs over levels observed in vehicle control animals. The positive control, CP, induced a significant increase in micronucleated PCEs in both males and females. MPH was negative in the mouse bone marrow micronucleus assay.

1072 N7-(1-(HYDROXYMETHYL)-2,3-DIHYDROXYPROPYL) GUANINE IS THE MAJOR DNA ADDUCT AFTER EXPOSURE TO 1,3-BUTADIENE BUT NOT 1,2-EPOXY-3-BUTENE.

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Mice are more susceptible to the rodent carcinogen 1,3-butadiene (Bd) than rats. Genotoxic epoxides of Bd, 1,2-epoxy-3-butene (BMO), 1,2-epoxy-3,4-butanediol (EBD) and 1,2:3,4-diepoxybutane (DEB), have been suggested as the ultimate carcinogens. Reaction of DNA or guanosine (dG) with BMO gives equal amounts of *N*7-(2-hydroxy-3-butenyl)guanine (**G1**) and *N*7-(1-(hydroxymethyl)-2-propenyl)guanine (**G2**). When DEB was reacted with DNA or dG mainly *N*7-2,3,4-trihydroxybutylguanine (**G3**) was formed. EBD exists as different stereo-isomers. Racemic EBD reacted with dG gave equal amounts of **G3** and *N*7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine (**G4**), but when S-EBD was reacted with dG **G4** was the predominant adduct (> 90%) with only traces of **G3** (~5%). In rats and mice exposed to [4-^{14}C]-BMO by i.p. administration (1-50 mg/ kg) DNA adduct profiles in rat and mouse were similar in liver and lung with **G1** and **G2** as main adducts (857 and 368 per 10^8 bases in rat and mouse liver, respectively, after 20 mg/kg) and **G4** as minor adduct (101 and 50 per 10^8 bases). In rats and mice exposed to 200 ppm [2,3-^{14}C]-Bd by nose-only inhalation for 6 h, **G1** and **G2** were only minor adducts in liver (1.9 and 8.0 per 10^8 bases for rats and mice, respectively) and lung (1.6 and 6.6 per 10^8 bases). **G3** was present in mouse, but not rat, liver and lung at levels of 20 and 12 adducts/10^8 bases. The major adduct was **G4** which accounted for 13 and 90 (liver) and 11 and 139 (lung) adducts/10^8 bases in rats and mice, respectively. These results indicate that the major DNA adduct following exposure to Bd is from EBD, and not from BMO or DEB. In addition, the results suggest that there is a strong stereospecific preference for the formation of S-EBD in mice. These findings have important implications for the understanding of the mechanism of carcinogenicity of Bd.

1073 THE PRODUCTION OF MICRONUCLEI BY DI-*N*-BUTYL PHTHALATE IN MALE F-344 RATS.

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Di-n-Butyl phthalate (DPB) is an odorless and colorless liquid used in the manufactoring of plastics. The annual global production is estimated to be in the billions of pounds. These compounds have been found in sugar, salt, dairy products, meat, fish, shell fish, instant soups, cereal, and edible plant products. Due to the ubiquitous nature of DBP and the enormous potential of human exposure, it is of utmost importance to investigate the toxic potential of DBP. Although there is little carcinogenic and genotoxic data for DBP, other phthalate esters such as di (2-ethylhexyl) phthalate (DEHP)have been shown to be carcinogenic and genotoxic. Based on this data we hypothesize that DBP is genotoxic. To test this hypothesis male F344 rats were dosed with various doses of DBP (10g/kg, 5g/kg, & 1g/kg) by gavage. Twenty-four hours after dosing, the rats were sacrificed and the testes removed. Slides were prepared from the testes, stained with 33258 Hoechest, and analyzed by fluorescence microscopy. One-thousand cells from each group were analyzed for micronuclei formation. The results revealed that DBP produced micronuclei in a dose dependent fashion. The high dose (10g/kg) produced micronuclei in 174 cells, the 5g/kg dose produced mironuclei in 133 cells, and the 1g/kg dose produced micronuclei in 56 cells. Eight cells in the control group that recieved only corn oil formed micronuclei. The formation of micronuclei indicates that chromosome breakage has occurred. This is direct evidence that DBP is genotoxic *in vivo*. This genotoxicity can lead to cancer or other adverse effects.

1074 COMPARISON OF THE MUTAGENICITY OF METHYLEUGENOL (ME) *IN VIVO* IN THE LIVER OF BIG BLUE® TRANSGENIC RATS AND MICE.

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Methyleugenol (ME) is a naturally occurring allylbenzene found in some foods and spices and is also used as a flavoring agent. A primary metabolite of ME, 1'-hydroxymethyleugenol, forms protein and DNA adducts. Although ME is hepatocarcinogenic in rodents, it is not mutagenic in most standard assays. To explore whether ME is mutagenicity *in vivo*, it was administered by gavage for 90 days at a carcinogenic dose to transgenic Big Blue® male mice (300mg/kg/day, 5 days/week) and Big Blue® female rats (1,000mg/kg/day 5 days/week), harboring the lacI target transgene. Despite the finding that the mutant frequency (MF) of lacI in the liver of ME-treated mice ($4.27\pm1.09\times10^{-5}$) was the same as that recorded in control mice ($4.20\pm2.15\times10^{-5}$), the mutation spectrum from the ME-treated group was significantly different ($p<0.034$) from that in controls. In contrast to the findings in mice, the MF of lacI was significantly higher ($p<0.05$) in the liver of ME-treated female rats ($8.69\pm3.09\times10^{-5}$) compared to controls ($1.20\pm0.73\times10^{-5}$). These results illustrate the importance of interspecies comparisons in evaluating the mutagenic potency of a compound. This species difference for ME is most likely due to metabolic differences between rats and mice, an important consideration in the extrapolation of risk of ME to man.

1075 FREQUENCY AND SPECTRUM OF MUTATIONS INDUCED IN THE LAC I GENE OF TRANSGENIC MICE AFTER COMPLEX EXPOSURES TO GENOTOXIC CARCINOGENS.

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Transgenic rodents harboring shuttle vectors allow mutation analyses in any tissue of interest. These models also enabled the characterization of mutational spectra for several individual carcinogens. However, there is limited mutational data on agents given in combination. We have determined the mutant frequency of benzo[a]pyrene (BaP, 250 mg/kg/day), nitrosodimethylamine (NDMA, 7 mg/kg/day), and ethylnitrosourea (ENU, 50 mg/kg/day) in 12 week-old male Big Blue B6C3F1 mice administered either singly (3 daily oral doses) or in combination (BaP-day 1, NDMA-day 2, and ENU-day 3). All genotoxic agents, alone or in combination, increased mutant frequencies in the liver (3- to 6-fold) and kidney (2- to 4-fold). ENU alone and in combination increased mutant frequencies in the brain (1.5 fold) while BaP and NDMA alone had no effect. None of the treatments increased mutant frequencies in the testes. Mutational spectrum analyses of liver DNA revealed a high percentage of G:C to A:T transitions in the negative control (88%) and the NDMA (64%) groups. In contrast, BaP, ENU and the combination treatment induced a high percentage (>50%) of transversions. G:C to C:G (3 out of 4), A:T to T:A (3 out of 5), and G:C to T:A (10 out of 24) were the most common type of transversions induced by BaP, ENU, and the combination treatment, respectively. Finally, 46% (19 out of 41) of the mutations in the combination treatment group occurred at CpG islands compared to less than 22% in the other treatment groups. These results suggest that high doses of genotoxic agents in combination vs. singly while not affecting mutant frequency may change mutational spectrum.

1076 APPLICATION OF BIG BLUE® (BB) TRANSGENIC RAT FOR THE STUDY OF MUTATION INDUCTION IN NASAL MUCOSA: AN INHALATION STUDY WITH GLYCIDYL METHACRYLATE.

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The introduction of transgenic technology enhanced our ability to study mutagenicity in virtually every tissue of rats and mice. We have utilized the transgenic BB Fischer-344 rat model for the study of *in vivo* mutagenic potential of inhaled glycidyl methacrylate (GMA) - a chemical used in a variety of industrial polymer chemistry applications. Previous studies have shown that GMA is mutagenic *in vitro* in the Ames and CHO/HGPRT assays but was negative in a bone marrow micronucleus assay for cytogenetic damage following i.p. doses of up to 300 mg/kg bw. In order to evaluate its mutagenicity in a target tissue relevant to potential occupational exposure, respiratory and olfactory mucosa of GMA-exposed BB rats (1, 10, and 25 ppm; 6 h/d, 5 d/wk for 2 wks followed by a 4-wk manifestation period) were analyzed for lacI mutations. The highest concentration induced significant body weight depression and histopathological changes in both olfactory and respiratory mucosa. However, there were no significant increases in lacI mutant frequency (per 100,000 plaque forming units) vs. negative control either in the olfactory (15.7 vs. 15.1) or the respiratory (18.1 vs. 12.6) mucosa of rats (N=5) exposed to 25 ppm GMA. The positive control dimethylnitrosamine (p.o., 6 mg/kg/d, 5d/wk for 2 wks followed by 3 wks manifestation) induced a significant elevation in mutant frequency (194.3) in the olfactory mucosa. These results are suggestive of a critical role for metabolic processes in affording a significant level of protection against the potential *in vivo* genotoxicity of GMA.

1077 INVESTIGATION OF THE RADIOADAPTIVE RESPONSE IN BRAIN AND LIVER OF PUR288 *LacZ* TRANSGENIC MICE.

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The radioadaptive response (RA), where a small priming dose of ionizing radiation can lessen the effects of subsequent exposure to a higher radiation challenge dose, was investigated in multiple tissues within a transgenic organism. Well characterized in *in-vitro* models, current RA research has focused on particular cell types (i.e., lymphocytes), and does not provide comparative data for responses of multiple tissues within an organism. Transgenic animals are useful for such comparisons, because the transgene is integrated into all cells in the body. The pUR288 *LacZ* plasmid based transgenic mouse model utilizes a plasmid vector allowing highly efficient recovery of mutation targets, and is robust to large size change mutations that result from radiation exposure. Female C57Bl/6 pUR288 *LacZ* mice were exposed to priming doses ranging from 7.5 to 37.5 R x-rays over a three day period. After three weeks they received an acute challenge dose of 250 R x-rays. Animals were euthanized three weeks post-challenge, and brain and liver were flash frozen in liquid nitrogen, and stored at -80°C until analysis. Spontaneous mutant frequencies (MF) were significantly higher in liver than in brain (6.62×10^{-5} vs. 3.51×10^{-5}). In the absence of a priming dose, the 250 R challenge doubled the MF of both liver and brain (13.38×10^{-5} and 7.63×10^{-5} respectively). There were no apparent radio-protective effects from any priming dose in liver, while priming doses of 15, 22.5, and 37.5 R significantly reduced (by 40%) the mutagenic effects of the 250 R challenge in brain. Restriction analysis of mutants revealed a significant decrease in large size-change mutations at the three priming doses in brain. This study demonstrates the utility of this model for the investigation of radiological processes of large size-change mutations, as well as demonstrating a radioadaptive response in brains, but not livers of mice *in vivo*. (Supported by U.S. DOE DE-AC09-756R000 and the American Foundation for Pharmaceutical Education.)

1078 MUTATION FREQUENCIES OF MINISATELLITE REPEAT NUMBERS IN HUMAN SPERM BEFORE AND AFTER CANCER CHEMOTHERAPY WITH ALKYLATING AGENTS.

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The spontaneous frequency of minisatellite repeat number changes in the human germline is relatively high. We chose minisatellites as mutation targets to determine whether they can be used as sensitive indicators of heritable genetic damage caused by chemical mutagens. We compared the mutation frequencies in sperm of the same Hodgkin's disease patients pre- and post-treatment with alkylating agents. Small pool polymerase chain reaction (SP-PCR) (PCR on DNA equivalent to approximately 100 sperm) and Southern blotting techniques were used to detect mutations and quantify the frequency of repeat number changes at the minisatellite MS205 locus. At least 7900 sperm in each sample were screened. The mutation frequencies of pre- and post-treatment for the two patients treated with 3 to 4 cycles of Cytoxan, Vinblastine, Procarbazine, and Prednisone (CVPP) / Adriamycin, Bleomycin, Dacarbazine, CCNU, and Prednisone (ABDIC) were 0.22 and 0.23%; and 0.94 and 0.98%. The mutation frequencies for one patient treated with 6 cycles of Mechlorethamine, Oncovin, Procarbazine, and Prednisone (MOPP) were 0.79 and 1.14%. The mutation frequency of the patient after MOPP treatment was 1.44 times as high as that before treatment, which was statistically significant. Since the samples were collected 9 to 15 years after receiving alkylating agent chemotherapy, we conclude that there is no effect of CVPP/ABDIC regimens on the mutation frequency in spermatogonia. However, the higher doses of the alkylating agent procarbazine in the MOPP treatment may increase the mutation frequency at the MS205 locus in spermatogonia.

1079 ASSESSMENT OF DNA DAMAGE IN WORKERS EXPOSED TO ROOFING ASPHALT.

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To determine the potential for increased genetic damage due to asphalt-fume exposure, the single cell gel electrophoresis (comet) assay was used to measure DNA strand breaks in peripheral blood leukocytes at the start and end of a work week that involved exposure to asphalt fume, and in some cases coal-tar pitch. The study population consisted of 25 roofers exposed to hot asphalt and 12 construction workers not exposed to asphalt during the past 5 years. Air monitoring and urinary 1-OH-pyrene levels were used to assess PAH exposure. DNA damage is expressed as %Tail DNA (±S.E.M.). The average %Tail DNA at the start of the week was 15.1±0.6 among all roofers and 14.3±1.1 among controls. The average %Tail DNA at the end of the week was 16.7±0.6 among roofers and 14.5±1.0 among controls. In some cases roofers (n=18) were exposed to coal tar pitch during removal of existing roofing materials prior to applying hot asphalt. These roofers had higher air levels of total particulates and benzene solubles as well as urinary 1-OH-pyrene than a subset of roofers (n=7) not exposed to coal-tar pitch for at least 3 months. Start of the week levels of DNA damage were lower in the non-pitch exposed group of roofers (13.7±0.7) than in the pitch exposed group (16.1±0.6). End of the week levels of DNA damage were somewhat higher in the pitch-exposed roofers (16.9±0.8) compared to the group of roofers who were not exposed to pitch (16.1±0.6). Coal-tar pitch may contribute to higher basal levels of DNA damage than exposure to asphalt fume alone.

1080 ANALYSIS OF THE COMPONENTS OF EDIBLE OIL FUMES IN THE KITCHEN AND THEIR GENOTOXCITY IN *DROSOPHILA*.

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The objective of this study was to test the components of the condensation of edible oil kitchen fumes and their genotoxicity in Drosophila. Analytic method of the components was carried out by gas chromatography and mass spectra (GC/MS) and the genotoxicity of the condensate was detected using the sex-linked recessive lethal (SLRL) test in Drosophila. Results of GC/MS analysis showed that 74 kinds of organic compounds were detected out in the organic extracts of condensed oil from kitchen fumes. The compounds included hydroxylic acids, hydrocarbons, alcohols, esters, aldehydes, ketones, aromatic compounds, and steroids, etc. The total frequency rates of SLRL induced at the concentrations of 110, 320 and 960μg/ml were 1.732%, 4.306% and 1.707%, respectively. Meanwhile, their sterility rates of the first broods were 2.6%, 2.1% and 2.8%, respectively ($p<0.05$ as compared with the control). The frequency of SLRL in the second brood was 0.5% at 320μg/ml and 0.5% at 110μg/ml ($p<0.001$). We conclude that condensates of edible oil fume have many kinds of organic components which include hydroxylic acids, hydrocarbons, alcohols, esters, aldehydes, ketones, aromatic compounds, and steroids, and that the condensate of edible oil fume has genotoxic activity in Drosophila.

1081 SINGLE-STRAND DNA BREAKS FOLLOWING EXPOSURE TO COMBINED THERAPEUTIC HIV/AIDS AGENTS.

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The Single Cell Gel Assay (Comet Assay) was used to measure DNA single-strand breaks occurring in human lymphoblast cells (GM03798, NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ) exposed to therapeutic HIV/AIDS agents either individually or in combination. Azidothymidine (AZT) had been noted to cause such DNA damage by other detection methods. We wished to determine if the Comet Assay would also detect the damage by AZT and if more damage would occur with combined therapeutic agents. Therefore, we also examined zalcitabine (ddC) and didanosine (ddI) individually, in binary combinations with AZT, and as mixtures of all three chemicals. All three compounds are nucleoside analogs used to treat HIV/AIDS. Viability of the cell cultures was determined with trypan blue. For the Comet Assay, the cells were treated with each of the compounds for various times and concentrations to determine the optimal concentrations and times for testing. AZT caused increasing amounts of cell death and DNA single-strand breaks as the concentration was increased from 100 to 800 μM. The maximum loss of viability occurred at 48 hrs. When individually tested at 200 to 800 μM, neither ddC nor ddI caused the loss of cell viability or the DN single-strand breaks noted with AZT. Combinations of ddC at the highest concentration tested (800 μM) with AZT at 200 μM (a concentration producing minimal comets) increased the size of the comets noted with AZT alone indicating additional DNA damage. Similar combinations of ddI and AZT showed the same effect. Mixtures of ddC (800 μM), ddI (800 μM) and AZT (200 μM) produced the greatest amount of damage.

1082 A GENOTOXICITY STUDY WITH P-ARAMID RFP (RESPIRABLE-SIZED, FIBER-SHAPED PARTICULATES).

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The objective of this *in vitro* assay was to assess the potential for p-Aramid RFP (respirable-sized, fiber-shaped particulates) to induce chromosomal aberrations in cultured human peripheral blood lymphocytes without metabolic activation. The highest concentration tested in this assay was limited by the physical characteristics of p-Aramid RFP. The test article was suspended in fully supplemented RPMI culture medium with 1% Pluronic F68. All dosing was achieved using a dosing volume of 90% (900 µL/mL) and the vehicle control cultures were treated with 900 µL/mL of fully supplemented RPMI culture medium with 1% Pluronic F68.In the chromosomal aberrations assay, the treatment period was for 3.0 hours and 19.0 hours without metabolic activation, and cultures were harvested 22.0 hours from the initiation of treatment. Replicated cultures of human whole blood lymphocytes were incubated with 6.30, 12.6, 25.2, 50.4, 101, 201, and 401 µg/mL. Cultures treated with concentrations of 6.30, 12.6, 25.2, and 50.4 µg/mL for 3.0 hours and 6.30, 12.6, 25.2, and 201 µg/mL for 19.0 hours were analyzed for chromosomal aberrations. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. The results demonstrated that p-Aramid RFP was negative for inducing chromosomal aberrations in cultured human peripheral blood lymphocytes without metabolic activation.

1083 SPONTANEOUS HYDROLYSIS AND AN ACTIVE REPAIR PROCESS DETERMINE THE OVERALL RATE OF REMOVAL OF DNA-PROTEIN CROSSLINKS FROM FORMALDEHYDE-EXPOSED CELLS.

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Formaldehyde is an ubiquitous chemical with numerous sources of human exposure in both environmental and occupational settings. Formaldehyde is also a rodent carcinogen and it forms DNA-protein crosslinks (DPC) as the dominant form of DNA damage in exposed cells. Neither the mutagenic potential nor repair mechanisms of these important lesions are known at this time. In this study, we conducted detailed analysis of the stability of formaldehyde-induced DPC *in vitro* and in cultured cells. Both DNA-histone crosslinks formed *in vitro* and DPC induced *in vivo* exhibited a similarly high rate of spontaneous hydrolysis with the average half-life of 26.3 hr at 37°C and physiological pH/ionic conditions. The rate of the DPC removal from intact cells was even more rapid and was similar among three different human cell lines (HF/SV fibroblasts, Ad293 kidney and A549 lung cells). The average half-life was 12.5 hr; range 11.6-13 hr. After adjustment for the spontaneous loss, an active repair process was calculated to eliminate DPC from cells with the average t1/2=23.3 hr. Nucleotide-excision repair appears to play no significant role in the removal of DPC since repair-deficient XP fibroblasts had kinetics of the adduct elimination similar to other cells. Depletion of intracellular glutathione had no effect on the initial levels of DPC or the rate of their repair. A rapid loss of DPC from exposed cells determined in part by hydrolytic instability of these lesions suggests that application of DPC measurements as a biological dosimeter of formaldehyde exposure could be limited to situations with very recent exposures.

1084 MECHANISM OF METHYLENE DI-PHENYL DIISOCYANATE GLUTATHIONE CONJUGATE MICRONUCLEI INDUCTION DISTINGUISHABLE FROM THAT OF METHYLENEDINALINE.

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Methlyene di-phenyl diisocyanate (MDI) is widely used in the production of polyurethane products, such as wood binders and foams. Diisocyanates are very reactive compounds that can undergo nonenzymatic hydrolysis to form methylenedianiline (MDA), or react under physiological conditions with primary amines to form ureas and/or with thiols to form labile thiol acid esters. MDA is a carcinogen in animals and a suspected carcinogen in humans. We have previously reported that exposure of Brown Norway rats (BNR) to MDI induces micronuclei (MN) in bone marrow polychromatic erythrocytes. In vitro studies suggested that both MDI glutathione (MDI-GSH) conjugates and MDA are potential genotoxic metabolites of MDI. Addition of MDI directly to cell cultures did not induce micronuclei. The mechanism of micronuclei induction of these metabolites was explored in the present study. Chinese hamster lung fibroblasts were incubated with MDA or MDI-GSH. Micronuclei kinetichore from cytokinesis-blocked cells were labeled by immunofluorescent staining. Cells were washed, methanol fixed, treated with Tween 20 buffer, then labeled with antikineticore and fluoresceinated goat anti-human IgG antibodies to discern the presence of centromere within the micronuclei. Vincristine, DMSO and glutathone were used as positive, solvent and negative controls, respectively. MDA induced MN were negative with respect to anti-kineticore antibody binding. This is consistent with induction of chromosomal fragments by MDA binding to DNA. MDI-GSH induced MN had a significant increase in the number of anti-kineticore antibody labeled MN. MDI-GSH also increased the number of cells in metaphase. These results suggest that MDI-GSH MN induction was mediated through disruption of microtubules, whereas MDA MN induction was by a DNA-binding mechanism.

1085 *IN VIVO* DETECTION OF CHEMICALLY-INDUCED MUTATIONS IN TRANSGENIC FISH CARRYING CII/CI MUTATIONAL TARGETS.

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We examined the responses of the cII/cI transgenes in fish (medaka, *Oryzias latipes*) following treatment with either, the model mutagen, ethylnitrosourea (ENU), or a drinking water disinfectant by-product, potassium bromate ($KBrO_3$). The assay consisted of recovering the bacteriophage lambda vector which harbors the cII/cI target genes using *in vitro* packaging, followed by plating phage-infected indicator bacteria to quantify the frequency of cII/cI mutants. The assay is based on the role that the cII protein plays in the ability of the bacteriophage lambda to commit to either the lytic or lysogenic cycle using specialized E. coli hosts. The lysogens produced by the lambda phage with the wild-type cII/cI are favored, and indistinguishable in the E. coli lawn, while lambda phage that carry a cII/cI mutation are selected by forming plaques on the bacterial lawn when incubated at 24°C. Despite the small size of the fish, the vector was recovered with high efficiency (>0.3-2 $X10^6$ PFUs/reaction) which facilitated practical analyses of cII/cI mutant frequencies (MF) in whole fish, as well as in liver, and testes. Exposure-dependent inductions in MF (minimum two-fold induction above 2.9 $X\ 10^{-5}$) were observed in whole fish, testes, and liver after 15 days following 1 h exposures to 80 mg/L and 120 mg/L ENU. A ~two-fold induction of cII/cI mutants was observed in fish following a 28 day chronic exposure to 400 mg/L $KBrO_3$. These results further support the practical viability of transgenic fish as models for mutation detection in genotoxicity, and with distinct relevance to aquatic environmental health hazard assessment.

1086 RADICAL GENERATION CAPACITIES OF AMBIENT PARTICULATE MATTER IN RELATION TO ITS GENOTOXICITY.

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The purpose of this study was to investigate the generation of radicals by ambient particulate matter and to relate these data to (oxidative) DNA damage in alveolar type II cells. Particle morphology and chemical composition was determined by TEM/EDX and AAS analysis of transition metals. Fenton-driven radical formation by particles was evaluated by electron spin resonance (ESR) and by unwinding of supercoiled f X 174 RF1 DNA. DNA damage was measured by single cell gel electrophoresis and immunohistochemical analysis of 8-hydroxydeoxyguanosine. In line with previous observations, PM as well as PM supernatant cause depletion of supercoiled plasmid DNA. Both particles and supernatant produced hydroxyl radical-DMPO adducts in the presence of hydrogen peroxide, and this effect was completely abrogated upon pre-incubation with desferrioxamine (5 mM). PM supernatants also caused DNA damage in type II cells, and this damge was reduced upon pretreatment with desferrioxamine. Our data provide evidence that airborne particulate matter generates hydroxyl radicals in acellular systems, and that this may result in enhanced DNA damage in lung cells.

1087 SUPRA-ADDITIVE GENOTOXICITY OF A COMBINATION OF GAMMA-IRRADIATION AND ETHYL ETHANESULFONATE IN MOUSE LYMPHOMA L5178Y CELLS.

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While testing for genotoxicity is usually performed on single chemicals, exposure of humans always comprises a number of genotoxic agents. The investigation of potentially synergistic effects of combinations therefore is an important issue in toxicology. Combinations of a 511 keV gamma-radiation with the chemical alkylating agent ethyl methanesulfonate were investigated in the *in vitro* micronucleus test in mouse lymphoma L5178Y cells. With combinations in the low-dose-linear effect range for the individual agents (0.25-2 Gy and 0.8-3.2 mM, respectively), supra-additivity by 34-86% was seen. The synergism was more pronounced at the higher dose levels. Supra-additivity was confirmed in experiments using cytochalasin B and analyzing binucleate cells only, to control for putative effects on the cell cycle. Statistical significance was shown by a 2-factor analysis of variance with interaction. The results indicate that damage to DNA by gamma-radiation and alkylation could affect different rate-limiting steps in the formation of micronuclei. Further investigations will have to show whether the observations are of general validity, in particular, whether other endpoints of genotoxicity produce the same results and whether the degree of supra-additivity is always dose-dependent. The latter would have a strong impact on the risk assessment for mixtures at low dose.

1088 SATURATION OF BASE EXCISION REPAIR RESULTS IN PERSISTENT 5'-NICKED AP SITES IN HUMAN CULTURED CELLS EXPOSED TO METHYL METHANESULFONATE.

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It has been demonstrated that significant numbers of mutagenic apurinic/apyrimidinic (AP) sites are generated under physiological conditions in genomic DNA through spontaneous depurination. In addition, DNA glycosylases enzymatically introduce AP sites from a wide range of base adducts. We previously demonstrated that methyl methanesulfonate (MMS) induced an accumulation of AP sites in human cultured cells. We further hypothesized that this increase in AP sites may be derived from saturation of the deoxyribose 5-phosphate (dRp) lyase activity of DNA polymerase β (β-pol). In this study, we quantitated and characterized AP sites and heat-labile bases in H2E1 cells exposed to MMS for up to 24 hr. To measure heat-labile lesions such as N7-methylguanine (7MG) and N3-methyladenine, a depurination assay was further optimized by incubating the DNA at 70°C for 24 hr in PBS. The number of heat-labile lesions detected by the Aldehyde Reactive Probe-Slot-Blot method was slightly higher than the number of 7MG adducts. These heat-labile lesions increased in a time-dependent manner and reached a plateau at 4-h exposure. Based on the number of AP sites and heat-labile lesions, we estimated that ~700 heat-labile lesions/1,000,000 nucleotides induced an imbalance of base excision repair, resulting in an increase in the number of AP sites in cells. The AP sites were predominantly cleaved 5' to the lesions, as expected following AP endonuclease activity. These results suggest that dRp lyase activity of b-pol, the next enzyme in base excision repair, may be the rate-limiting step. (Supported in part by NIEHS Superfund Basic Research Program Grant P42-ES05948.)

1089 EVALUATION OF MUTAGENICITY OF BENZO[A]PYRENE, DIBENZOTHIOPHENE, ISOQUINOLINE AND CARBAZOLE USING A NEWLY IMPROVED A_L MUTAGENICITY ASSAY.

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The A_L mutagenicity assay has been shown to be highly sensitive compared to Ames and HPRT assays. The A_L human/hamster hybrid cell line (Jones *et al.*, 1975) contains one copy of human chromosome 11, encoding several cell surface antigens useful as markers for mutation. We have extended this assay by introducing a metabolic activation system. In the initial phase of studies exploring mutagenicity of benzo[a]pyrene (BaP), dibenzothiophene (DBT), isoquinoline (IQ) and carbazole (CZ), toxicity studies were conducted. Cell viability was determined by clonogenicity assay after 20 or 24 hr exposure to increasing concentrations of individual chemicals. Range of concentrations studied was from 1.25μM to 80μM; 6 or 7 concentrations were used for each dose-response curve. Chemical exposures were conducted with and without activation using Aroclor-induced rat liver S9 fraction. Optimal S9 concentration was 100μg/ml. In the absence of S9, CZ, IQ and BaP exhibited no toxicity toward A_L cells. IQ exhibited no toxicity with S9 activation. DBT exhibited toxicity both with and without metabolic activation; with addition of S9, a "rebound" dose-response curve at a lower dose range was consistently observed. Estimated LD_{50} for both BaP and CZ following activation was 5μM. Mutation rates at the S1 locus were determined in A_L cells exposed to each chemical. Consistent with previous findings, BaP exposure induced a dose-dependent increase in mutant fraction (M_f) with S9 activation only. At LD_{50} of BaP with S9, M_f was approximately $100/10^5$ surviving cells, a 7 to 12 fold increase above background. Preliminary results suggest that neither IQ nor CZ induce appreciable mutation at this locus. Following conclusive quantitation of mutagenicity, derivatives of those chemicals resulting in significant M_f will be examined in the same system to elucidate pathways leading to toxic and mutagenic interactions. (Supported by NIEHS 1 R01 ES09655.)

1090 *IN VITRO* STUDIES ON THE INDUCTION OF APOPTOSIS-RELATED DNA STRAND BREAKS (AR-DNASB) BY THE BENZENE METABOLITES MUCONALDEHYDE (MUC) AND HYDROQUINONE (HQ), SINGLY AND IN COMBINATION.

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The biological effects of benzene, a clastogen and human leukemogen, are mediated, in part, by ring-hydroxylated metabolites including hydroquinone (HQ) and ring-opened metabolites including trans,trans-muconaldehyde (muconaldehyde, MUC), a 6-carbon diene dialdehyde. We hypothesize that benzene hematotoxicity involves interactive effects by muconaldehyde and hydroquinone with respect to DNA. In the present studies, we used the TUNEL assay to study the effect of MUC and HQ, singly and in combination, on AR-DNASB in HL-60 cells and bone marrow cells of male CD-1 mice and transgenic mice overexpressing SOD (TgSOD). HL-60 cells treated with 5, 10 and 25 μM MUC had a TUNEL index (% labeled cells) of 4.3±0.6%, 56.5±15.3% and 95.0±1.0%, respectively, compared to 1.7±0.5% for control, while treatment with 5-25 μM HQ for 1hr resulted in no significant increase in TUNEL labeling. Co-treatment with MUC and HQ resulted in significant decreases in TUNEL labeling relative to treatment with MUC alone i.e. 48% for the 25 μM MUC/HQ combination compared with 95% for MUC alone. MUC was more effective than HQ in inducing AR- DNASB in bone marrow cells of CD-1 or SOD transgenic mice, i.e. the TUNEL index was 32.8±2.0% and 22.2±4.0% for 10 μM MUC or 10 μM HQ, respectively at the 18 hr time point in TgSOD mice. SOD overexpression appeared not to affect AR-DNASB levels in response to MUC or HQ. Treatment with binary mixtures of 5 μM MUC and 1-10 μM HQ (8-18 hr incubation) resulted in less than additive increases in AR-DNA strand break levels. These results indicate that interactions at low benzene metabolite concentrations can alter genotoxic outcome observed for individual metabolites. The relevance of our findings to benzene hematotoxicity remains to be determined. (Supported by NIH grants ESO2558 and ESO9676 from the National Institute of Environmental Health Sciences, and NIEHS Center grant ESO5022.)

1091 ASSESSMENT OF THE GENOTOXICITY OF THALOMID (THALIDOMIDE).

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Thalidomide has been approved for use in the US. The genotoxicity of Celgene's formulation of thalidomide, Thalomid, was determined in 3 *in vitro* assays. In the Ames assay, reverse mutations at the histidine loci of 5 strains of *S. typhimurium* (TA1535, 1537, 98, 100, 102) and the tryptophan locus of one strain of *E. Coli* (WP2 uvrA) were determined. The assay was performed in the presence and absence of Arochlor 1254-induced S9 using both liquid pre-incubation and plate incorporation methods. Concentrations of 50 - 10,000 mg Thalomid/plate were used. In the gene mutation assay, the xanthine-guanine phosphoribosyl transferase (XPRT) locus was examined for forward mutation in cultured AS52 Chinese hamster ovary (CHO) cells. Cells were incubated with Thalomid and S9 at 0.017 - 1000 mg/ml and without S9 at 0.50 - 1000 mg/ml. In the *in vivo* micronucleus assay, the potential for Thalomid to induce micronuclei in newly formed polychromatic erythrocytes (PCEs) in mouse bone marrow was determined. Mice were dosed ip with 0, 500, 2500 and 5000 mg/kg Thalomid in 1% carboxymethylcellulose (CMC)

and sacrificed at 24, 48 or 72 hours. Femur bone marrows were scored for micronucleated PCEs. A positive response is obtained if Thalomid induces a statistically significant dose-dependent increase for a single sacrifice time or sex in the frequency of PCEs at one or more concentration. There was no significant increase in revertant frequencies in the Ames assay. Thalomid did not produce any significant dose-dependent increase in the forward gene mutation frequencies. It was also negative in the micronucleus test. Celgene's formulation of thalidomide is therefore considered non-genotoxic in the assays used.

1092 THE INDUCTION OF BASE-SUBSTITUTION MUTATIONS BY DAUNOMYCIN IN *SALMONELLA TYPHIMURIUM*.

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Anthracyclines (primarily daunorubicin and adoxrubicin) are mutagenic antibiotics that are widely used in chemotherapy procedures for the treatment against a variety of malignant tumors, and these compounds arrest tumor cell growth by interacting with cellular DNA. However, clinical use of anthracyclines is hindered by tumor resistance and the adverse effects of these compounds (acute myelosuppression and cardiotoxicity) to healthy tissues. In spite of intensive investigations, the events leading to cell death and differential cytotoxicity in the presence of these antineoplastic compounds are not totally understood. A great many short term tests have been developed that allow compounds to be screened for potential gentotoxicity. The first such assay to be optimized for convenience and sensitivity is called the Ames test. Previous studies showed that daunomycin induced frameshift mutations in prokaryotic (*e.g.* TA98 in the Ames Test) and eukaryotic cells. More recently, a set of *Salmonella* strains (TA7001 to TA7006) was generated to easily identify base-substitution events by reversion of *his* point mutations. In this study, we show that daunomycin can also induce base-substitution mutations in *Salmonella*. Specifically, AT to GC transition mutations (TA7001) were induced 6-fold, GC to AT transition events (TA7004) were induced 11-fold, and GC to TA transversion mutations (TA7005) were induced 8-fold above spontaneous levels. Thus, it appears that daunomycin can induce a wide array of mutational events which include frameshifts and base-substitution mutations.

1093 INDUCTION OF MUTAGENIC DNA DAMAGE DURING REDUCTION OF CHROMATE BY CYSTEINE IS DEPENDENT ON FORMATION OF CHROMIUM(III)-DNA ADDUCTS.

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Mutagenic activity of carcinogenic chromium(VI) arises from its intracellular metabolism, a process which ultimately yields Cr(III). Cysteine (Cys) is considered to be one of the potential reducers of Cr(VI) *in vivo*, and this amino acid also forms crosslinks with DNA in Cr(VI)-exposed cells. In this work, we studied formation of mutagenic DNA damage during reduction of Cr(VI) by Cys with the aim of identifing types of lesions responsible for the genotoxic effects of Cr(VI). The mammalian pSP189 shuttle-vector was utilized for detection of mutagenic activity following replication of the plasmids in human diploid fibroblasts. Reduction of Cr(VI) by Cys led to an extensive Cr(III)-DNA binding which could be prevented either by occupation of DNA phosphates with Mg ions or by blocking Cr(III) reactions by addition of EDTA or inorganic phosphate. Incubation of Cr(VI)/Cys with DNA resulted in the formation of Cr(III)-DNA monoadducts, Cys-Cr(III)-DNA and DNA-DNA crosslinks. Reductive metabolism of Cr(VI) did not lead to the production DNA strand breaks or apurinic sites when trace amounts of iron were removed from all reagents. Reduction of Cr(VI) by Cys was found to lead to the dose-dependent formation of mutagenic DNA damage. Mutations were undetectable when Cr(III) binding to DNA was blocked. A substantial increase in the fluorescence of the oxidant-sensitive dye DCF was observed in Cr(VI)/Cys reactions, however, DCF fluorescence was not affected by conditions that eliminated mutagenic DNA damage. We concluded that the formation of mutagenic DNA lesions during reduction of Cr(VI) by Cys is determined by binding of Cr(III) to DNA.

1094 *SALMONELLA TYPHIMURIUM* YG7104 AND YG7108 CO-EXPRESSING HUMAN CYP2E1 AND NADPH-P450 REDUCTASE ARE SENSITIVE TO PROMUTAGENIC CYP2E1 SUBSTRATES IN THE AMES ASSAY.

M. T. Cooper and T. D. Porter. *University of Kentucky, Lexington, KY.* Sponsor: M. Vore.

We have developed an Ames mutagenicity test that utilizes cloned human cytochrome P450 2E1, rather than a rat liver homogenate (S9 fraction), to activate promutagenic CYP2E1 substrates, including nitrosamines, to their reactive forms. Nitrosamines can cause mutagenicity by alkylating DNA at the O^6 position on guanine residues. Nitrosamines are not mutagenic, *per se*, but must be activated by liver enzymes, namely CYP2E1, in order to react with the DNA. Nitrosamines have been categorized as human mutagens, yet yield poor results in the traditional Ames assay. We postulated this lack of sensitivity in the Ames assay to nitrosamine mutagenicity might be due to the DNA repair capabilities in the commonly used Ames bacterial strains such as TA1535. TA1535 is capable of repairing alkylation damage to DNA via two native O^6-methylguanine methyltransferases. These two enzymes are encoded by genes *ogt* and *ada*. Deletions derivatives of TA1535 have been developed: YG7104 (*ogt*) and YG7108 (*ogt* and *ada*). To examine the role of these methyltransferases in mutagenicity we co-expressed human CYP2E1 and P450 reductase in strains TA1535, YG7104 and YG7108. CYP2E1 and reductase expression was verified using Western blot, cytochrome *c* reduction and CO-difference spectra. The non-expressing TA1535, YG7104 and YG7108 strains yield no increase over spontaneous revertants when exposed to nitrosamines, chloroform or CCl_4. TA1535 co-expressing CYP2E1 and reductase shows no increase in sensitivity to nitrosamines, chloroform or CCl_4; however, both YG7104 and YG7108 co-expressing strains show increased sensitivity to various nitrosamines (dimethyl-, diethyl-, dipropyl-, dibutyl-), chloroform and CCl_4. The increased sensitivity in these strains was blocked by ethanol, an inhibitor of CYP2E1. (Supported by NIH grant ES-06420 and fellowship ES-05852.)

1095 UTILITY OF AN AMES SCREENING ASSAY FOR THE EARLY EVALUATION OF PHARMACEUTICAL COMPOUNDS FROM A COMBINATORIAL LIBRARY.

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Genotoxicity testing of pharmaceutical compounds traditionally has been performed once a drug development candidate is selected. An Ames screening assay that requires only 13 mg of compound, compared to 1 to 2 gms for a FDA/OECD-compliant Ames assay (full Ames), has been developed. Therefore, genotoxicity testing can be performed much earlier in the drug selection process. Five Salmonella typhimurium strains (TA98, TA100, TA102, TA1535 and TA1537) are used in the screening assay in a liquid preincubation format with and without S9 metabolic activation. Strains TA98 and TA100, and strains TA1535 and TA1537 are evaluated as mixed cultures. Approximately 50 substituted heterocyclic compounds from a combinatorial chemistry library were evaluated in the screening assay. Compounds were tested at six dose levels up to 500 mg/plate with TA98/TA100, TA1535/TA1537 and TA102. Significant structure-activity relationships were observed. Compounds containing a nitropyridyl substituent induced a statistically significant, dose-dependent, at least two-fold increase in revertant frequencies (positive) in strains TA98/TA100 and in strains TA1535/TA1537. In contrast, compounds with an aminonitropyridyl or a cyano substituent at the same position on the molecule were negative or equivocal in the same strains. A change from an imidazole to a pyridone substituent at a different position on the scaffold also was associated with positive test results. Selected molecules that were tested in the screening assay also were tested in a full Ames assay. Compounds with the nitropyridyl substituent also were positive in the full Ames, and increased the revertant frequencies of strains TA98 and TA1537. The screening Ames assay showed good concordance with the full Ames assay. Only one compound gave a different result, being equivocal in the screening Ames assay and negative in the full Ames assay. Thus, the screening Ames assay is predictive for the full Ames assay and has been an invaluable tool in directing medicinal chemistry efforts.

1096 GENOTOXIC INTERACTIONS OF MODEL COMPOUNDS AND BINARY MIXTURES.

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Although human exposure typically occurs to complex mixtures, the vast majority of toxicology literature has been developed for individual compounds. Information is needed to describe potential chemical interactions to facilitate the preparation of human health risk assessments for complex mixture exposures. This study investigated the genotoxic interactions of model compounds and binary mixtures representing two classes of compounds. The model chemicals included the polycyclic aromatic hydrocarbons benzo(a)pyrene (BAP), chrysene and anthracene; and the chlorinated aromatic hydrocarbons pentachlorophenol (PCP) and tetrachlorophenol. Genotoxicity was measured using either the Salmonella/microsome mutagenicity assay, or the *E. coli* prophage induction assay. Generally, the Salmonella assay is more sensitive to benzo(a)pyrene, while *E.coli* responds to chlorinated phenols. Chemicals were dissolved in DMSO and tested as individual compounds, as well as binary, and in some cases tertiary mixtures. Individual chemicals were tested at a minimum of five concentrations and the concentration of each component of the binary mixtures was the same as was used with the individual chemcials. For all mixtures, the response appeared to be very dose dependent. Some inhibition of BAP mutagenicity was observed at higher concentrations of chrysene. In the first experiment with BAP and PCP, BAP induced a 9.5 fold increase in plaques at a concentration of 0.95 mM, while PCP induced an 18.3 fold increase and the BAP-PCP mixture induced an 87 fold increase in plaque formation. In a second experiment, BAP induced a 9.3 fold increase, PCP induced a 14.5 fold increase, and the BAP-PCP mixture induced a 60.4 fold increase in plaque formation. The enhanced genotoxic response was highly dose dependent, and some inhibition was observed at the highest dose tested. Additional studies are planned to investigate the effect of the mixture on metabolite formation.

1097 PRETREATMENT OF *SALMONELLA TYPHIMURIUM* WITH INTERCALATORS INCREASES THE MUTAGENICITY OF N-ACETOXY-2-ACETYLAMINOFLUORENE.

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Exposure of DNA to a genotoxin can result in non-additive damage by subsequent exposure to a second genotoxin. An on-going study has examined effects of DNA intercalators, benzo(a)pyrene diol epoxide (BPDE) and ethidium bromide (EB) on the mutagenicity of N-acetoxy-2-acetylaminofluorene (AcO-AAF) in the T98 frameshift strain of Salmonella typhimurium. T98 was incubated with BPDE (0.075 microgram/plate) for 15 min, then with AcO-AAF (0.25 microgram/plate); the mutagenic potency of AcO-AAF was increased 4-6 fold above the theoretical additive effect. In a parallel experiment EB (0.02 microgram/plate) increased the mutagenic potency of AcO-AAF 5-fold. Increases were a linear function of concentration of BPDE and EB. Pretreatment with increasing concentrations of BPDE (up to 0.3 microgram/plate) or EB (up to 3 microgram/plate) alone did not produce significant numbers of revertants, indicating enhancement was due to an increase in AcO-AAF mutagenic potency. Enhancement was not observed when treatment order was reversed. Pretreatment of T98 with DNA cross-linking agent, cis-platin, before EB treatment blocked the EB-dependent enhancement of AcO-AAF mutagenicity. Pretreatment of cells with BPDE, EB, or aflatoxin B1-8,9-epoxide enhanced primarily -2 frameshift mutations induced by AcO-AAF in the his D3052 allele. The results are discussed in terms of modulation of DNA damage by carcinogenic mixtures potentially due to changes in helix conformation by the first genotoxin followed by non-additive damage by subsequent exposure to a second genotoxin. (Supported by EPA grant R825809.)

1098 OXIDATIVE DNA DAMAGE AND MUTAGENICITY OF CYANOETHYLENE OXIDE.

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Cyanoethylene oxide (CNEO), the epoxide of acrylonitrile (ACN), has been prepared and tested for its mutagenicity in the Ames assay using *S. typhimurium* strains TA1535 and the TA7000 series. These effects are being investigated to provide an understanding of the reports of genotoxicity of ACN with metabolic activation. Mutants from strain TA1535, capable of growing in the absence of histidine, have been reported to occur most commonly with GC®AT transitions or GC®TA transversions. In the TA7000 series, only one type of base substitution can produce mutants capable of growing in the absence of histidine. CNEO exposures produced dose-related increases in mutants in both TA1535 and TA7004, which corresponds to a GC®TA transversion. This mutation is consistent with base mispairing of 5-hydroxycytosine or the formation of apurinic sites resulting from repair of 8-oxodeoxyguanosine. A dose-related positive response was also obtained in one of two experiments with TA7002, which may reflect AT®TA transversions. Other strains did not give a positive response, including TA7001, which is consistent with negative results for TA102. In a parallel experiment exposing TA1535 to CNEO, DNA was extracted and the levels of 8-oxodeoxyguanosine and 5-hydroxycytosine were determined by HPLC with electrochemical detection. Both oxidized DNA nucleosides increased in a dose-dependent manner. Since no increases were seen in calf thymus DNA treated with CNEO, the oxidative damage seen in the bacteria appear to result from stimulation of reactive oxygen species or inhibition of DNA repair.

1099 COMPARATIVE MECHANISMS OF ACTIVATION OF ESTROGEN RECEPTOR α BY ESTROGEN AND 4'-HYDROXYTAMOXIFEN.

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Seventeen β-Estradiol (E2) and 4'-hydroxytamoxifen (4-OHT) exhibit estrogen receptor α (ERα) agonist and antagonist activities in mammary cancer cells, whereas both compounds exhibit agonist activity in the endometrium. This study compared the estrogenic effects of E2 and 4-OHT in ECC-1 and HEC1A endometrial cancer cells and other cell lines using constructs containing E2-responsive inserts from the creatine kinase B (pCKB), cathepsin D (pCATH) and complement C3 (pC3) gene promoters linked to luciferase (luc) or chloramphenicol acetyltransferase (CAT) reporter genes. Cells were also cotransfected with wild-type ERα variants expressing activation function 1 (ERα-AF1) or AF2 (ERα-AF2). Using pCKB as a model construct, E2 significantly induced CAT activity (15- to 30-fold) in both endometrial cell lines as well as HepG2 (liver) and ER-negative MDA-MB-231 (breast) cancer cell lines cotransfected with wild-type ERα expression plasmid. 4-OHT also acted as an ERα agonist in the four cell lines cotransfected with ERα; however, the magnitude of the induction response (4- to 14-fold) was lower. Similar results were obtained using pC3 and wild-type ERα in the same cell lines. In contrast, using the pCKB construct, E2 but not 4-OHT activated reporter gene expression in cells cotransfected with ERα-AF1 or ERα-AF2, and results obtained with pC3-luc varied with different cell lines, wild-type or variant ERα. The data indicate that the ERα-AF1-dependent activation by 4-OHT is cell context- and gene promoter-dependent, and this is consistent with the increasingly complex interactions associated with ligand-mediated activation of ERα. (ES04917 and ES09106.)

1100 EVALUATION OF AGONISTIC AND ANTAGONISTIC EFFECTS OF SEVERAL CHEMICALS, MAINLY PYRETHROID INSECTICIDES, USING THREE *IN VITRO* ASSAYS WITH HUMAN ESTROGEN, ANDROGEN OR PROGESTERONE RECEPTOR MEDIATED MECHANISMS.

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Agonistic or antagonistic effects of xenoestrogens (BisphenolA, *p*-nonylphenol and *o,p'*-DDT), putative antagonists (4-hydroxytamoxifen, hydroxyflutamide and RU486) and pyrethroid insecticides (*d-trans*-allethrin, cypermethrin, empenthrin, fenvalerate, imiprothrin, permethrin, *d*-phenothrin and prallethrin) were evaluated using three *in vitro* assays with human estrogen α(ERα),androgen (AR) or progesterone (PR) receptor mediated mechanisms. Mammalian cell-based luciferase reporter gene assays were developed for analyzing the effects on receptor-mediated gene activation. Receptor-independent effects on consutitutive luciferase activation in control cells were examined for determining the appropriate dose levels of chemicals. Effects on ligand-dependent interaction between receptors and a coactivator (TIF2: Transcriptional Intermediary Factor 2) were examined by yeast two-hybrid assays. Moreover, binding capacity of test chemicals was investigated by competitive ligand binding assays. Significant ($p0.05$) positive effects of cognate hormones (E2:17β-estradiol, DHT:dihydrotestosterone and progesterone) were detected in all assays. Xenoestrogens showed significant agonistic effects in ERα assays. Putative antagonists inhibited the cognate hormone-mediated transactivation and interaction between the receptors and TIF2 through the receptor binding. However, none of pyrethroids tested showed

significant agonistic or antagonistic effects (100nM-10μ) in the present assays, suggesting that pyrethroids tested do not exert hormonal or anti-hormonal effects through human ERα, AR or PR-mediated mechanisms.

1101 ESTROGEN-MEDIATED ACTIVATION OF c-*Fos* PROTO-ONCOGENE THROUGH PROTEIN BINDING THE SERUM RESPONSE ELEMENT.

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Analysis of the c-fos protooncogene promoter has identified a distal GC-rich element (-1168 to -1161) required for estrogen (E2)-responsiveness in MCF-7 breast cancer cells. More proximal downstream sequences (-354 to -296) contain a *sis*-inducible element (SIE) and a serum response element (SRE) required for transactivation by growth factors. Surprisingly, in MCF-7 cells transfected with constructs containing the -354 to -296 (pSS) or SRE (pSRE) from the fos gene promoter, E2 induced a 2- to 4-fold increase of chloramphenicol acetyltransferase (CAT) (reporter gene) activity, and this response was dependent on cotransfection with estrogen receptor α (ERα). Hormone-dependent induction was inhibited by cotransfection with dominant negative ras or MAPK expression plasmids; the response was also inhibited by the MAPKK inhibitor PD 98059 and the pure antiestrogen ICI 182,780. Analysis of proteins interacting with SRE was determined by gel electophoretic mobility shift assays using MCF-7 cell nuclear extracts, and both the serum response factor and Elk-1 transcription factor were identified as SRE-bound proteins. Retarded band intensities were enhanced after treatment with E2 or growth factors. Thus, results of this study show that ligand-activated ERα mediates the ras → MAPK signaling pathway in MCF-7 cells and activates c-fos expression through the SRE. This represents a unique mechanism of ERα in which E2 and growth factors activate proteins binding the same response elements. (ES09253 and ES09106)

1102 EFFECTS OF LIGAND-STRUCTURE ON ESTROGEN/ANTIESTROGEN INDUCTION VIA ESTROGEN RECEPTOR/SP1 INTERACTIONS WITH GC-RICH PROMOTER ELEMENTS.

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Estrogen receptor (ER)-mediated transactivation is dependent on ER-subtype, gene promoter, cell context and ligand structure. In ER-positive human breast cancer cells, 17β-estradiol (E2) induces several genes through ERα/Sp1 interactions that involve interactions with GC-rich sites, and for some genes, other DNA-bound factors may also be required. Ligand-dependent ERα activation of heat shock protein 27 gene expression gave a unique induction response; 10 nM E2 and 1000 nM of the steroidal pure antiestrogen ICI 164,384 induced heat shock protein (Hsp) 27 mRNA levels (3.2- and 2.3-fold), whereas the structurally-related steroidal antiestrogen ICI 182,780 did not affect mRNA levels. E2 and both steroidal antiestrogens caused a 2- to 5-fold increase in reporter gene activity using constructs containing E2-responsive inserts from the Hsp 27 gene promoter (pHsp27) or a consensus GC-rich Sp1-binding site (pSp1). Estrogen/antiestrogen-induced gene expression was observed in cells cotransfected with wild-type human ERα expression plasmid; however, only E2 induced reporter gene activity in cells cotransfected with a DNA-binding domain-deficient ERα mutant (HE11). In contrast, other Sp1-dependent constructs from the c-fos and adenosine-deaminase gene promoter were responsive to E2 but not the steroidal antiestrogens suggesting that specific promoter elements may contribute to antiestrogen-dependent activation, and this is currently being investigated. (CA76637 and ES09106).

1103 CELL AND PROMOTER-SPECIFIC INTERACTIONS OF STEROID RECEPTOR COACTIVATORS WITH ESTROGEN RECEPTOR α (ER_α) AND ER_α/SP1.

T. A. Nguyen and S. Safe. *Texas A&M University, Department of Veterinary Physiology & Pharmacology, College Station, TX.*

Steroid receptor coactivators (SRCs) are a family of proteins that interact with steroid hormone receptors and other members of the nuclear receptor family resulting in enhanced ligand-induced transactivation. SRC family members are expressed in multiple tissue and cancer cell lines, and their coactivator effects have primarily been characterized in non-mammary cancer cell lines. This study investigates the effects of SRCs on estrogen receptor α (ER_α) activation of estrogen response element-dependent constructs (pERE) and GC-rich elements (pSp1) that bind ER_α/Sp1. Initial results show that in CHO-1 and COS-1 cells treated with E2 and transfected with pERE and ER_α, there was a 4- to 8-fold induction of reporter gene activity. Cotransfection with SRC-1 and SRC-3 enhanced (30-50%) the induction response as previously reported. In contrast, enhancement of ER_α action by SRCs was not significant in MCF-7 human breast cancer cells. In a parallel study, MCF-7 cells were transfected with ER_α and pSp1, and estrogen induced a 2- to 3-fold increase in reporter gene activity; however, after cotransfection with SRC-3, there was a subsequent 100% decrease in ER_α/Sp1-dependent transactivation. In gel mobility shift and coimmunoprecipitation assays, SRC-3 decreased ER_α/Sp1 binding to [^{32}P]Sp1 oligonucleotide, and both ER and Sp1 antibodies coimmunoprecipitated [^{35}S]SRC-1 incubated with ER or Sp1 alone or in combination, respectively. These data reveal complex cell-specific functional differences in ER_α and ER_α/Sp1 interactions with SRC family members. Results of a systematic study in different cell lines with SRC-1, SRC-2, SRC-3 and other coactivators will also be described. (CA76637 and ES09106).

1104 CELL CONTEXT-DEPENDENT ESTROGEN RECEPTOR α (ERα) AGONIST AND ERβ ANTAGONIST ACTIVITIES OF METHOXYCHLOR METABOLITES.

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Structure-activity relationships among a series of methoxychlor metabolites have identified four congeners that exhibit estrogen receptor α (ERα) agonist activity but partial ERβ antagonist responses in Hep G2 cells using the C3-luc construct containing an estrogen (E2)-responsive insert from the complement C3 gene promoter. Using the C3-luc assay system (cotransfected with ERα or ERβ), the effects of cell context on the ERα (agonist) and ERβ (antagonist) activities were determined for 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), 2,2-bis(*p*-hydroxyphenyl)-1,1-dichloroethylene (HPDE), 2-(*p*-hydroxyphenyl)-2-(*p*-methoxyphenyl)-1,1,1-trichloroethane (HPTE-OMe), and 2-(*p*-hydroxyphenyl)-2-(*p*-methoxyphenyl)-1,1-dichloroethylene (HPDE-OMe). Initial studies utilizing HPTE as a prototype showed that this methoxychlor metabolite exhibits ERα agonist and ERβ antagonist activities in several cell lines transfected with C3-luc and ERα or ERβ including PE06 and PE04 human ovarian cancer cells, HeLa cells (cervical cancer) and SAOS human osteosarcoma cancer cells. Although HPTE was an ERα agonist in MDA-MB-231 breast cancer cells, no inhibition of E2-activated ERβ-dependent luciferase activity was observed at HPTE concentrations as high as 10 ∝M. The comparative ERα (agonist)/ERβ (antagonist) activities of the four methoxychlor metabolites were investigated in SAOS cells, and all compounds inhibited (100%) ERβ activation of C3-luc. The ERα agonist activities of these compounds were variable; only HPTE was a full agonist at the highest concentration (10 ∝M); however, potencies were cell context-dependent. (ES09106 and ES04917)

1105 TRANSCRIPTIONAL ACTIVATION OF ORNITHINE DECARBOXYLASE GENE EXPRESSION BY ESTROGENS IN MCF-7 BREAST CANCER CELLS.

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Ornithine decarboxylase (ODC) is a key enzyme in the biosynthesis of polyamines and important for cell proliferation and growth of cancer cell lines, including MCF-7 human breast cancer cells. 17β-Estradiol (E2) induces proliferation and ODC gene expression in estrogen receptor (ER)-positive MCF-7 cells, and this study investigated the molecular mechanisms of ODC induction by E2. A series of constructs containing -164 to +29 (pODC1), -122 to +29 (pOCD2), -93 to +29 (pODC3), and -74 to +29 (pODC4) ODC gene promoter inserts were transiently transfected into MCF-7 cells, cotransfected with ERα expression plasmid, and treated with 10nM E2. Luciferase (reporter gene) activities were induced 2- to 3-fold with constructs pOCD1, pOCD2 and pODC3, but not pODC4, suggesting that the -93 to -74 region of the promoter was required for ERα-mediated transactivation. This region contains a CCAAT motif, and in transient transfection studies using a construct (pODC5) containing this site and upstream sequences (-164 to -71), E2 did not induce luciferase activity suggesting that the CCAAT binding site was required but not sufficient for hormone-mediated induction. The -164 to +29 region of the ODC gene promoter multiple (5) recognition sites for nuclear transcription factors and the requirement for each motif was determined by

site-directed mutagenesis followed by transfection of the mutant constructs, and the results showed that both a CCAAT and an adjacent CP2 binding site were required for E2-mediated transactivation. This response did not require direct ERα protein-promoter interactions, and the mechanisms of transcription factor activation via ERα-induced kinase-dependent and -independent pathways will be discussed. (CA76636 and ES09106)

1106 LIGAND-ACTIVATED ESTROGEN RECEPTOR α (ERα)/Sp1 ACTION IN BREAST CANCER CELLS IS DEPENDENT ON THE ACTIVATION FUNCTION 1 DOMAIN OF ERα.

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Estrogen receptor α (ERα)/Sp1 is an estrogen-inducible transcription factor complex that interacts with specific GC-rich sites in several target genes. In contrast, ERβ/Sp1 exhibits minimal ligand-induced activity, and using a series of chimeric proteins containing activation function-1 (AF-1) of ERα fused to AF-2 of ERβ ($ERα_{/β}$) and AF-1 of ERβ fused to AF-2 of ERα ($ERβ_{/α}$), it was shown that E2-induced transactivation via ER/Sp1 was dependent on the AF-1 domain of ERα. Deletion mutants of the AF-1 domain of wild-type human ERα and chimeric $ERα_{/β}$ were generated, and E2-induced transactivation was determined in MDA-MB-231 breast cancer cells cotransfected with deletion mutants and constructs contain consensus GC-rich (pSp1) or estrogen response element ($pERE_3$) inserts. Induction of reporter gene activity was observed for cells transfected with $pERE_3$ and wild-type ERα (3.4-fold) or $ERα_{/β}$ (3.90-fold) and significant induction was observed for all deletion mutants. In cells transfected with pSp1 and wild-type ERα or $ERα_{/β}$, there was a 2.6- and 4.4-fold induction of reporter gene activity, respectively, and the loss of induction in cells transfected with ERα-AF-1 deletion mutants was associated with aa 79 to 117. Subsequent studies with a newly reported p68 (helicase) AF-1-dependent coactivator of ER and other ligand-activated nuclear receptors showed that coexpression of this protein decreased ERα/Sp1 action in breast cancer cells. (CA76636 and ES09106)

1107 DOWNREGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN HEC1A ENDOMETRIAL CANCER CELLS THROUGH INTERACTIONS OF ESTROGEN RECEPTOR α AND Sp3 PROTEINS.

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Vascular endothelial growth factor (VEGF), a potent angiogenic factor, is downregulated by 17β-estradiol (E2) in HEC1A endometrial cancer cells, and initial studies identified a region of the VEGF gene promoter (-131 to +54) required for hormone-decreased expression. Subsequent deletion analysis identified a minimal active construct (pVEGF6) that contained the -66 to +54 promoter region, and the E2 response was subsequently lost by mutation of a G-rich -66 to -52 sequence. Nuclear extracts from HEC1A cells bound the G-rich sequence in gel mobility shift and footprinting assays, and results of oligonucleotide competition and antibody supershifts confirmed that both Sp1 and Sp3 proteins, but not early growth response-1 (Egr-1) protein, bound this critical promoter sequence. The mechanism of estrogen receptor α (ERα)-mediated downregulation of pVEGF6 was investigated in *Drosophila* Schneider SL-2 cells that do not express endogenous Sp1, Sp3 or ERα proteins. In SL-2 cells transiently transfected with pVEGF and Sp1 expression plasmids, cotransfection with ERα (and treatment with E2) significantly increased the reporter gene activity. In contrast, cotransfection with ERα decreased transactivation in SL2 cells transfected with pVEGF6 and Sp3 expression plasmids. These data, coupled with preliminary results of coimmunoprecipitation and other Sp3-ERα interaction studies, suggest a unique mechanism of E2-decreased gene expression through ERα-Sp3 interactions. (CA76636 and ES09106)

1108 REGULATION OF TRANSFERRIN GENE EXPRESSION BY 17β-ESTRADIOL IN HUMAN BREAST CANCER CELLS.

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Transferrin is an iron binding protein that transports ferric iron between sites of absorption, storage, and utilization in vertebrate serum. Transferrin is an essential growth factor involved in growth regulation and differentiation. Transferrin is predominately synthesized in the liver; however, its expression has been identified in several cell types including the MCF-7 human breast cancer cell line. Secretion of transferrin by MCF-7 cells is induced in response to 17β-estradiol (E2). Treatment with 10 nM E2 of MCF-7 cells transfected with 3.6 Kb of the transferrin 5'-promoter sequence (Tf-3600/+39-CAT) linked to a chloramphenicol acetyltransferase (CAT) reporter resulted in a 20-fold increase in CAT activity. However, E2 had no effect on CAT activity from cells transfected with a reporter construct containing only 620 bases (Tf-620/+39-CAT) of the 5'-regulatory region of transferrin. Transfection of MCF-7 cells with a CAT reporter containing bases -1600 to -620 resulted in a 6-fold increase in CAT activity in response to E2. Sequence analysis of the 1000 bases between -1600 and -620 did not reveal any perfect, palindromic estrogen response elements (EREs); however, there is one imperfect palindromic ERE at -795 as well as several half palindromic ERE sites (ERE1/2). Putative binding sites for AP-1, CREB, and Sp1 also are found within this region, and previous studies have shown E2 responsiveness through direct or indirect interactions of estrogen receptor α with proteins bound to similar sequence motifs. Current studies are focused on identifying the sequence elements within the -1600 to -620 region of the transferrin gene promoter responsible for induction of transcription by E2. (CA76636 and ES09106)

1109 TRANSCRIPTIONAL ACTIVATION OF CATHEPSIN D GENE EXPRESSION BY GROWTH FACTORS.

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Insulin growth factor I (IGF-I), transforming growth factor α (TGFα) and epidermal growth factor (EGF) induced cathepsin D gene expression and reporter gene activity in MCF-7 human breast cancer cells transiently transfected with a construct (pCD1) containing a -2576 to -124 cathepsin D gene promoter insert. In contrast, IGF-I, but not TGFα or EGF, induced reporter gene activity in cells cotransfected with wild-type estrogen receptor (ER) expression plasmid and a construct (pCD2) containing estrogen-responsive downstream elements from -208 to -101. Promoter deletion and mutational analysis experiments identified four GC-rich sites and an imperfect palindromic estrogen responsive element required for IGF-I activation of the ER (ligand-independent). Subsequent studies with the mitogen-activated protein kinase (MAPK) inhibitor, PD98059, and a $serine^{118}$-ER mutant confirmed the role of the MAPK pathway for IGF-I activation of the ER in MCF-7 cells. Thus, growth factor activation of ER can mediate transactivation vs. ER/Sp1 binding to GC-rich sites and represents a novel pathway for ligand-independent ER action. The divergent pathways for IGF-I and TGFα/EGF activation of the ER observed in MCF-7 cells contrast with previous data indicating that pathways for growth factor activation of the ER are dependent on the gene and/or gene promoter and on cell context. (ES09106 and ES09253)

1110 INSULIN-LIKE GROWTH FACTOR-1 INDUCES ADENOSINE DEAMINASE IN MCF-7 HUMAN BREAST CANCER CELLS THROUGH ESTROGEN RECEPTOR-SP1 INTERACTIONS.

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Adenosine deaminase (ADA) catalyzes the deamination of adenosine and deoxyadenosine, and 17β-estradiol (E2) induces ADA gene expression in MCF-7 human breast cancer cells. Analysis of the ADA gene promoter has shown that only one of six proximal (-211 to +11) GC-rich Sp1 binding sites in the promoter at -79 to -73 is responsible for E2-inducibility via estrogen receptor α (ERα)/Sp1 interactions. In transient transfection studies with pADA211, which contains the -211 to +11 ADA gene promoter linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene, E2 and insulin-like growth factor 1 (IGF-1) induced a 3.2- and 2.5-fold increase in CAT activity, whereas transforming growth factor α and epidermal growth factor were not active. The induction response was only observed in cells cotransfected with ERα expression plasmid suggesting a unique ligand-independent ERα/Sp1 activation by IGF-1. The mechanism of this response was further investigated in MCF-7 cells treated with 10 nM IGF-1 cotransfected

with pADA211, ERα expression plasmid, and dominant negative expression plasmids for ras or MAPK. The 2.5-fold induction response by IGF-1 was totally blocked by both dominant negative plasmids; moreover, in cells cotransfected with variant ER expression plasmids mutated in key phosphorylation sites (serine 118 or 167), IGF-1 activation of ERα was not observed. These results demonstrate that IGF-1 activates ERα through the ras-MAPK pathway and ERα/Sp1 induces ADA through interaction with GC-rich sites in the promoter. The failure of other growth factors to activate ERα/Sp1 is being investigated. (ES09253 and ES09106)

1111 LIGAND STRUCTURE-DEPENDENT DIFFERENCES IN ACTIVATION OF ESTROGEN RECEPTOR α IN HUMAN HepG2 LIVER AND U2 OSTEOGENIC CANCER CELL LINES.

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Differences in ligand-activation of estrogen receptor α (ERα) were investigated in human HepG2 liver carcinoma and U2 osteogenic sarcoma cells transfected with wild-type ER (ER-wt) and variants expressing only activation function 1 (ER-AF1) or AF2 (ER-AF2). The estrogen-responsive C3-luc construct containing the complement C3 gene promoter linked to a bacterial luciferase reporter gene was used to determine ligand-induced transactivation by wild-type or variant ERα. The qualitative pattern of ER-dependent responses was similar in both cell lines for a series of weakly estrogenic hydroxy and dihydroxyaromatic compounds including *p*-octylphenol, *p*-nonylphenol, 2′,4′,6′-trichloro-4-biphenylol, 2′,3′,4′,5′-tetrachloro-4-biphenylol, bisphenol A and 2,2′-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane. The weakly estrogenic pesticide, kepone, and the phytoestrogens, resveratrol (a trihydroxystilbene) and naringenin (a flavanone), induced distinctly different patterns of responses; induction by these compounds was not observed in either cell line cotransfected with ER-wt or ER-AF2. In contrast, naringenin activated ER-AF1 in HepG2 cells and resveratrol activated ER-AF1 in U2 cells. In HepG2 cells cotreated with E2 plus the estrogenic compounds, only BPA and resveratrol exhibited ERα antagonist activity. Structure-dependent differences in ERα activation and inhibition are consistent with the increasingly complex tissue-specific patterns of ERα action indicating that the estrogenic activity of an individual compound can only be determined using an extensive testing protocol. (ES09106 and ES04917)

1112 ACTIVITY OF BENZO[A]PYRENE AND ITS HYDROXYLATED METABOLITES IN AN ER-α REPORTER GENE ASSAY.

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This study investigated benzo(a)pyrene (B[a]P), a substrate for CYP1A, 2C and 3A, to characterize the metabolic competence of an ER-α transactivation system and to explore potential interactions of cellular metabolism with ER-mediated responses. In a system utilizing MCF-7 cells transiently transfected with a chimeric estrogen receptor (Gal4-HEG0) and a luciferase reporter (17m5-G-Luc), B[a]P (>1 μM) produced responses comparable to that of 0.1 nM 17-β estradiol (E2). Metabolic profiles of B[a]P-treated MCF-7 cultures showed endogenous formation of the 3- and 9-hydroxy (3-OH and 9-OH) and the 7,8- and 9,10-dihydroxy (7,8-OH and 9,10-OH) B[a]P metabolites. Alpha-Napthoflavone (NF), an Ah receptor antagonist and P450 inhibitor, also decreased the response to B[a]P but not to E2. In the ER-α reporter assay, the 3-OH and 9-OH, but not the 7,8-OH and 9,10-OH metabolites produced maximal responses comparable to E2 with EC50 values of 1.2 and 0.7 μM, respectively. ICI inhibited responses to the 3-OH and the 9-OH species, while NF inhibited the response to the 9-OH, but not the 3-OH metabolite. These results suggest that MCF-7 cells are capable of metabolically activating BaP to an estrogenic substance in an *in vitro* gene expression assay. Funded by The Chemical Manufacturers Association (Arlington, VA).

1113 INCORPORATION OF S-9 ACTIVATION INTO AN ER-α REPORTER GENE ASSAY.

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The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) recommended that the evaluation of chemicals in the *in vitro* transcriptional activation assays be performed in the presence and absence of metabolic activation in order to enhance the ability to detect compounds with pro-hormonal activity. Therefore, the feasibility of incorporating an exogenous metabolic activating system into an estrogen receptor-α (ER-α) transactivation assay was investigated. 17βide methoxychlor (MXC), were evaluated in the presence and absence of Aroclor-1254 induced rat liver S-9 fractions. In the absence of S9, both E2 and MXC responded consistently, with average EC50 values of 9.6 x 10E-11 M and 1.2 x 10E-5 M, respectively. Following 24h exposures to 0.1% S-9 fractions with NADP and isocitrate cofactors, the EC50 value for E2 increased to 1.4 x 10E-9 M. By comparison, the EC50 value for MXC decreased to 4.9 x 10E-7 M. In the presence of S-9, both E2 and MXC demonstrated increased secondary metabolite formation as evidenced by HPLC analysis. Consistent with these data, E2 metabolites evaluated in the ER-α reporter assay exhibited decreased potencies and the MXC metabolite HPTE, increased potency, relative to parent. These results show that S-9 is not required for detection of the pro-estrogen, MXC, apparently due to low, but sufficient, intrinsic metabolic activity of MCF-7 cells. However, 0.1% S9 was shown to be compatible with the MCF-7 cell reporter gene assay and has the potential to enhance detection of pro-estrogenic materials. (Funded by The Chemical Manufacturers Association, Arlington, VA.)

1114 A COMPARISON OF (ANTI-) ESTROGENICITY AMONG TEN POLYCHLORINATED BIPHENYL (PCB) METHYLSULFONES AND THEIR PRECURSOR PCBs IN TWO HUMAN CELL LINE-BASED ER-CALUX ASSAYS.

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Polychlorinated biphenyls (PCBs) with *meta-para* chlorine-unsubstitution are biotransformed to persistent 3- and 4-methylsulfone ($MeSO_2$-) metabolites in humans. The potential for $MeSO_2$-PCBs to modulate estrogen receptor- (ER-) mediated induction of luciferase activity *in vitro* was investigated using two ER-CALUX assays with stably transfected human breast T47D.luc adenocarcinoma cells (T47D-ER) and 293 human embryonal kidney cells (293HEK-ERα). (Anti-) estrogenic activity was determined for ten 3- and 4-$MeSO_2$-PCBs (i.e., of CB-49, -52, -70, -87 and -101), their precursor PCBs, the analog $MeSO_2$-2,5-Cl_2benzene (2,5-Cl_2Bz), 4′-OH-CB30 (2,4,6-Cl_3), and anti-estrogenic tamoxifen and ICI 182,780. In both assays 4′-OH-CB30 (10 μM) was the only estrogenic compound, resulting in a 50- to 60-fold induction. 17β-estradiol (E_2) had a similar efficacy, but with much greater potency (EC_{50}, 10 pM). In the T47D-ER, PCBs and 2,5-Cl_2Bz were not (anti-) estrogenic up to 10 μM. The $MeSO_2$-PCBs were not estrogenic up to 10 μM. The 3-$MeSO_2$-PCBs did not antagonize of E_2-induced activity (EC_{50}, 10 pM) up to 10 μM;, except for 3-$MeSO_2$-CB70 and -CB-101. $MeSO_2$-PCBs having a 4-$MeSO_2$-group, mono-*ortho*-chlorination and 3,4-dichloro-substitution on the non-$MeSO_2$-containing phenyl ring were the most potent antagonists. Inhibition was as great as 80% at concentrations as low as 1.0 μM;. Inhibition by tamoxifen (EC_{50}, 0.1 μM) and ICI 182,780 (EC_{50}, < 0.01 μM) was essentially 100%. Similar compound- and concentration -dependent trends for (anti-) estrogenicity were observed in the 293HEK-ERα assay. Anti-estrogenicity was not due to cytotoxic or cell proliferation effects. The present results suggest that depending on the structure, persistent $MeSO_2$-PCBs are anti-estrogenic *in vitro* via interaction of the ER.

1115 ESTABLISHMENT OF *XENOPUS LAEVIS* AS A MODEL FOR INVESTIGATING *IN VITRO* AND *IN VIVO* ENDOCRINE DISRUPTION IN AMPHIBIANS.

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In this study, *in vitro* estrogen receptor (ER) competitive binding and gene expression assays, and the induction of vitellogenin mRNA *in vivo* were used to assess *Xenopus laevis* as an amphibian model for examining potential endocrine disruptors. Competitive binding to *Xenopus* ER was investigated using a bacterially expressed fusion protein consisting of glutathione-S-transferase (GST) linked to the ER ligand binding domain (LBD) of *Xenopus* (GST-xER). Equilibrium analysis revealed saturation was reached at a concentration of 10 nM [^{3}H]17β-estradiol (E2) with a dissociation constant (K_d) of 6.4±1.3 nM. In a competitive binding assay, the IC_{50} value for E2 was 12.2±0.34 nM. The ability of E2 to induce *Xenopus* ER-regulated gene expression was assessed in MCF-7 human breast cancer cells transiently transfected with a chimeric receptor consisting of the Gal4 DNA binding domain linked to the *Xenopus* ER LBD (Gal4-xER) and a Gal4-regulated luciferase reporter gene, 17m5-G-Luc. Treatment of E2 resulted in approximately 30-50 fold maximal induction of reporter gene activity with an EC_{50} of 0.67±0.31 nM. Adult male *Xenopus* were intraperitoneally treated for 3 consecutive days with E2 at 0, 0.05, 0.1, 0.5, 1, and 5 mg/kg for a period of 12 days. The level of vitellogenin induction was determined using semiquantitative RT-PCR. E2 treatment showed a dose response increase in vitellogenin mRNA with the maximal induction at an accumulative dose of 3 mg/kg E2 ($p < 0.001$, $n = 35$). In summary, we have developed three complementary methods to detect estrogenic effects of E2 using *Xenopus* as a model. These methods can be used to assess the estrogenicity of a substance in *Xenopus* as a model amphibian species.

1116 *IN VITRO* ESTROGENIC POTENCY OF POLYBROMINATED DIPHENYL ETHERS.

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Polybrominated diphenyl ethers (PBDEs) are used in large quantities as additive flame retardants in e.g. plastics and textile materials. PBDEs have been detected in biota, human adipose tissue and plasma samples. The present study investigated the possible (anti-)estrogenic potency of PBDEs in an estrogen receptor mediated, chemical activated luciferase reporter gene assay (ER-CALUX). Human T47D breast cancer cells, stably transfected with an estrogen responsive luciferase reporter gene construct (T47D.luc), were exposed to 17 different PBDEs and 3 hydroxylated PBDEs (concentrations varied from 0.01 to 50 μM) in present or absence of 10 pM estradiol (E2). In total 8 PBDEs showed estrogenic potencies, with EC50 values varying from 2.5 to 7.3 μM. The EC50 value of the most potent hydroxylated PBDE (2-bromo-4-(2,4,6-tribromophenoxy)phenol) was 0.5 μM, with a maximum fold induction of luciferase activity exceeding that of E2, though at concentrations 50,000 times higher. In an ER-α transfected human embryonic kidney cell line (293 HEK.luc), 4-(2,4,6-tribromophenoxy)phenol was a highly potent estrogen (EC50 < 0.1 μM and a maximum 35- to 40-fold induction similar to E2) relative to the estrogenic 2,4,6-tribromodiphenyl ether and 2,2',4,4',6-pentadiphenyl ether (EC50 < 5.0 μM, maximum < 15 fold induction). These results indicate that several pure PBDE congeners, but especially hydroxylated PBDEs are inducers of the estrogen receptor signal transduction pathway *in vitro*. (This study was supported by the commission of the European Communities [grant ENV-CT96-0170].)

1117 DIFFERENCES IN (ANTI) ESTROGENICITY BETWEEN TECHNICAL TOXAPHENE AND FOUR OF ITS ENVIRONMENTALLY IMPORTANT CONGENERS IN THE ER-CALUX ASSAY.

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Among the endocrine disrupting contaminants, the organochlorine pesticide toxaphene has been identified as a chemical class possessing estrogenic activity. Toxaphene is a mixture consisting of at least 670 constituents which has mainly been used on a large scale in the US. Due to aerial and aquatic transport, toxaphene is an ubiquitous contaminant, thus relatively high concentrations are found in the environment and human foodstuffs. Because of selective degradation and transport, the congener pattern of the technical mixture is changed markedly in environmental matrices and biota. Three congeners CHB26, CHB50, and CHB62, according to the Parlar nomenclature, are detected in almost every sample. Together with CHB32, which is readily degraded, these comprise the four toxaphene indicator compounds. Until now, only the technical mixture has been used to assess estrogenic effects. However, in the present study we also used the four pure congeners in the ER-CALUX assay. In this *in vitro* assay, luciferase activity can be induced in stably transfected human breast cancer cells via the estrogen receptor pathway. Estradiol (positive control) and technical toxaphene induced luciferase activity maximally 49- and 22-fold, respectively. The calculated EC50 values are 4.81 pM for estradiol, and 18.7 μM for technical toxaphene. None of the four pure congeners showed any effect on luciferase activity in the same concentration range as the technical mixture. Based on these observations, it can be concluded that most probably other constituents are responsible for the observed effects of technical toxaphene. The present results show that the estrogenic risk of toxaphene exposure in humans is possibly overestimated if the technical mixture is solely used in *in vitro* assays.

1118 *IN VITRO* STUDIES OF POTENTIAL DEVELOPMENTAL TOXICITY OF SOME AGROCHEMICALS COMMONLY USED IN RED RIVER VALLEY, MN.

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Epidemiologic studies by our group show increased frequency of birth defects in the pesticide applicators and general population of the Red River Valley, Minnesota. Based on frequency of use and known biology, certain herbicides, pesticide additives, fungicides, and mycotoxins are suspect agents. To define whether these agents affect certain developmental endpoints *in vitro*, we examined twelve selected agrochemicals using MCF-7 breast cancer cell line. In the flow cytometric assay we used, cell proliferation in this estrogen-responsive cell line indicates xenobiotic-mediated estrogenic effects. Cell viability, morphology, ploidy and apoptosis were incorporated in this assay. The data showed that adjuvants X-77 and Activate Plus induced significant cell proliferation at 0.1 and 1 μg/ml. Commercial grade herbicides 2,4-D LV4 and 2,4-D Amine induced cell proliferation at 1 and 10μg/ml. The reagent grade 2,4-D products failed to induce proliferation over the same concentration range, suggesting that other ingredients in the commercial products, presumably adjuvants, could be a factor in these results. Fungicide triphenyltin and mancozeb provoked apoptosis that peaks at 4.1μg/ml (10^{-5}M) and 50μg/ml, respectively. Triphenyltin also induced aneuploidy at 0.41μg/ml (10^{-6}M). These data suggest potential developmental toxicity of several test agrochemicals, and are a first step in assessments of human effects and *in vivo* studies.

1119 ACETAMINOPHEN ALTERS ESTROGEN RECEPTOR-REGULATED PROCESSES IN DIFFERENT CELLS IN A LIGAND-BINDING-INDEPENDENT MANNER.

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Previous studies demonstrated that acetaminophen stimulates proliferation of estrogen receptor (ER) positive breast cancer cells, and this acetaminophen-induced proliferation is inhibited by anti-estrogens. These studies indicate acetaminophen alters breast cancer cell proliferation by an ER-mediated pathway. The next goal was to determine if acetaminophen binds ERα or ERβ and

to determine the extent to which acetaminophen alters expression of specific ER-regulated genes in different cells. The ability of acetaminophen to compete with binding of 3H-estradiol to purified ERα or ERβ was determined. Acetaminophen (106-fold molar excess) did not compete with 3H-estradiol binding ERα or ERβ, indicating this drug does not bind ER in the same manner as E2. The effect of acetaminophen on expression of specific genes was determined in ER + breast cancer cells (MCF-7) and endometrial carcinoma cells (Ishikawa). In MCF-7 cells, c-myc is induced by estradiol. Ribonuclease protection assays were used to examine c-myc gene expression in MCF-7 cells; c-myc expression was normalized to expression of 18S rRNA. Estradiol induced an ~3-fold increase in c-myc expression 1 hr after addition to cells, and acetaminophen induced c-myc RNA ~2-fold 2 hr after addition to cells. In Ishikawa cells, estradiol induces alkaline phosphatase activity, which is measured spectrophotometrically. Acetaminophen did not induce alkaline phosphatase activity; rather, acetaminophen inhibited estradiol-induced alkaline phosphatase activity in a dose dependent manner. The acetaminophen-mediated reduction of estradiol-induced alkaline phosphatase activity was not due to direct inhibition of this enzyme activity or to general toxicity. These findings demonstrate that acetaminophen can exhibit estrogenic or anti-estrogenic activity on different ER-regulated processes in different cells in a manner that appears to be independent of ligand binding to ER.

1120 INVESTIGATING THE MECHANISM OF METHOXYCHLOR TOXICITY THROUGH ESTROGEN RECEPTORS ALPHA AND BETA USING CDNA ARRAY TECHNOLOGY.

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The reproductive effects of the pesticide methoxychlor during utero exposure is similar to, but not identical to that observed for the sex steriod estrogen. The dissimilarities may be due to distinct patterns of estrogen receptor α (ERα and estrogen receptor β (ERβ expression in selected targer tissues. We have shown previously that the *in vivo* metabolite of methoxychlor, 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), has selective agonist activity through ERα and antagonist activity through ERβ and androgen receptor. To investigate the mechanism of methoxychlor-induced toxicity, we subcutaneously dosed C57BL/6 male and female weanling mice daily with either vehicle (corn oil), 17β-estradiol (E2) (50μg/kg), HPTE (500mg/kg), E2+HPTE, or flutamide (45mg/kg) for three days. On day 4, several target organs were harvested. We observed an increase in uterine wet weight of 3.8-fold with E2, 2.6-fold with HPTE, and 8.9-fold with E2+HPTE. Flutamide treatment had no effect on uterine wet weight, and neither E2, HPTE, nor flutamide significantly altered ovarian or testicular wet weights. RNA from prostates, testes, uteri and ovaries was isolated and used to probe Clontech Atlas Mouse cDNA expression arrays. HPTE altered the expression of several of the same genes as E2, such as glutathione peroxidase, insulin-like growth factor binding protein-6, and cathespin D, while other genes were differentially regulated, such as interleukin-11, insulin-like growth factor binding protein-4, and integrin beta. Quantitation of gene changes will be required using standard molecular techniques. These data will provide a better understanding of the roles of ERα and ERβ in both normal endocrine function and its alteration by environmental chemicals. (ES09106 & ES04917)

1121 EFFECTS OF OCTYLPHENOL ON TESTOSTERONE BIOSYNTHESIS BY CULTURED PRECURSOR CELLS (PC) AND IMMATURE LEYDIG CELLS (ILC) FROM RAT TESTES.

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4-Tert-octylphenol (octylphenol, OP) is a degradation product of alkylphenol ethoxylates, widely used as a surfactant additive in the manufacture of detergents, plastics and pesticides. OP has been reported to mimic estrogen in many cellular systems and, thus, to potentially alter normal sexual growth/maturation. In the present studies, the direct effects of OP on 10 mIU/ml human chorionic gonadotropin (hCG)- stimulated testosterone (T) biosynthesis by cultured PC and ILC were examined. PC are mesenchymal cells that differentiate into ILC, mainly through the actions of luteinizing hormone, between ~ 14 to 28 d after birth, and ILC are converted to adult Leydig cells during the next 4 to 5 weeks of maturation. PC and ILC were isolated by elutriation and Percoll gradient centrifugation of collagenase-dispersed testes from 23-day-old Sprague-Dawley rats. Increasing concentrations of OP (1-2000 nM) progressively decreased hCG-stimulated T starting at 100 or 500 nM, to maximal declines of 30 to 70% below control at the highest concentration in both PC and ILC following exposure for 24 h. OP, similarly, decreased 1 mM 8-Br-cAMP-stimulated T in both PC and ILC, suggesting that the main site(s) of action occur after cAMP formation. To further localize the site(s) of action of OP, the conversion of 1 μM 22 (R) hydroxycholesterol, pregnenolone, progesterone or androstenedione to T after exposure to increasing OP concentrations and hCG was evaluated. Progressive declines in 22 (R) hydroxycholesterol, pregnenolone and progesterone conversion to T were observed, but not androstenedione, suggesting that P450c17 activity, which converts progesterone to androstenedione, is inhibited by OP but not 17β-hydroxysteroid dehydrogenase activity, which converts androstenedione to T. OP also may inhibit cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase-isomerase activities, but additional studies are necessary to confirm this possibility. Interestingly, 17β-estradiol (1-1000 nM) had no effect on hCG-stimulated T formation in both PC and ILC, and concomitant exposure to 100 nM ICI 182,780, a pure estrogen antagonist, did not alter the inhibitive effects of OP. These results suggest that OP effects on both PC and ILC do not mimic the actions of estrogen and are not mediated through the classic estrogen receptor -α or -β pathway.

1122 MONO-(2-ETHYLHEXYL) PHTHALATE SUPPRESSES ESTRADIOL BY DECREASING AROMATASE MRNA EXPRESSION LEVEL AS SHOWN BY REAL TIME RT-PCR.

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Di-(2-ethylhexyl) phthalate (DEHP) is a commercially important plasticizer that is ubiquitous in the environment. DEHP and its active metabolite mono-(2-ethylhexyl) phthalate (MEHP) are reproductive and endocrine toxicants in animal models. MEHP has a direct effect on the granulosa cells of the rat ovary to suppress estradiol production by affecting aromatase, the rate limiting enzyme for the conversion of testosterone to estradiol. To better understand the mechanism of action of MEHP, we tested the hypothesis that MEHP decreases transcription of the aromatase gene. Granulosa cells were obtained from 28 day old Fisher 344 rats that had been treated with PMSG either 24 or 48 hours earlier to induce follicle development. Cells were cultured for 48 hours with FSH (2.5 ng/mL) and testosterone (500nM) and 0mM, 0.001mM, or 100mM MEHP (in DMSO). As previously found, 100mM MEHP significantly decreased mean estradiol levels in media compared to control and low dose with $p<0.0001$. Moreover, MEHP had a greater effect on estradiol production from cells obtained 24 hours after PMSG treatment compared to cells obtained 48 hours after PMSG treatment. This is consistent with the understanding that MEHP affects the differentiating granulosa cell. RNA was analyzed using the TaqMan real-time PCR assay to measure aromatase message. In 4 separate experiments, samples treated with 100mM MEHP had half ($p<0.005$) as much aromatase message compared to control samples when normalized to GAPDH. The 0.001mM treatment group was not statistically different from controls. This was the case for both the 24 hour and 48 hour post-PMSG treatment. These results indicate that the suppression of estradiol by MEHP may occur at a transcriptional level. Future experiments will examine the effects of MEHP on the aromatase protein. We anticipate that these studies will uncover the molecular mechanisms by which MEHP inhibits granulosa cell differentiation and ovulation.

1123 OXIDATIVE STRESS BY SODIUM ARSENITE INHIBITS ADRENAL CHOLESTEROL METABOLISM WHILE SHOWING BOTH STIMULATION AND SUPPRESSION STEROIDOGENIC ACUTE REGULATORY OF PROTEIN.

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The steroidogenic acute regulatory protein (StAR) is a key participant in the hormonally activated transfer of cholesterol to cytochrome P450scc. Here we show that two activators of stress activated protein kinase pathways (SAPK), arsenite and anisomycin, each potentiate the maximum stimulation of StAR mRNA (3.5 kb) by Br-cAMP at low concentrations (respectively, 20 μM, 2.5 fold and 0.2 μM, 5 fold). Hydrogen peroxide (100 μM), a strong activator of NFκB, was ineffective in this stimulation. At higher concentrations (>40 μM) of each activator StAR mRNA and protein expression was reduced sharply, reaching almost complete suppression by arsenite. None of these agents changed basal StAR expression indicating potentiation and suppression of the protein kinase A stimulation as opposed to a direct effect on transcription. Time courses for StAR stimulation by Br-cAMP indicated that at low concentrations of arsenite rates of transcription were elevated after a 1 hr lag. At higher concentrations an early increase was followed after 2 hr by a rapid decrease indicating onset of increased degradation of StAR mRNA. Hydrogen

peroxide was only weakly inhibitory even at high concentrations (500 μM). StAR protein levels were elevated at low levels of anisomycin and arsenite and decreased at higher levels in parallel with the changes in mRNA. Arsenite substantially changed the distribution of StAR forms. Notably there was an elevation of the p37 metabolism in both Y-1 and MA10 cells was inhibited by arsenite at high concentrations. Cholesterol metabolism in both Y-1 and MA10 cells was inhibited by arsenite at high concentrations. There was no significant stimulation of pregnenolone production at low concentrations of arsenite where StAR mRNA and protein expression were potentiated. Stimulation of StAR expression by arsenite and anisomycin corresponded to enhanced activation of p38 kinase. Inhibition by arsenite corresponded in dose and time to a threshold for activation of JNK. Anisomycin at higher levels directly inhibit protein translation. Arsenite potentiated 20α-hydroxycholesterol metabolism by P450scc indicating that mitochondrial function and cytochrome turnover are not compromised. In vivo studies indicates that ACTH-stimulated blood corticosterone (60 min/ACTH) were lowered by two-fold after intragastric administration of sodium arsenite (20 mg/kg body weight, 24 hr prior to stimulation with ACTH). This inhibition is remarkably similar to what we have seen in cultured adrenal cells. These studies show that StAR expression is highly sensitive to activation of these SAPK by both arsenite and anisomycin through effect at multiple levels of regulation.

1124 EFFECTS OF CHLORO-S-TRIAZINE HERBICIDES AND METABOLITES ON AROMATASE (CYP19) ACTIVITY IN VARIOUS HUMAN CELL LINES AND ON VITELLOGENIN PRODUCTION IN MALE CARP HEPATOCYTES.

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A potential mechanism for the estrogenic properties of the chloro-*s*-triazine herbicides was investigated *in vitro* in several cell systems. Effects on aromatase, the enzyme that converts androgens to estrogens, were determined in H295R (adrenocortical carcinoma), JEG-3 (placental choriocarcinoma) and MCF-7 (breast cancer) cells; effects on the estrogen receptor-mediated induction of vitellogenin were determined in freshly isolated hepatocytes of a genetically uniform strain of adult male carp (*Cyprinus carpio*). Aromatase activity was measured by the conversion of [1β-^{3}H]-androstenedione to estrone and ^3H_2O; both the release of ^3H_2O and the formation of estrone were quantified as measures of catalytic activity. Atrazine, simazine, propazine and two of the six tested common metabolites of these herbicides induced aromatase activity in H295R cells concentration- (0-30 μM) and time- (0-72 h) dependently. At a 30 μM concentration an apparent maximum induction of about 2 to 2.5 fold was achieved after exposure to the triazines for 24 h. The induction responses were confirmed by similar increases in CYP19 mRNA levels, determined by reverse-transcriptase polymerase chain reaction. In JEG-3 cells the induction responses were less pronounced, while aromatase expression in MCF-7 cells was not detectable under our culture conditions. None of the triazine herbicides or their metabolites (0-30 μM) were capable of inducing vitellogenin production in carp hepatocytes. They were also not capable of antagonizing the induction of vitellogenin by 20 nM (EC_{50}) 17β-estradiol. These findings together with those of other investigators indicate that the estrogenic effects associated with the triazine herbicides *in vivo*, are not estrogen receptor-mediated, but may be partly explained by their ability to induce aromatase.

1125 DIETHYLSTILBESTEROL REGULATES COX-2 EXPRESSION IN VAGINAL EPITHELIAL CELLS.

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Exposure of the human fetus to the synthetic estrogen diethylstilbesterol (DES) is associated with a high incidence of vaginal adenosis later in life. This condition, characterized by the appearance of glandular tissue within the vagina, has been attributed to altered differentiation of vaginal epithelial cells (VEC). However, the molecular events leading to this condition are poorly understood. To explore this process we exposed primary cultures of human vaginal ectodermal epithelial cells to DES. These cells proliferate in medium containing low levels of calcium (<0.05 mM). The addition of calcium (1.2 mM) to the cells causes the cells to flatten, elongate and take on characteristics of differentiating vaginal epithelial cells. In low calcium conditions these cells constitutively express cyclooxygenase 2 (Cox-2), an immediate early mediator of inflammation expressed in secretory tissues including gastrointestinal mucosa and kidney. Calcium-mediated differentiation downregulates Cox-2 expression in VEC. We found that treatment of undifferentiated, but not differentiated VEC, with DES (1 nM) dramatically upregulates Cox-2 expression. These findings suggest that regulation of Cox-2 is differentiation-dependent. We hypothesize that regulation of Cox-2 expression in undifferentiated VEC may reflect events important in DES-mediated pathology.

1126 8-BROMO CYCLIC-AMP AND DEXAMETHASONE (DEX) FAIL TO REVERSE THE DECREASE IN CATECHOLAMINES (CA) INDUCED BY ATRAZINE (ATZ) AND SIMAZINE (SIM) IN PC12 CELLS.

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Previously we reported that the chlorotriazines (CTZs), in particular ATZ, disrupt ovarian function, suppress luteinizing hormone release and decrease hypothalamic norepinephrine concentration in the rat. Using PC12 cells, we also showed that the CTZs reduce catecholamines concentration and release *in vitro*. The present study was designed to verify i) the altered intracellular concentration and pattern of release of CA by ATZ and SIM in PC12 cells and ii) whether cAMP and DEX, which are known to regulate the enzymes invovled in CA synthesis, can reverse the effects of ATZ and SIM. PC12 cells were incubated with ATZ or SIM alone or in combination with 8-bromo cAMP (1 mM) or DEX (1 μM) for up to 36 h. Both ATZ and SIM (12.5, 25, 50, 100, and 200 μM) significantly inhibited intracellular dopamine in a dose-dependent manner. ATZ (100, 200 μM) and SIM (200 μM) significantly inhibited intracellular norepinephrine and caused a dose-dependent decrease in norepinephrine release at 6-24 h. Cell viability was not altered by any treatment. Both 8b-cAMP and Dex stimulated intracellular dopamine and norepinephrine concentrations and norepinephrine release over time, however these activators failed to reverse the decreases in catecholamines induced by the CTZs. These data suggest that both ATZ and SIM suppress catecholamines synthesis in PC12 cells but not through the same regulatory pathways effected by 8b-cAMP or Dex. (This abstract does not reflect U.S. EPA policy.)

1127 GENE INDUCTION BY PHENOBARBITAL AND CELL SIGNALING IN THE HEPATOCYTE.

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The induction of biotransformation processes has substantial impact on an individual's response to pharmaceutical and toxicant exposures. Phenobarbital (PB) is a prototypical inducing agent for a variety of drugs and other xenobiotic compounds that exhibit pleiotropic effects in the liver of mammalian organisms. Biotransformation genes that are markedly up regulated by this "class" of compounds include certain glutathione transferases, UDP-glucuronosyl transferases, aldehyde dehydrogenases, and cytochrome P450 monooxygenases. The molecular mechanisms and signaling pathways responsible for the transcriptional activation effects of PB inducers remain poorly defined. To further investigate the induction paradigm, one research thrust of our laboratory has focused on the development of transgenic mouse and primary hepatocyte *in vitro* models enabling investigation of the biology of PB induction together with that of liver-specific gene expression. Initially, results form our transgenic models largely pointed the way to the subsequent discovery of a PB responsive unit or module (PBREM) localized ~2200 bp upstream of the transcriptional initiation site for the rat CYP2B2 and mouse Cyp2b10 genes. This module is centered on a nuclear factor 1 site and is flanked by sequence motifs for putative nuclear receptor interactions. Further transgenic mouse studies by our laboratory indicated that NR1 is not the key regulator of PB responsiveness within the PBREM; rather, a CAR nuclear receptor protein was identified recently by Dr. Negishi's group as a critical modulator of PB transcription. Since this transcriptional response is largely lost in transformed cell lines, we have developed a defined primary hepatocyte culture model and investigated liver specific signaling cascades required for maintaining the highly differentiated cell phenotype. Using this model, we demonstrated that de novo protein synthesis is not required for PB induction. Arrays of pharmacological agents have been evaluated for their effects on hepatocyte signaling networks and potential modulation of the PB induction process. PKC, MAPK, CAMKII and P13K pathways have been largely ruled out as PB signaling mediators. In contrast, important roles were established for protein kinase A (PKA) and protein phosphatase pathways (PP2A) as regulators of the induction response, although recent experiments have established that PB exposures per se do not increase cAMP levels or PKA activity in hepatocytes. Further experiments with the developed transgenic and *in vitro* models are poised to delineate the cell signaling networks and associated extracellular matrix interactions that define the hepatic phenotype, regulate genetic induction, and modulate the toxicological responses characteristic of this organ.

1128 ROLE OF CO-REPRESSORS AND CO-ACTIVATORS IN REGULATION OF SOLUBLE RECEPTOR MEDIATED TRANSCRIPTION.

G. H. Perdew. *Penn State University, University Park, PA.*

One of the primary areas of interest to toxicologists is to understand the mechanisms underlying tissue-, cell-, and species-specific expression of genes involved in a given toxic response. Recently it has become increasingly apparent that a major contributing factor to transcriptional activation and repression of target genes is the recruitment of co-activators and co-repressors. In addition, there are several examples of tissue-specific expression of co-activators (e.g. ARA70) that may contribute to a unique pattern of gene expression. Transcriptional regulation by co-activators/co-repressors has been predominantly studied in the context of steroid receptors, such as Estrogen and PPARg γ receptors. Many of the coactivators such as p300 and SRC-1, which have been extensively characterized, bind to a growing list of enhancer binding transcription factors, this may indicate that these factors compete for a limiting pool of available coactivators. It has also been shown that even small differences in expression of transcription factors and coactivators can lead to threshold effects during growth and development. In addition to affecting steroid hormone receptors, recent studies revealed that co-activators play a role in regulating transcriptional activation by the Ah receptor/ARNT heterodimer. Studies presented in this symposium will examine the ability of co-activators/co-repressors to alter expression of enzymes by the Ah receptor and several steroid receptors in a tissue- and ligand-specific manner.

1129 HORMONES, TRANSCRIPTIONAL REPRESSION, AND THE ROLE OF CO-FACTORS IN THE FUNCTION OF NUCLEAR HORMONE RECEPTORS.

Z. Yang, S. H. Hong and M. Privalsky. *University of California, Davis, CA.*
Sponsor: G. H. Perdew.

The nuclear hormone receptors, such as the estrogen, androgen, retinoid, vitamin D3, and thyroid hormone receptors, play many crucial roles in vertebrate physiology and development. Nuclear hormone receptors have also been implicated as targets of endocrine disrupters, such as environmental estrogens, and it is clear that aberrant receptor activity can play a causal role in a variety of human endocrine and neoplastic diseases. Nuclear hormone receptors function by regulating the expression of specific target genes in response to binding of cognate hormone. These transcriptional properties are mediated through the ability of these nuclear hormone receptors to physically associate with coactivator and corepressor proteins. In this presentation, we will describe our studies elucidating how different hormone-derivatives, natural and artificial, regulate the interaction of thyroid hormone and retinoid receptors with the SMRT/N-CoR corepressors, and with the SRC-1 coactivators. We will also describe our recent results demonstrating how other signal transduction systems operating within target cells, such as the MAP kinase cascade, can modulate the actions of these nuclear hormone receptors and thereby modify the actions of the hormone ligand itself. We also will report our newest studies that detail how aberrant cofactor/receptor interactions can contribute to several human endocrine and neoplastic disorders.

1130 A PHARMACOLOGICAL DISSECTION OF RXR SIGNALING PATHWAYS WITH REXINOIDS.

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Sponsor: G. H. Perdew.

Receptor X receptor (RXR) plays a central role in the regulation of many intracellular receptor signaling pathways. To examine signaling pathways mediated by RXR and its partners we have designed and characterized a series of synthetic ligands that bind RXR and modulate its transcriptional properties. These RXR ligands, referred to as rexinoids, are dimer selective in that they can function as agonists and/or antagonists depending upon the context of the homodimer or heterodimer partner. These rexinoids have been used as pharmacological tools in a multitude of molecular cellular and animal models. In animal models of non-insulin dependent diabetes mellitus (NIDDM) and obesity, RXR agonists function as insulin sensitizers significantly decreasing hyperglycemia and hyperinsulinemia. At the molecular level, these activities are mediated by the RXR: PPARγ heterodimer. Binding of agonists to these nuclear receptors results in a conformational change in receptor structure that promotes interaction between activated receptors and co-activators. Analysis of RXR: PPARγ heterodimers and cofactors suggests that RXR and PPARγ can distinguish among co-activators by recognizing distinct structural features of nuclear receptors. RXR ligands selectively recruits SRC-1 to the dimer whereas, CBP selectively recruits CBP. These differences may account for the gene and tissue selectivity of these pharmacological agents. Thus, the ability of RXR to dimerize with distinct receptors allows rexinoids to alter transcriptional signaling of distinct endocrine signaling pathways and offers new opportunities for the effective treatment of certain pathophysiological states.

1131 ROLE OF TRANSCRIPTIONAL CO-ACTIVATOR PROTEINS IN FUNCTIONING OF THE Aryl HYDROCARBON RECEPTOR/ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR (AHR/ARNT) DIMER.

T. V. Beischlag, K. Muhammad, W. E. Nelson and O. Hankinson. *University of California, Los Angeles, CA.*

AHR binds a variety of pollutants, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo(a)pyrene, and mediates the carcinogenic and toxic effects of these compounds. After binding ligand, AHR dimerizes with the ARNT protein. The AHR/ARNT dimer activates transcription of CYP1A1 and certain other genes. Induction of CYP1A1 is involved in carcinogenesis by benzo(a)pyrene. In contrast, the mechanisms of TCDD carcinogenesis and toxicity is obscure. However, they probably depend (at least in part) upon transcriptional activation of certain other (perhaps unidentified) genes. We are studying the role that know coactivator proteins play in transcriptional activation by the AHR/ARNT dimer. We are particularly interested in the three members of the Steroid Receptor Cactivator (SRC) family, SRC-1 (also called NCoA-1), SRC-2 (NCoA-2, TIF-2, GRIP-1), and SRC-3 (p/CIP, ACTR, AIB1, TRAM1) because they contain bHLH-PAS (basic helix-loop-helix-per/arnt/sim homology region) domains towards their amino termini. Each of the SRC proteins stimulates TCDD-dependent induction of a luciferase reporter gene by the AHR/ARNT dimer. SRC-1 and SRC-2 bind ARNT *in vitro* and in mammalian cells *in vivo*. Thus the SRC proteins appear to act as transcriptional coactivators for the AHR/ARNT dimer. Thyroid receptor/retinoblastoma protein interacting protein (Trip230), another coactivator protein, was also found to stimulate transcriptional activation by the AHR/ARNT dimer and associate with ARNT in mammalian cells *in vivo*. Trip230 therefore may be another coactivator for AHR/ARNT. Finally, we have isolated several novel ARNT interacting proteins, some of which may represent transcriptional coactivators. Future studies will probe the mechanism of activation of the various coactivator proteins.

1132 NUCLEAR RECEPTOR CO-ACTIVATORS RIP140 AND SRC-1 INTERACT WITH GLUTAMINE-RICH DOMAIN OF THE Aryl HYDROCARBON RECEPTOR AND MODULATE ITS TRANSCRIPTIONAL ACTIVITY.

M. B. Kumar and G. H. Perdew. *The Pennsylvania State University, State College, PA.*

The Aryl hydrocarbon Receptor (AhR), a soluble cytosolic protein mediates many of the toxic effects of TCDD and related chemicals. The toxic effects are both cell-, tissue-, and species dependent. Although the mechanism of action of ligand-mediated AhR has been elucidated, the sequence of events following the binding of AhR/Arnt to DRE has yet to be understood. The possible role of Nuclear Receptor (NR) coactivators RIP140 and SRC-1 in the modulation of transcriptional activity of AhR/Arnt was examined. RIP140 and SRC-1 enhanced TCDD-mediated, dioxin response element-driven reporter gene activity in three cell lines. Co-immunoprecipitation and co-localization assays revealed that RIP140 and SRC-1 interacted with AhR has a complex transactivation domain composed of an acidic, Q-rich, and P/S/T subdomains. Mapping of the interaction sites revealed that RIP140 and SRC-1 were recruited by the AhR was mapped to a location between amino acid residues 154-350, which is distinct from those involved in Estrogen Receptor binding. The signature motif, LXXLL, which is responsible for binding of several coactivators to NR, is not required for RIP140 binding to the AhR. These results suggest that the AhR may be capable competing with steroid receptors for a common coactivator pool.

1133 INTRODUCTION.

S. B. Pruett[1] and T. R. Jerrells[2]. [1]*LSU Medical Center, Shreevport, LA and* [2]*Omaha VA Medical Center, Omaha, NE.*

Excessive ethanol (EtOH) consumption is a pervasive problem, and it is associated with an increased risk of infectious disease and cancer. Recent studies in animal models and human subjects have revealed a number of mechanisms by which EtOH decreases immune function. A mouse model of acute EtOH exposure has revealed decreases in NK cell function (and in resistance to B16F10 melanoma cells) and decreases in MHC class II proteins on B cells. These actions are mediated at least partly by an EtOH-induced stress response. Results from a mouse model of chronic EtOH consumption demonstrate a delayed suppression of antibody responses that is not mediated by direct action of EtOH, but by an indirect mechanism. Results from a similar mouse model indicate that early suppression of innate immunity to intracellular bacterial or viral infections contributes to a subsequent over production of proinflammatory cytokines. These cytokines represent a major pathogenic mechanism. A potential molecular mechanism for suppressed innate immunity has been identified in human subjects following acute exposure to EtOH in a controlled experimental setting. Monocytes from these subjects exhibit diminished NFκB activation associated with preferential induction of inhibitory p50/p50 homodimers. Human alcoholics have increased numbers of activated T cells, and this may result from upregulation of CD40L. There also seem to be alterations in the Th1/Th2 balance. Thus, EtOH alters a number of immune functions through several different mechanisms. Some common effects and mechanisms of action have been identified in acute and chronic studies in rodents and humans, but additional comparisons are needed. Completion of the *in vitro/in vivo*, human/animal parallelogram seems feasible and could provide valuable information for risk assessment in immunotoxicology.

1134 SUPPRESSION OF NK CELL ACTIVATION AND MHC CLASS II EXPRESSION ON B CELLS BY ACUTE ETHANOL EXPOSURE IN MICE: MECHANISMS OF ACTION AND INVOLVEMENT OF ENDOGENOUS CORTICOSTERONE.

S. B. Pruett. *LSU Medical Center, Shreveport, LA.*

In a mouse model of acute EtOH exposure, among the most striking immunological changes are a decrease in NK cell lytic function (with a consequent decrease in resistance to B16F10 melanoma metastases) and a decrease in the expression of MHC class II proteins on B cells. The glucocorticoid antagonist RU 486 partially prevents suppression of NK cell function and completely prevents the loss of MHC II proteins. This suggests that corticosterone produced as part of the EtOH-induced stress response is involved in suppression of NK cell function and completely responsible for the decrease in MHC class II expression. Administration of exogenous corticosterone and evaluation of the area under the corticosterone vs. time curve in EtOH and corticosterone-treated mice support these conclusions. Furthermore, similar blood EtOH levels are required to increase glucocorticoid levels in mice and in humans. The mechanism of suppression of NK cell activation and resistance to B16F10 tumor cells seems to involve decreased upregulation of type I interferon and granzyme B and decreased perforin levels. The mechanism by which MHC class II expression on B cells is suppressed involves down regulation of transcription of the MHC class II gene. In addition, *in vitro* studies at relevant corticosterone levels indicate a novel mechanism that is mediated by caspase activation and is associated with apoptosis of B cells. These results indicate that the importance of the EtOH-induced neuroendocrine stress response depends on the immune parameter evaluated and that EtOH acts by different mechanisms to alter different immune parameters within the same experimental model. (This work was supported by grants from NIAAA [AA09505] and NIEHS [ES09158], and the author is supported by a Career Development Award from NIAAA [AA00201].)

1135 THE EFFECTS OF SUB-CHRONIC ETHANOL FEEDING ON IMMUNE-MEDIATED HOST DEFENSES TO INTRACELLULAR BACTERIA AND VIRUSES.

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Human beings who consume large amounts of alcohol have been shown to have a significantly higher incidence of infections. Of interest is the incidence of tuberculosis and hepatitis C virus infections in populations of human beings who chronically consume large amounts of alcohol. With the use of a mouse feeding model my laboratory has investigated the effects of ethanol (ETOH) consumption on parameters of immunocompetence, especially cellular immune responses to intracellular bacteria and a murine virus that causes a hepatitis. The studies have been done with a pair-feeding protocol where C57B1/6 mice were fed a liquid diet containing ETOH to supply 37% of the calories from ETOH, an isocaloric control liquid diet, or laboratory chow. The results of these studies have shown that animals that consume the ETOH diet are significantly more susceptible to a systemic infection with Listeria monocytogenes and an oral infection with Salmonella typhimurium. We have also shown that a more severe hepatitis following infection with murine cytomegalovirus is associated with consumption of ETOH in this model. Interestingly, data have been collected that support the suggestion that a more robust inflammation is the major mechanism of pathogenic effects following infection in the ETOH-consuming animals. This apparently is the result of over production of proinflammatory cytokines. The common mechanism of susceptibility seems to be an inability of the early, innate immune response to control the initial replication of the infectious organism. (Supported by AA07731 and AA12450.)

1136 RESULTS FROM A CHRONIC LIQUID DIET MODEL—INDIRECT, TIME-DEPENDENT EFFECTS ON THE HUMORAL IMMUNE RESPONSE.

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The overall objective of these studies was to characterize the effects of chronic exposure to ethanol using a pair-feeding regimen. Adult female B6C3F1 mice were exposed to a liquid diet containing 5% ethanol (v/v) that provided 36% of the caloric intake. There was a definite temporal relationship to the consequent suppression of the antibody response to a T-dependent antigen with no suppression observed after 14 days of exposure. The magnitude of suppression increased from 18% after 21 days to 70% after 42 days. In contrast with our results with the *in vivo* antibody response, we saw no suppression of the *in vitro* antibody responses by splenocytes from ethanol-treated mice to a variety of antigens after any length of exposure. We speculated that the basis of the suppression of the *in vivo* antibody response was an indirect consequence of exposure to ethanol. We subsequently determined that when splenocytes from untreated mice were cultured in 5% serum from ethanol-exposed mice (42-day group), there was a >80% suppression of the *in vitro* antibody response relative to the serum from the pair-fed controls. As an important comparative control, we showed that there was no difference between the responses of splenocytes from untreated mice when cultured in either 5% normal mouse serum or 5% serum taken from the pair-fed controls. A determination of the ethanol content in the serum from the treated mice (42-day group) indicated that the amount of ethanol present in the cultures (<0.003%) could not account for the observed suppression. These results will provide the framework for the discussion of a model which is centered around an indirect mechanism of ethanol-induced immunosuppression, which is mediated in part by a serum factor.

1137 ROLE OF NF-κB IN INHIBITION OF INFLAMMATORY MEDIATOR PRODUCTION BY ALCOHOL IN HUMAN MONOCYTES.

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Sponsor: S. B. Pruett.

Chronic alcohol use is associated with impaired immunity and host defense. We showed that even *in vitro* acute ethanol treatment results in decreased production of inflammatory cytokines that are major contributors to host defense including tumor necrosis factor-α (TNFα), IL-1β, and chemokines IL-8 and MCP-1. Here we show that acute consumption of alcohol (2 ml vodka/kg body weight; BAL:.1g/dl) by non-alcoholic healthy individuals significantly decreases TNFα and IL-1β production in ex vivo challenged isolated blood monocytes ($P<0.01$). SEB-induced IL-8 and MCP-1 levels were also reduced in whole blood obtained 18 hrs after alcohol consumption ($p<.01$). However, the intracellular mechanisms for down-regulation of bacterial stimulation-induced inflammatory cytokine production in MØ after acute alcohol treatment are yet to be dissected. Here, NF-κB/Rel was tested as a potential target of ethanol in regulation of inflammatory cytokine gene expression. We have previously reported a rapid decrease in mRNA and protein levels of the NF-κB-regulated inflammatory cytokines in MØ after acute ethanol treatment. Minimal NF-κB binding was seen in unstimulated MØ from non-alcoholic donors. MØ activation with 1μg/ml LPS or SEB substantially increased NF-κB binding of both the p65/p50 and p50/p50 dimers as early as 30 min and as late as 24 hrs after stimulation. However, ethanol (25mM) prevented p65/p50

heterodimer induction by LPS or SEB in MØ. NF-κB inhibitor by ethanol required de novo protein synthesis and was not mediated by inhibition of SEB or LPS-induced IkB degradation. Finally, preferential induction of p50/p50 homodimer was seen by ethanol in MØ. These results imply that inhibition of NF-κB activation is likely to be one of the intracellular mechanisms for the ethanol-induced inhibition of inflammatory mediator production in monocytes. (Supported by NIH AA08577 and A11576 grants.)

1138 CHRONIC ETHANOL EXPOSURE—COMPARISONS OF HUMAN AND EXPERIMENTAL DATA.

R. T. Cook. *University of Iowa & Veterans Administration Medical Center, Iowa City, IA.* Sponsor: S. B. Pruett.

Chronic alcoholism is associated with signs of immune deficiency; there are significant increases in serious infections such as bacterial pneumonia, septicemia, tuberculosis and others. It is unknown whether alcohol congeners influence any of the immune alterations, but animal models of alcohol abuse demonstrate that pure dietary ethanol at 25+% of total calories is immunosuppressive, with increased mortality from experimental bacterial pneumonia, more extensive infections by Listeria and Salmonella, and increased numbers of Mycobacterium avium in chronic ethanol animals. Evaluation of lymphocyte immunophenotype and function in human alcoholics has shown persistent activation of CD8+ T-cells both in alcoholics with and those without liver disease, and very substantial activation of both CD8+ and CD4+ T-cells in alcoholics with liver disease. The concept has arisen that the immune deficiency in alcoholics may result from an increase in TH2 type lymphocyte function. Recent work in the author's laboratory has demonstrated that most alcoholics have an increase in a phenotype (CD57+ T-cells) that show a rapid TH1 response after *in vitro* stimulation through the T-cell receptor (TCR). These cells are end-stage effectors which produce significant IFNγ and TNFα, and are thought to then rapidly undergo apoptosis. Cytoplasmic IL-4 can also be demonstrated in the fresh CD4+ T-cells of some alcoholics, so the balance of TH1/TH2 may be altered. Stimulation through the TCR rapidly upregulates CD40 ligand (CD40L) on CD4+ T-cells *in vitro*, and there is some data suggesting its persistent upregulation in the alcoholic, consistent with an increase in T-dependent B-cell activation, and an increase in T-cell-monocyte interactions. These data and that of the previous speakers will be summarized and an integrated view of current questions in the field will be offered.

1139 MYCOTOXINS: RECENT ADVANCES AND THEIR RELEVANCE TO CARCINOGENESIS AND TOXICOLOGY.

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Fumonisins are produced by *Fusarium moniliforme* (recently reclassified as *F. verticillioides*) and *F. proliferatum*, fungi commonly found on corn. They are suspected human carcinogens which affect populations utilizing contaminated corn as a dietary staple. Fumonisin B_1 (FB_1), the most thoroughly studied fumonisin, causes the species-specific toxicities elicited by *F. verticillioides* in farm and laboratory animals. On the molecular level, fumonisins disrupt sphingolipid metabolism by inhibiting ceramide synthase. Apoptosis is an early cytotoxic event following fumonisin exposure. These findings are significant not only for mycotoxicology, but also for understanding fundamental toxicological principles. The aim of this symposium is to explore how fumonisins can be used to investigate connections between carcinogenicity, cytotoxicity and apoptosis, and specific biomolecular events. Specific topics to be covered are: (a) the liver and kidney tumorigenic effects of FB_1 in rats and mice; (b) the role of apoptosis as an early event in fumonisin toxicity; (c) the use of FB_1 as a model for the role of apoptosis in nongenotoxic carcinogenesis and its implications for evaluating risk; (d) the use of fumonisins as research tools for studying sphingolipid-mediated cell regulatory functions, including those governing apoptosis and mitosis; and (e) to show how an integrated strategy combining biochemical, physiological and pathological approaches can be used to study *in vivo* mechanisms of toxicity and interspecies differences.

1140 CARCINOGENICITY OF FUMONISIN B_1 IN F-344 RATS AND B6C3F1 MICE.

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Fumonisin B_1 (FB_1) is a toxin produced by Fusaria fungi that contaminate crops worldwide. The tumorigenicity of FB_1 was determined in male and female F344/Nctr rats and B6C3F1/Nctr mice. The highest concentration of FB_1 in the diets (NIH-31) were 100 and 150 ppm for female and male rats, and 80 and 150 ppm for female and male mice, respectively. FB_1 induced renal tubule adenomas and carcinomas in male rats (50 and 150 ppm), and did not induce tumors in female rats. FB_1 increased hepatocellular carcinoma formation in female mice (50 and 80 ppm), while no increase in tumor formation was observed in male mice. FB_1-induced changes in cell proliferation, apoptosis, and sphingoid bases were consistent with the observed target tissues. These results support the conclusion that FB_1 is a rodent carcinogen. This data is currently being used for human cancer risk estimations.

1141 APOPTOSIS AND ITS IMPLICATIONS FOR TOXICITY, CARCINOGENICITY AND RISK: FUMONISIN B_1 AS AN EXAMPLE.

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The balance between the rates of cell proliferation and cell loss in conjunction with the differentiation status of the tissue are among the many factors contributing to carcinogenesis. Nongenotoxic (non-DNA reactive) chemicals may affect this balance by increasing proliferation through direct mitogenesis or through a regenerative response following loss of cells through cytotoxic (oncotic) or apoptotic necrosis. In male rats, the mycotoxin fumonisin B_1 (FB_1) caused hepatic toxicosis and hepatocellular carcinomas in one study but caused renal carcinomas in a second. It has also been shown to cause liver cancer in female mice. An early effect of FB_1 exposure in these target organs is apoptosis. Induction of apoptosis may be a consequence of ceramide synthase inhibition and disruption of sphingolipid metabolism by FB_1. FB_1 is not genotoxic in bacterial mutagenesis screens or in the rat liver unscheduled DNA synthesis assay. Thus, FB_1 may be the first example of an apparently nongenotoxic carcinogen which exerts its effects through apoptotic necrosis, atrophy, and consequent regeneration. If so, selection of damaged cells which are resistant to apoptosis, but susceptible to cancer development, would have important implications for neoplastic development and evaluation of cancer risk.

1142 INHIBITORS OF SPHINGOLIPID BIOSYNTHESIS AS RESEARCH TOOLS FOR UNDERSTANDING THE MECHANISM OF ACTION.

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The first reported fungal inhibitor of *de novo* sphingolipid biosynthesis was fumonisin B_1 (FB_1). Since this discovery, 40 known or suspected microbial inhibitors of sphingolipid biosynthesis have been reported. These include metabolites that inhibit serine palmitoyltransferase, ceramide synthase (including FB_1), and inositol phosphoceramide synthase. In addition to the microbial inhibitors, there are also inhibitors of glycosylceramide synthase, ceramidase, and sphingosine kinase. These inhibitors are used to reveal the role of sphingolipids in cellular regulation and the importance of ceramide synthase inhibition in the diseases associated with fumonisins. FB_1-inhibition of ceramide synthase increases the intracellular concentration of free sphingoid bases and their 1-phosphates, and decreases the *de novo* biosynthesis of ceramide and more complex sphingolipids. Free sphingoid bases, sphingoid base metabolites, ceramide, and glycosphingolipids are known modulators of cell function, survival, and growth. In short-term experiments, inhibition of ceramide synthase by fumonisins can inhibit cellular effects induced by ceramide generated *de novo* or increased glycosylceramide synthesis. In longer term experiments, ceramide synthase inhibition promotes the effects induced by free sphingoid bases/metabolites, and inhibits the processes that require more complex sphingolipids. Inhibitors of other enzymes in the *de novo* sphingolipid biosynthetic pathway have proven useful in dissecting the contribution of the changes in specific sphingolipid pools in the cellular effects of fumonisins. These studies have revealed new information about the mechanisms of action of fumonisins and the importance of sphingolipids in cellular regulation.

1143 RELATING MOLECULAR MECHANISMS TO PATHOPHYSIOLOGICAL EFFECTS: FUMONISIN AS A CASE STUDY.

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Fumonisins, mycotoxins produced by *Fusarium verticillioides* (= *F. moniliforme*) and *F. proliferatum*, inhibit sphingosine N- acyltransferase, a key enzyme in the pathway of *de novo* sphingolipid biosynthesis, resulting in increased concentrations of sphingosine (So) and sphinganine (Sa) in serum and tissues of all species. Fumonisins cause lethal pulmonary edema in pigs and leukoencephalomalacia in horses; renal injury in rabbits, sheep, and rats; and liver injury in all species. Our recent studies have focused on the pathogenesis of fumonisin B_1 induced pulmonary edema, which is unique to swine, taking into consideration pathological, physiological and biochemical alterations. Interstitial pulmonary edema was accompanied by membranous accumulations in the pulmonary capillary endothelial cells which appear specific to this cell type and to swine. Fumonisin B_1 increased mean pulmonary arterial pressure and decreased heart rate, cardiac output and mixed venous oxygen tension. Additionally fumonisin B_1 decreased cardiac contractility as assessed by left ventricular end-systolic elastance and maximal rate of change of pressure. However, the permeability of the alveolar-capillary membrane was not altered. Fumonisin increased the concentrations of So and Sa in the kidney>liver>lung>heart, as well as in serum. Neither pulmonary edema nor cardiovascular physiologic alterations occurred in calves given purified fumonisin B_1 intravenously at the same dose; however, the increases in serum Sa and So in calves were much lower than in pigs. The changes in pigs are compatible with the inhibition of L-type calcium channels by increased So and/or Sa concentration. Therefore, fumonisin-induced pulmonary edema in swine presumably results from acute left-sided heart failure mediated by inhibition of sphingolipid biosynthesis.

1144 THE INFLUENCE OF CO-POLLUTANTS ON THE TOXICITY OF AIRBORNE PARTICULATE MATTER.

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Recent epidemiological reports have shown an association between ambient air particulate matter (PM) concentrations and human mortality and morbidity, such as increased hospitalizations. PM coexists in the ambient air with many other pollutants, and therefore complicates the assessment of the contribution of PM to the affected health endpoints. In the National Research Council's Report in 1998 that concerned Airborne PM Research Priorities, one of the research aspects emphasized was performance of controlled toxicological studies and epidemiological studies related to examining effects of co-pollutants on possible PM-induced health endpoints. In this session, evidence of the support for co-pollutant influences on PM-associated health effects will be examined from epidemiological studies. Evidence of co-pollutants affecting PM-induced responses will be will drawn from findings of controlled *in vivo* and *in vitro* exposure studies that examine cardiopulmonary effects of PM. These controlled co-pollutant studies can potentially suggest which pollutants are likely to influence PM-induced lung and extrapulmonary toxicity, and potential mechanisms for these interactions. Additionally, the characteristics and components of PM likely to be influenced by other ambient pollutants can be identified in these studies. Oxidants including ozone, inorganic components such as sulfates, organic constituents such as aromatic compounds, and biological substances (i.e., endotoxin) can all play an important role in modifying PM-induced effects by allowing co-pollutant interactions. Data presented from gaseous pollutant interaction with cigarette smoke, more typically an indoor pollutant, will compliment the understanding of outdoor PM toxicology.

1145 EPIDEMIOLOGIC EVIDENCE OF CO-POLLUTANT INFLUENCE ON PARTICULATE-INDUCED TOXICITY.

C. A. Pope. *Brigham Young University, Provo, UT.* Sponsor: M. C. Madden.

Epidemiologic evidence suggests that airborne particulate matter (PM), especially primary and secondary combustion-related PM, is a risk factor for cardiopulmonary disease and mortality. Most epidemiological studies have focused on effects of acute exposure. Chronic exposure, however, may be more important in terms of overall public health relevance. Chronic exposure likely increases the risk of cardiopulmonary disease and mortality. Acute exposure can exacerbate existing cardiopulmonary disease and increase the number of persons in a population who become symptomatic, require medical attention, or die. There is also evidence of health effects due to exposure to other air pollutants-including O_3, CO, SO_2, and NO_2. Basic approaches to evaluating the contributions of co-pollutants to PM-induced toxicity include: 1) estimate regression models with multiple pollutants as covariates and use statistical criteria such as significance levels or coefficient size and stability to evaluate the relative impact; 2) evaluate estimated pollution effects in areas with different levels and combinations of co-pollutants. These epidemiologic approaches reveal evidence of combustion-source PM toxicity that is independent of co-exposure to SO_2, NO_1, or O_3. It remains unclear if the relative toxicity of combustion-related PM is due to the relative small size of the particles, their chemical composition, or their associations with other combustion related pollutants. Epidemiologic studies are limited due to the use of people living in uncontrolled environments with complex mixtures of air pollution. An improved understanding of the influence of co-pollutants on PM-toxicity requires continued contributions from toxicological studies.

1146 EFFECT OF CO-POLLUTANTS (SO_2, NO_2, AND NH_3) ON THE ACUTE PULMONARY TOXICITY OF PARTICLES AND PARTICULATE MATTER (PM)—ASSOCIATED METALS.

K. L. Dreher. *USEPA, Research Triangle Park, NC.* Sponsor: D. L. Costa.

Exposure to ambient air PM occurs in the presence of a complex and dynamic mixture of gaseous air pollutants. The ability of co-pollutants to influence the adverse health effects associated with ambient air PM exposure represents a critical risk assessment issue. Toxicology studies have employed surrogate aerosols and mixtures to examined the extent to which co-pollutants (SO_2, NO_2, and NH_3) may influence the ability of particles to alter lung physiology, immune function and injury or modulate the toxicities of causal PM components. Exposure of animals to aerosol mixtures containing SO_2 and soluble metal salts or insoluble metal oxides was found to enhance the ability of SO_2 to induce alterations in pulmonary function through the formation of H_2SO_4. Additional studies involving the exposure of animals to carbon black (CB) particles in the presence of sulfuric acid or SO_2 with high relative humidity were found to produce immunotoxic and cellular cytotoxic effects that were not induced by individual exposures to these substances. Similar studies have detected increased cell proliferation in epithelial cells at airway bifurcations in rats exposed to a mixture of NH_4NO_3 and CB particles. Surrogate mixture studies have examined the potential interactions between PM-associated metals (Fe and Zn) and other PM co-constituents (H_2SO_4, NO_3-, and NH_4+) resulting from the transformation of gaseous air pollutants (SO_2, NO_2 and NH_3). Dilute sulfuric acid was found to dramatically increase ZnO bioavailability and pulmonary toxicity. Nitrate and ammonium were found to enhance Fe and attenuate Zn pulmonary toxicities. Metal bioavailability could account for some but not all of the observed effects of NO_3-, and NH_4+ on either Fe or Zn pulmonary toxicities. These results demonstrate the ability of SO_2, NO_2 and NH_3 co-pollutants to influence PM toxicity through: (i) the surface formation and/or deposition and enhanced pulmonary delivery of toxic substances such as acids; and (ii) the surface formation and/or deposition of acids, NO_3-, and/or NH_4+ which can modify the toxicity of other PM constituents such as metals by enhancing their bioavailability or biopotency, and/or altering their cellular specificity.

1147 EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE (ETS) ENHANCES THE SENSITIVITY OF THE LUNGS TO OZONE-INDUCED INJURY.

K. E. Pinkerton, M. Yu and H. P. Witschi. *University of California, Davis, CA.*

To examine the effects of ETS on the sensitivity of the lungs to ozone, A/J and B6C3F1 mice were exposed to filtered air, ETS, ozone or ETS followed by ozone. ETS exposure was for 6 hr/day for 3 days at a total particulate concentration of 30 mg/m³; ozone exposure was for 24 hrs at 0.5 ppm. Proliferating cells were identified by bromodeoxyuridine (BrdU) immunolabeling. The percent of BrdU labeled cells within the centriacinar regions of the lungs was found to be significantly elevated in both strains of mice following exposure to ozone (3-fold above control) and further augmented in mice exposed to ETS and ozone (4-fold above control). In contrast, exposure to ETS alone did not change BrdU labeling compared with filtered air control mice. Cytokine release *in vitro* from alveolar macrophages obtained from the lungs by bronchoalveolar lavage was also examined. In both strains of mice, there was a significant decrease in IL-6 production with or without

lipopolysaccharide (LPS) stimulation following exposure to ozone alone or to ETS plus ozone. There was also a significant elevation in LPS-stimulated TNF-α release following exposure to ETS plus ozone. We conclude that (1) ETS exposure enhances the sensitivity of mice to ozone-induced lung injury and (2) the increase in susceptibility to ozone injury observed may be associated with a reduction in the levels of IL-6 and concomitant enhancement in LPS-stimulated release of TNF-α from alveolar macrophages. (Supported by TRDRP and NIH ES00628.)

1148 THE EFFECTS OF OXIDANTS ON MODIFYING THE TOXICITY OF AMBIENT PM.

A. J. Ghio, J. H. Richards, L. A. Dailey, G. E. Hatch and M. C. Madden. *USEPA, Research Triangle Park, NC.*

Exposure to ambient air particulate matter (PM) is associated with increased human morbidity and mortality due to cardiopulmonary toxicity. It has been proposed that PM presents an oxidative stress to the lower respiratory tract. Interactions between PM and oxidants such as ozone (O_3) are possible as a result of a chemical reaction and/or shared mechanism of biological effect. We used diesel exhaust particles (DEP) to examine for an interaction between PM and O_3 in inducing lung toxicity. The effects of ozonation on DEP-induced lung toxicity were examined. Rats were intratracheally instilled with saline vehicle, DEP, or DEP previously exposed *in vitro* to O_3. At 24 hr post instillation, lungs were lavaged, and total protein (TP) levels, LDH activity, and cell differential counts determined. With up to 500 μg instilled, both particle types induced significant dose-dependent increases in TP, LDH, and neutrophils (compared to vehicle). However, OzDEP was more potent at 10 μg than DEP in the induction of neutrophilia and an LDH increase. The increased toxicity of the OzDEP was not due to oxidation by air, as air-exposed DEP had the same activity as unexposed DEP. These data suggest that low, ambient concentrations of O_3 can react with DEP to produce an altered, stable particle that is more toxic in a rat model than the unexposed DEP. O_3 and at least some PM may therefore chemically react, and play a role in PM-induced morbidity and mortality.

1149 INTERACTION OF ENDOTOXIN, OZONE, AND ULTRAFINE PARTICLES.

G. Oberdörster, A. Elder, J. N. Finkelstein and C. Cox. *University of Rochester, Rochester, NY.*

Exposure to endotoxin (LPS) can occur by multiple routes including inhalation of ambient particles contaminated with LPS, respiratory tract infection with gram negative bacteria, and from gastrointestinal sources via increased mucosal permeability. LPS induces the LPS exposures the response of the organism to other subsequent stimuli could be significantly altered, resulting in a greater susceptibility towards experiencing adverse effects. Thus, depending on the sequence and mode of exposure. LPS can be viewed both as a co-pollutant and as a priming agent of target cells. Epidemiological observations of an association of increased morbidity with low urban particulate levels still require proof of causality. There is some controversy as to whether particles alone at low environmental concentrations can cause significant adverse effects. Several hypotheses have been proposed and are currently being tested to examine causality including the suggestion that the presence of host susceptibility factors such as age in addition to co-pollutants is essential for inducing health effects related to low particulate levels. Our hypothesis that particle size also plays a crucial role in that ultrafine particles have a greater toxic potential than larger particles has been supported by results from several laboratories in the past. Subsequent studies in rats and mice with inhaled low-level carbonaceous ultrafine particles in combination with ozone and priming of the respiratory tract with LPS showed that ultrafine particle-induced pulmonary inflammation can be amplified by prior LPS and by simultaneous ozone exposure. Age also plays an important role since an oxidative stress response appears to be more likely in the aged organism.

1150 ARE DIETARY SUPPLEMENTS SAFE?

R. E. Osterberg. *USFDA, Rockville, MD.*

Most dietary supplements are regulated under the Dietary Supplement Health and Education Act (DSHEA) and are not subjected to the same standards and labeling requirements as medicines and foods. With over half of the U.S. public now consuming dietary supplements, (Americans spent almost $4 billion in 1998 on herbal remedies), more are turning to "natural" products than ever before for many reasons. They want something less toxic than prescription drugs and/or they want to prevent sickness and/or improve their quality of life. The demand for dietary supplements necessitates that the industry makes efforts to ensure that its products are safe and that its claims are substantiated. Manufacturers must also become more vigilant because international groups (i.e., Codex Alimentarius Commission) and federal and state regulators are becoming more concerned about product safety and standards. Although most dietary supplements have extensive marketing histories, the published literature contains relatively few documented safety studies in humans, including information on supplement-drug or supplement-food interactions. This roundtable will discuss the concerns that regulators have with dietary supplements and the steps that the dietary industry is taking to address those concerns within the context of DSHEA. Also discussed will be the information that is known and assumed about the safety/toxicities of selected dietary supplements.

1151 DIETARY SUPPLEMENT SAFETY—IMPROPER USE OF THE ADVERSE EFFECTS REPORTING SYSTEM.

T. M. Farber. *ToxaChemica, International, Rockville, MD.*

A proposed rule has been issued by the FDA, which would have a detrimental effect on the marketing and use of ephedra-containing dietary supplements. The proposed rule was issued primarily on the basis of a large number of Adverse Event Reports (AERs) gathered by the FDA.The FDA and the Texas Department of Health, through intensive multimedia warnings and manipulation, have turned a passive alerting system into an active data-gathering process through the active recruitment of cases and the exaggeration of the possible hazards of ephedra products. The AER database is flawed to a significant degree; lacking critical information in almost 90% of the cases. In spite of the fact that a causality analysis could not be performed on this poor database, the FDA has made invalid epidemiological projections based on this information. The AER system for dietary supplements needs to be significantly improved so that causality can be demonstrated before actions are taken against dietary supplements. The proposed rule on ephedra-containing dietary supplements should be withdrawn.

1152 DIETARY SUPPLEMENTS—HUMAN HEALTH RISK STANDARDS AND METHODS OF ASSESSMENT.

M. Bolger. *USFDA, Washington, DC.*

The statutory mandate for assessing the safety/risk of dietary supplements is contained in the federal Dietary Supplement Health and Education Act of 1994. A dietary supplement is intended for ingestion and is a vitamin, mineral, herb or botanical or amino acid. It is used to supplement the diet by increasing the intake of a concentrate, metabolite, constituent, extract or combination of any of the aforementioned ingredients. If a dietary supplement (A) presents a significant or unreasonable health risk under conditions of use or (B) is a new dietary ingredient for which there is inadequate information to provide reasonable assurance of safety, it is deemed to pose an imminent hazard to public health or safety. The burden of proof to satisfy these safety/risk standards reside with the FDA. Current approaches for quantifying the hazards/risks of dietary supplements will be described through several case studies. The types of information/issues considered in the safety/risk assessment of dietary supplements include product identification (e.g. botanicals plant identification), history of use, dietary supplement mixtures and interactive effects (additive, synergistic, antagonistic), patterns of animal and human intoxications (e.g., differences in routes and frequency of exposure, retrospective, case-control, prospective studies), use of adverse reporting information (e.g., passive vs active reporting systems), uncertainty in extrapolation (route to route, species to species, duration and frequency of exposure), uncertainty in dose-response information (e.g. laboratory animal vs. veterinary and human case reports), severity of response and adverse effect, and risk characterization-safety assessment versus quantitative risk assessment.

1153 BOTANICAL DIETARY SUPPLEMENTS—ISSUES OF SAFETY.

J. Cardellina. *Council for Responsible Nutrition, Washington, DC.* Sponsor: R. E. Osterberg.

Questions about the safety of botanical dietary supplements have garnered increasing attention in the past two years. While most herbs with a well-documented historical use are generally regarded as safe, based on sustained human consumption over a long period of time, little is known about the interaction of today's dietary supplements with other herbs or with prescription and over the counter medications.At a minimum, a functional, effective adverse effect reporting system is required. It is insufficient merely to collect and collate reports of potential adverse effects; it is necessary that follow up investigation and evaluation of such reports take place, so that causality can be established or negated. This would be a critical step in developing meaningful data about the safety of such products.

1154 DIETARY SUPPLEMENT SAFETY.

L. C. Totman. *Consumer Healthcare Products Association, Washington, DC.*

The U.S. market for dietary supplements has greatly expanded in recent years. Dietary supplements do, however, have an extensive history of use. They are marketed with reasonable expectation that no harm will occur, as are other regulated food products. The largest segment of the dietary supplement market comprises vitamins, minerals, and a few botanicals. Concerns about safety mostly involve products outside that core of the market. FDA has authority to take action against any dietary supplement product that is found to be unsafe or that makes unsubstantiated claims or unapproved drug claims. FDA also has legal authority to move against any dietary supplement that presents a significant or unreasonable risk of injury or illness. The agency can prevent a product from being marketed if insufficient information is submitted to show the basis for determining it is "reasonably expected to be safe". Adverse event reports (AERs) on marketed products can signal safety problems that warrant further investigation and perhaps action to assure public safety. FDA's AER system for dietary supplements should be strengthened. Good manufacturing practices (GMPs) are critically important for dietary supplement product safety. FDA needs to have strong, practical GMP regulations and enforcement. Dietary supplement safety depends on responsible manufacturers and FDA's use of its enforcement powers.

1155 EFFECTS OF CO-EXPOSURE OF TIMOTHY GRASS POLLEN AND DIESEL EXHAUST PARTICLES ON SPECIFIC POLLEN ANTIBODY PRODUCTION (IGE AND IGG1) AFTER INTRANASAL AND INTRATRACHEAL APPLICATION IN BROWN NORWAY RATS.

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During the last decades, the prevalence of allergy has increased world-wide. To investigate the adjuvant effect of DEP in pollen allergy, Brown Norway (BN) rats were sensitized intranasally or intratracheally with timothy grass pollen (Phleum pratense) with or without DEP (3 mg/ml). Intranasal sensitization (200 µl, 10 mg/ml) was performed daily for 5 consecutive days and intratracheal sensitization (200 µl, 10 mg/ml) was performed once. Challenge with pollen was performed at day 21 similarly to the sensitization protocol. Blood samples were taken at day 28 after the first sensitization. The binding of DEP to pollen grains was studied by scanning electron microscopy and the inflammatory response in the lung was studied by light microscopy. IgE and IgG1 responses against pollen grains were measured by digoxigenin (DIG)-ELISA. Scanning electron microscopy revealed a mixture of free DEP and DEP associated with pollen grains. Both intranasal and intratracheal routes of administration of pollen grains induced inflammatory reactions in the lung with an influx of macrophages, eosinophilic granulocytes and granuloma formation. Pollen grains were localized in the alveoli both after intranasal and intratracheal administration and were surrounded by macrophages. The number and localization of pollen grains were similar for both routes of administration. After co-exposure with DEP, DEP loaded macrophages were found around the pollen. Localization, inflammatory reaction and integrity of pollen were similar as seen without DEP. At day 28 specific IgE and IgG1 antibodies were found in serum of rats immunized intranasally or intratracheally. IgE antibody response was higher in rats immunized with pollen grains and DEP than in rats immunized with pollen only. The IgG1 antibody response was much higher compared to the IgE response, but the level of IgG1 antibodies was slightly affected by DEP.

1156 THE ADJUVANT ACTIVITY OF PARTICULATE POLLUTANTS IN AN INTRANASAL MODEL.

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The prevalence of respiratory allergy is increasing. Particulate pollutants like diesel exhaust particles (DEP) may play a role by acting as adjuvants, stimulating Thelper 2 (Th2) immune responses to common allergens. We studied the influence of particulate pollutants *on the sensibilisation process* in an intranasal model. The defined reporter antigen TNP-OVA (2,4,6 trinitrophenylphosphate-ovalbumin) (10µg) was given intranasally on 3 consecutive days together with DEP, carbon black particles (CBP) or amorphous silica particles (SIP) (total dose: 200µg) to female BALB/C mice (6-8 weeks). At day 10 an intranasal challenge with TNP-OVA alone was given and at day 15 ELISPOT (on draining lymph nodes of the lung (PBLN), spleen and bone marrow) and ELISA (on serum) were performed. Data on IL-4 and IFNγ were obtained by flowcytometry on PBLN cells and ELISA on supernatant of overnight culture. After CBP exposure an increase in number of T cells and B cells, the latter producing increased levels of IgG1, was observed in the PBLN. The ratio of IL-4+ over IFNγ+ T cells increased, as well as overnight IL-4 production. In serum elevated levels of IgG1 and IgE were observed, IgG2a levels did not change. DEP exposure caused elevation of IgG1 levels, while IgE levels and IL-4 production showed a trend to be increased. SIP administration induced no significant changes. No effects were observed in spleen and bone marrow. CBP adjuvate the immune response most clearly, skewing the response in Th2 direction. The adjuvating activity of DEP is less strong but also shows some Th2 characteristics. SIP do not adjuvate in this model. Findings are in accordance with epidemiological studies showing that DEP and fine particles in general are involved in respiratory allergy.

1157 BETA-1,3-GLUCAN ADJUVATES THE ALLERGIC RESPONSE TO OVALBUMIN IN MICE.

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The polyglucose beta-1,3-D-glucan is a major structural component of the cell wall of yeasts and fungi. There is an association between exposure of the airways to β-1,3-glucan and increased prevalence of atopy and decreased expiratory capacity. We have investigated the effect of the soluble beta-1,3-glucan SSG from Sclerotinia sclerotiorum on the allergic immune response to the model allergen ovalbumin (OA) by using the popliteal lymph node assay (PLNA)in mice. In PLNA allergens and adjuvants are injected repeatedly into one hind footpad. End point measurements are PLN weight and cell number and proliferation, and serum levels of IgE, IgG1 and IgG2a anti-OA. The mice were given SSG+OA, SSG or OA alone and either sacrificed on D20, or reinjected with OA on D21 before sacrifice on D26 or D33. SSG+OA increased PLN weight and cell number ($p \leq 0.023$) compared with OA or SSG alone on D26. On D26 and D33, SSG+OA strongly increased IgE and IgG1, but not IgG2a, anti-OA IgE levels ($p<0.001$)compared with OA or SSG alone on. Our results show that β-1,3-glucan has an adjuvant activity on the allergic immune response in a mouse model. This supports the reported observations of inhaled β-1,3-glucan as an enhancer and inducer of allergic disease in humans.

1158 SYSTEMIC ADMINISTRATION OF *BORDETELLA PERTUSSIS* ENHANCES PULMONARY SENSITIZATION TO DUST MITE IN JUVENILE RATS.

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Studies have shown that the incidence of allergy and asthma has increased significantly, especially in children, in the past few decades. Inactivated *B. pertussis*, which has been routinely given to children as a vaccine component, has also been used as an adjuvant for enhancing IgE production in experimental models of allergy. The objective of this study was to establish an aller-

gy model in young rats and address the hypothesis that systemic administration of *B. pertussis* could augment allergic immune responses after intra-pulmonary house dust mite (HDM) sensitization. Three-week old female Brown Norway rats were sensitized with 10 µg HDM intratracheally or intraperitoneally with or without intraperitoneal injection with 10^8 whole killed *B. pertussis* organisms. Ten days later, the rats were challenged with 5 µg HDM intratracheally. Bronchial lymph nodes and bronchoalveolar lavage fluid (BAL) were collected 0, 2, and 4 days post challenge. For both systemically and locally sensitized rats, co-administration of pertussis enhanced HDM-specific lymphoproliferative responses in cells from the pulmonary lymph nodes and eosinophil, total protein, lactate dehydrogenase, and HDM-specific IgE and IgG levels in BAL. These responses in the locally sensitized rats given pertussis were comparable to those in systemically sensitized rats given pertussis. Regardless of adjuvant, local lymphoproliferative responses and eosinophilia were greater in locally sensitized rats, whereas BAL IgE and IgG were greater in systemically sensitized rats. Unlike studies of adult rats, we were unable to demonstrate immediate airway hyperreactivity to HDM challenge in any of the treatment groups. The data show that intratracheal instillation of HDM induces a mild allergic sensitization in juvenile rats and that co-administration of *B. pertussis* enhances this sensitization process to levels seen in animals immunized with antigen and *B pertussis*. These results suggest that simultaneous exposure to Th2 type vaccines and common antigens may be a risk factor for allergic sensitization and asthma. (This abstract does not necessarily reflect EPA policy.)

1159 RESPONSES OF BALB/C MICE TO RESPIRATORY SENSITIZATION WITH THE BIOPESTICIDE, *METARHIZIUM ANISOPLIAE*.

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Metarhizium anisopliae, an entomopathogenic fungus licensed for indoor use, has been shown to elicit allergic responses in BALB/C mice intraperitoneally (IP) sensitized and intratracheally (IT) challenged with a *M. anisopliae* crude antigen (MACA) preparation. In this study the IT route of sensitization is compared to IP sensitization and to an IT vehicle control. The IT route consisted of 4 IT exposures of 10 µg MACA in 50 µl HBSS over a four-week period (vehicle controls received HBSS only). While the IP route consisted of IP sensitization with 25 µg MACA in 0.2 ml of 1.3% alhydrogel followed 14 days later with an IT challenge (10 µg MACA/50 µl HBSS). Mouse airway reactivity was assessed by methacholine challenge and serum and bronchoalveolar lavage fluid (BALF) samples were collected and the lungs fixed in 10% formalin at 1, 3, and 8 days following the MACA IT challenge (DPIT). Both total BALF cellularity and differential cell counts were significantly greater in IT sensitized mice compared to both IP and vehicle controls at 1 DPIT with total cellularity and eosinophilia still significant at 3 DPIT. Both IT and IP mice had significantly elevated levels of IL-5 (1 and 3 DPIT) but only IP mice had significant IL-4 (1 DPIT). Airway reactivity was significantly increased for both IP and IT mice (1 and 8 DPIT). However, IT mice also demonstrated significant airway reactivity at day 3 compared to both IP and vehicle controls. Analysis of lung pathology showed that the types of lesions in both IP and IT mice were similar in nature but more severe in IT mice. Additionally, 4 protein bands have been identified by serum IgE on western blots. These proteins compromise ~12.3% of the MACA proteins. In summary, sensitization to MACA was achieved with the IT protocol. Local lung responses including total IgE, inflammation and airway hyperreactivity were greater in the IT sensitized mice whereas the systemic total IgE response was greatest in the IP sensitized mice. (This abstract does not reflect EPA policy.)

1160 NATURAL RUBBER LATEX ALLERGY: A CRITICAL REVIEW.

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Several researchers have suggested that occupational contact with natural rubber latex (NRL) gloves caused an epidemic of type I NRL allergic responses, especially among health care workers. However, the risk factors, temporal trends, and natural history of the illness have not been well described. We critically reviewed the published epidemiologic literature on NRL allergy, summarizing the weight of evidence supporting latex gloves as a cause of NRL allergy. We excluded over 200 case reports of individual subjects (mostly health care workers) as they contained little epidemiologic information that related disease to exposure. The remaining studies (approximately 20) were assessed using standard epidemiologic criteria for study quality, bias, validity and causation. None met all of these criteria. Limitations included: lack of control groups, lack of blinded outcome assessment, the potential for selection bias, comparison of risks based upon prevalence rather than incidence differences, lack of control for duration of exposure and atopy (the genetic tendency for allergic reactions), and lack of a standardized method for diagnosis. Four studies (Gryzbowski, 1996; Ownby, 1996; NHANES III; NIOSH, 1999) showed the least potential for the biases mentioned above. Most notably, the study by NIOSH (NIOSH, 1999) satisfied most of the criteria for a well conducted epidemiology study, comparing current health-care workers with a matched control population. Overall, these studies indicated that the prevalence of latex sensitization in health care workers is not significantly higher than in the general population that has far less contact with latex gloves. In summary, the weight of evidence is inconsistent with the occurrence of an epidemic in health care workers. Further studies that improve upon the quality of previous work should be performed.

1161 ORAL SENSITIZATION TO PEANUT PROTEINS; A BROWN NORWAY RAT FOOD ALLERGY MODEL.

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We developed an oral sensitization protocol for food proteins for the rat. To further validate this model rats were exposed to various concentrations of peanut protein-extracts and immune-mediated effects after an oral and respiratory challenge were investigated.Young Brown Norway (BN) rats (n=8) were exposed to different doses of a crude peanut extract (CPE; 0.1, 1, 10 or 20 mg protein/ml/rat/day) or roasted (RPE; 1 mg protein/ml/rat/day) peanut-protein extract by daily gavage dosing during 42 days without the use of an adjuvant. Blood samples were obtained from the orbital plexus at weekly intervals. Peanut-specific IgE and IgG responses were determined by PCA and ELISA, respectively. After a resting period the animals received first an oral (100 mg protein) and one week later a respiratory (0.6% or 1% protein) challenge with peanut proteins and effects on the breathing frequency were studied. Preliminary results show that animals orally exposed to the different doses of both peanut-protein extracts developed specific antibodies. Almost all animals developed specific IgG antibodies against peanut-proteins except some animals exposed to RPE. The responses observed in the animals dosed with 1 mg of raw-peanut-protein extract were higher compared to the animals exposed to 1 mg of roasted peanut extract. Although not all PCA measurements have been performed yet, preliminary results show a more variable specific IgE response between the different treatments. Both oral and respiratory challenges with peanut proteins resulted in a temporary decrease in breathing frequency in a minority of animals. We show that Brown Norway rats develop specific IgG and IgE responses upon daily intra gastric dosing of peanut proteins without the use of an adjuvant. In this model, CPE seem to induce better antibody responses compared to RPE. Moreover, systemic immune-mediated effects after an oral and respiratory challenge are observed in a few animals. Additional analyses will be performed to compare the induced immune responses observed in this study with studies performed previously at our laboratory with other food allergens (hen's egg white and cow's milk) to obtain information on the relative allergenicity of these products to support that the BN rat may provide a suitable animal model to study the allergenicity of (novel) food proteins.

1162 DIVERGENT ANTIBODY RESPONSES INDUCED IN MICE BY FOOD PROTEINS.

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We have shown previously that systemic (intraperitoneal; ip) exposure of BALB/c strain mice to the protein allergen ovalbumin stimulates the production of both specific IgG and IgE. Treatment under identical conditions with bovine serum albumin, a food protein considered to have a relatively limited allergenic potential, elicits vigorous IgG but no, or only low titer, IgE antibody. We have now examined antibody responses provoked in mice following exposure to other food proteins: acid phosphatase derived from the potato, a common food protein which is suspected of having only very low allergenic potential, and the non-mitogenic agglutinin peanut lectin, an important allergenic constituent of peanut extracts. Mice received ip or intradermal (id) injections of protein in phosphate buffered saline on days 0 and 7. Fourteen days after the initiation of exposure, serum samples were prepared and analyzed for IgG antibody by enzyme-linked immunosorbent assay and for IgE content by homologous passive cutaneous anaphylaxis assay. Administration of potato acid phosphatase by id or ip routes stimulated IgG antibody pro-

duction but failed, even at doses of 10%, to elicit detectable IgE. In contrast, treatment with as little as 0.2% peanut lectin via either route of exposure provoked vigorous IgG and IgE antibody responses. The ability of peanut lectin to induce IgE antibody was not a general property of lectins, as administration of equivalent amounts of the agglutinin derived from potato, a non-mitogenic lectin with comparable molecular weight to peanut lectin, failed to stimulate detectable IgE. Taken together these data suggest that these proteins display in mice a divergent ability to stimulate IgE antibody following id or ip exposure to native protein which is consistent with their varying allergenic potential in man.

1163 THE ROLE OF THE LIVER IN THE MECHANISM OF ORAL TOLERANCE.

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It is currently believed that most drug-induced allergic reactions (DIARS) are caused by immunogenic conjugates formed from the reaction of a reactive metabolite of a drug with cellular proteins. However, it is not clear why more people do not develop immune reactions against protein adducts of drugs. One possible mechanism that may explain the low incidence of DIARS in many cases is oral tolerance, an antigen-specific immunological hyporesponsiveness induced by the oral administration of antigens. Although recent evidence suggests that the liver may have a role in the induction of oral tolerance against protein antigens, relatively little work has been done to explore the role of the liver in tolerance caused by the oral administration of drugs and other sensitizing chemicals. As an initial step to address this problem we fed the skin-sensitizing agent 2,4-dinitrochlorobenzene (DNCB) (250 mg/kg) to mice prior to sensitizing and challenging them by painting DNCB on the skin of the abdomen and ears, respectively. In another set of animals, DNCB was fed to mice and protein adducts were measured in the liver immunohistochemically and by immunoblotting with the use of polyclonal antibodies raised against the dinitrophenyl (DNP) hapten. It was found that the feeding of DNCB to mice inhibited the ear swelling effects of DNCB and led to the formation of protein adducts in hepatocytes, Kupffer cells and endothelial cells of the liver. Since these cells have been suggested to have a role in the induction of oral tolerance against protein antigens, they may also inhibit immune reactions against protein adducts of xenobiotics that are either transported to liver from other sites or are formed in the liver.

1164 LACK OF ALLERGENICITY OF RECOMBINANT LACTOFERRIN: ROLE OF GLYCOSYLATION.

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It has been shown previously that recombinant allergenic proteins such as the profilins are less allergenic than native proteins with respect to IgE binding and that such may be as a result of differential glycosylation of the recombinant and native materials. In the current experiments we have compared the ability of human native and recombinant forms of the iron-binding protein lactoferrin (LF) to induce IgE responses following systemic (intraperitoneal; ip) exposure of BALB/c strain mice. Mice received 250 µl of 1% w/v of protein by ip injection on days 0 and 7 and were exsanguinated 14 days after the initiation of exposure. Pooled sera were analyzed for IgE content by homologous passive cutaneous anaphylaxis assay and IgG antibody was measured by enzyme-linked immunosorbent assay. Administration to mice of native LF provoked vigorous anti-LF IgG and IgE antibody responses. Identical exposure to the recombinant material failed to elicit detectable IgE antibody and resulted in somewhat less vigorous IgG antibody responses. The failure of recombinant LF to induce IgE was not due to a loss of allergenic epitopes, since IgE antibody raised against native LF recognized equally both forms of LF. The ability of native, but not recombinant, LF to provoke IgE production was independent of the degree of iron saturation, with apo and iron-saturated forms of native LF stimulating high titer IgE responses. It was demonstrated that native and recombinant LF have identical amino acid sequences and 3D X ray crystallographic structure. Thus, the divergent IgE responses stimulated by the two forms of LF are likely to be a function of their differential glycosylation status.

1165 THE INVOLVEMENT OF EPIDERMAL GAMMA DELTA T CELLS IN THE INITIATION PHASE OF A CONTACT HYPERSENSITIVITY.

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Components of the natural immune system are crucial in immunosensitization to allergic and autoimmunogenic chemicals, because they deliver both adjuvant as well as immunodeviating signals. In contact hypersensitivity, epidermal gamma delta T cells may play a role in this respect. Previously, we have shown that epidermal application of the contact allergen 2,4-dinitro-1-fluorobenzene (DNFB) induces migration of murine gamma delta T cells from the epidermis of the ear to the local auricular lymph node (ALN). In this study, the kinetics of the migration, cytokine production and localization in the lymph node of gamma delta T cells were characterized in C57Bl6 mice that were ear-painted with 1% (w/v) DNFB in a mixture of acetone and olive oil (AOO, 4:1). In a separate study, the BrdU staining method was used to detect proliferating cells in the epidermis. Earsheets and lymph node cryosections were prepared and stained for gamma delta T (mAb GL3). Lymph node cells were used for cytokine detection in gamma delta T cells by flowcytometry. Results show that gd T cells: -home to the medullary region of the ALN; -localize in the vicinity of antibody forming B cells; -produce IFNgamma and hardly if any IL4; -reappear in the epidermis from day 10 to form a normal staining pattern again by day 20. The reappearance is not accompanied by BrdU+ gamma delta T cells in the epidermis. Combined with findings of others that epidermal gamma delta T cells are formed only during prenatal phase of murine life we suggest that reappearing gamma delta T cells remigrate back from the lymph node. Results together indicate that gamma delta T cells, by producing the type-1 cytokine IFNgamma, may be involved in skewing of the contact hypersensitivity response towards a type 1-dependent DTH-reaction. In concordance, C57Bl6 are Th1-responding mice and Th2-responding BALB/c mice contain very few gamma delta T cells in the epidermis.

1166 CHEMICAL STRUCTURE ACTIVITY RELATIONSHIPS IN SKIN SENSITIZATION.

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Knowledge of the underlying mechanisms of skin sensitization has enabled the development of (quantitative) structure activity relationships {(Q)SARS} for skin sensitizing chemicals. Typically these are based on physicochemical parameters representing the ability of the chemical to partition into the skin and its ability to react with skin proteins. In our most recent studies, fragrance chemicals containing aromatic and aldehydic groups and a series of diketones have been evaluated using a range of SAR techniques. Assessment of the "aromatic aldehydes" demonstrated that sensitizing ability was not related to the dipole across the aldehydic carbonyl group. This indicated that Schiff's base formation was less important than other reaction mechanisms, such as Michael addition. With the family of diketones, initial development of a QSAR suggested that an electrophilicity parameter based on Taft substituent constants derived for hard electrophiles having a reactive carbonyl group could be used as a measure of their chemical reactivity. Quantitative estimates of the skin sensitisation potential of these reactive hard electrophilic carbonyl compounds in the local lymph node assay (LLNA) showed a good correlation with their relative alkylation index (RAI) - an expression incorporating terms related to chemical reactivity, skin permeation and dose. Overall the findings reaffirm our view that physical organic chemistry is the key to understanding why some chemicals sensitise more strongly than others, while some do not sensitise at all, and provide further evidence of the value of the LLNA for SAR studies.

1167 COMPARATIVE SAR MODELING OF THE MOUSE LYMPHOMA ASSAY: THE NTP AND GENE-TOX DATABASES.

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The *in vitro* mouse lymphoma assay (MLA) measures somatic mutation at an autosomal diploid locus heterozygous for a drug resistance marker. This assay has been evaluated as a possible short-term assay for identification of carcinogens. We have constructed structure-activity relationship (SAR) models

of MLA data from two sources, the U.S. National Toxicity Program (NTP), and the Gene-Tox working group (GT), using the CASE/MultiCASE expert system. The two models differed significantly in predictivity, complexity, inferred mechanisms and in concordance with carcinogenicity. Based on an evaluation of the different criteria used to 'accept' data into the two data sets, it was expected that the NTP assay was measuring primarily gene-specific mutation, which would result in a fairly simple model with high concordance with other mutational assays. The GT data, on the other hand, were expected to be measuring somatic segregation, or 'loss of heterozygosity', which is known to occur by a variety of mechanisms. We therefore expected this model to be more complex, and perhaps less predictive, owing to potential antagonism between chemicals acting by distinct molecular mechanisms. Instead, we found the GT MLA model to be the simpler and more predictive of the two, and to have a very high concordance with carcinogenicity. These data suggest that loss of heterozygosity is the most important event in the carcinogenic process, and that, despite the wide variety of mechanisms that have been shown to be possible for this step in carcinogenesis, in reality only one or a very few commonly occur.

1168 THE ROLE OF OUTDOOR DUST IN EXPOSURES TO CHEMICALS IN SOIL: CASE STUDIES FOR ARSENIC.

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The development of risk-based remediation goals for contaminated soils is generally based on the assumption that human exposures are driven by direct contact with soil in the upper 3 to 6 inches. In the case of lead, exposures to the chemical in indoor dust are also considered. Less attention has been focused on the role of outdoor dust in such exposures. At most contaminated sites chemical concentrations in dust will reflect the concentrations in the underlying soil, and the relative contributions of outdoor dust and soil will be difficult to discern. However, at sites with active deposition of chemicals to soil from air, a marked concentration gradient may arise. Studies of arsenic exposures in children from two communities affected by copper smelters were used to examine the relative contribution of outdoor dust to the exposures. The arsenic concentrations in soil in Anaconda, MT and Ruston, WA were similar at the time of the studies; however, outdoor dust concentrations were approximately 10 times higher in Ruston due to the recent operation of the smelter. Urinary arsenic concentrations in Ruston children were also much higher. Prediction of urinary arsenic concentrations based on soil ingestion rates, and fractional arsenic absorption and urinary excretion indicated that only the outdoor dust could account for the observed urine concentrations in the Ruston children. This finding has significant implications for the design of studies of soil ingestion, and for remediation of contaminated soils.

1169 NONMONOTONIC DOSE RESPONSE TUMOR INCIDENCES USING STANDARD MODELS RESULTING FROM THE COMPENSATION OF INDUCED INCREASED MUTATION RATES BY CHANGES IN PARAMETERS INDUCED BY BODY WEIGHT DECREASES.

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Nonmonotonic dose-response curves, especially U- or J-shaped curves, are common results in tumor bioassays. There are a number of mechanisms that have been postulated to explain this, e.g., induced delays in proliferation rates resulting from DNA damage; induced DNA repair. However, the most common observation associated with these nonmonotonic curves is a loss in body weight (BW). The loss in BW results in a decrease in proliferation and an increase in apoptosis in organs that change their size with BW, such as liver. Using standard models for carcinogenesis, such as the Moolgavkar-Venzon-Knudson two-stage model, the impact of these changes on the tumor risk associated with agents that increase mutation rates (such as genotoxic agents) was evaluated. It was found that a 10% BW loss would compensate for at least a ten-fold increase in mutation rate, a 20% BW loss for at least a sixty-fold increase in mutation rate, and a forty percent BW loss for over a 500-fold increase in mutation rate. The competing effects on proliferation and mutation rates can result in U- or J-curves when mutagenic agents are administered, especially at the lower doses. Also, given the relative impact of the effects of BW and any mutagenic effect, these results also suggest that it is the effects of mutagens on proliferative parameters, not a direct effect on mutagenesis, that are most important in inducing tumors by these agents.

1170 ENHANCING THE RISK ASSESMENT PEER REVIEW PROCESS THROUGH STAKEHOLDER CONTRIBUTIONS.

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The EPA hazardous waste program has implemented a mechanism which enables stakeholders to highly controversial issues to contribute to the peer review process of associated risk assessment activities. All identified stakeholders are requested to submit nominations of highly qualified experts in the scientific disciplines identified in a notice published in the Federal Register. An independent peer review contractor selects from among the nominated scientists, making sure that none of the selected scientists has any real or perceived conflicts of interest. The peer review is in the form of a workshop open to the public at which stakeholders have the opportunity to make brief opening remarks and submit written comments. Depending on the complexity of the risk assessment issues to be addressed, the reviewers may then break into smaller discipline-specific subgroups. Following the meeting, the peer reviewers prepare a final report. To date, this mechanism has been used for four peer reviews of contentious risk assessment issues. Two of the peer reviews evaluated draft risk assessments of emissions from two facilites that burn hazardous waste, one evaluated the draft toxicity review document and proposed reference dose for a groundwater contaminant at a number of sites, and the fourth, which is still in process, will evaluate the toxicity testing strategy and associated protocols for a wastestream contaminant at one Superfund site. Stakeholder participation to date has been significant, both in terms of nominating experts and participating in the peer review workshops. Most stakeholders have supported the peer review process regardless of their positions on the risk assessment issues. This paper evaluates the lessons learned thus far and makes recommendations for future improvements. Such a model could be adopted by other governmental and private organizations for controversial risk assessment issues.

1171 CONSIDERATION OF ALTERNATE EXPOSURE PATHWAYS IN THE POSSIBLE RELATION TO PREVALENCE CHRONIC BERYLLIUM DISEASE.

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The association between airborne beryllium concentrations and prevalence of chronic beryllium disease (CBD) has not shown a clear dose-response relationship. It is recognized that individuals become sensitized to beryllium (Be) before developing CBD. Studies have primarily focused on the respiratory route, although prior skin patch tests have shown that individuals can be sensitized by Be exposure to the skin. Therefore, the purpose of this study is to evaluate whether evidence exists for a multiple Be exposure pathway model. Our proposed model integrates inhalation, dermal and oral Be exposure pathways associated with the worker task, clothes, hands, gloves, workplace surfaces and ambient air. The study design considered alternative exposure pathways that may contribute to Be sensitization by comparing potential routes of exposure in two different Be manufacturing facilities. In a qualitative evaluation, worker activity and personal protective equipment use in a Be manufacturing facility in Elmore, Ohio (which have reported cases of CBD) were compared to a Be processing facility in Cardiff, United Kingdom (which have not reported cases of CBD). It was found that the worker activities and protective control measures varied significantly between the two Be manufacturing facilities. Skin exposures were likely higher at the Elmore facility compared to the Cardiff facility since workers in the Elmore facility had frequent skin contact and clothing contamination with Be. These observations suggest that if an extrapulmonary route of exposure can cause sensitization, less than complete controls on skin contact and ingestion can be the reason why attempts to control Be respiratory exposure do not alone reduce the occurrence of sensitization and disease. Once sensitization is achieved, inflammation in the lung may occur with very small incremental lung burdens of Be. Other factors such as size distribution of airborne particles and Be form may also explain the differences in CBD prevalence between the two facilities, but air levels alone may not be an adequate measure of dose.

1172 THE PERSISTENT, BIOACCUMLATIVE TOXICS (PBT) PROJECT: RISK-BASED INTENTIONS—TOXICOLOGICAL LIMITATIONS.

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US EPA has established the Multimedia PBT Strategy with the intention of making persistent, bioaccumulative toxics (PBTs) a regulatory priority. In addition, several other programs in the U.S. and abroad are in the process of establishing criteria by which additional compounds can be added to a PBT list. These programs have generally recognized the need for risk-based goals, i.e., global actions to restrict or ban specific compounds should be based on an expected benefit to human health; yet, there is currently no unified plan for the consideration of toxicology in establishing PBT criteria. We assert that formulae are needed that can be used to derive a combined measure of persistence (P), bioaccumulation (B), and toxicity (T) in order to establish criteria for identifying new PBTs. These formulae should be based on a matrix of the three P, B, and T parameters wherein a range of criteria values are available for each parameter, varying in accordance with the values of the other two parameters. However, a key limitation in the consideration of toxicity information in the categorization scheme is the dearth of available data for assessing human health risks from very low-level, long-term exposures to environmental chemicals. Animal studies using high doses are clearly uninformative and epidemiological studies rarely have the power to detect effects from very low-level exposures. Therefore, while the consideration of human health risks is essential in developing PBT regulations, it is currently hindered by the current state of the science. We conclude that the existing epidemiological database should be used to examine how any human health effects from known PBTs could have been predicted by other toxicological data; and that such an evaluation should be considered a prerequisite to the use of toxicity data in a PBT categorization scheme.

1173 A PROBABILISTIC HUMAN HEALTH RISK ASSESSMENT FOR THE INTAKE OF POLYCHLORINATED BIPHENYLS (PCBS) IN ANGLERS OF THE LOWER FOX RIVER, WISCONSIN.

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Sediments and biota in the Lower Fox River (Wisconsin) have been shown to contain PCBs and, as a result, fish consumption advisories (FCAs) have been in place for the last several years. We recently conducted an assessment of the PCB-related health risks to recreational anglers of the Lower Fox River. Fish consumption rates were estimated from the recent Wisconsin Fishing and Outdoor Recreation Survey (WFORS) (TER 1999). A site-specific distribution of species-weighted, representative fish tissue PCB concentrations was derived based on the species that are caught and consumed by local anglers. Probabilistic risk estimates from this study suggest that fish consumption risks at the mean level of exposure are below applicable Wisconsin regulatory benchmarks— the incremental cancer risk was 1E-6, and the hazard index was 0.3. At the 95th percentile of exposure, the incremental cancer risk was 2E-5, and the hazard index was 3. The lack of a significant health risk might appear to conflict with the presence of the FCAs, particularly since a portion of our consumption rate distribution exceeds the FCA-recommended value. However, the FCA is based on assumptions regarding species-specific PCB concentrations and consumption rates that may not reflect the actual conditions measured in the WFORS survey and the biota analyses. Furthermore, the use of a probabilistic analysis minimizes the conservatism that is inherent in the point estimate assumptions used to establish the FCA. In summary, we conclude that the presence of an FCA does not necessarily imply that normal angler activities are associated with a health risk.

1174 IS SULFATE IN DRINKING WATER A HAZARD FOR INFANTS?

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Health advisories related to U.S. drinking water regulations for sulfate have focused on its laxative effects in infants and unacclimated travelers. Of greatest concern is whether this effect would cause serious dehydration in infants. Early, limited studies indicated that sulfate levels in water in excess of 500 ppm might cause diarrhea in infants. Recent data from ecological studies in areas of high sulfate in water, experiments with human volunteers, and animal studies indicate that higher levels may be tolerated without adverse effects. Studies of infants in areas of high sulfate in water have not shown an increased prevalence of diarrhea with increasing levels of sulfate in water; although, sample sizes at higher sulfate levels are small and mothers are reluctant to use water containing higher sulfate levels due to potentially unpleasant odor and taste. Adult human volunteers similarly showed no increase in diarrhea incidence with sulfate levels up to 1,200 ppm (the highest level tested). Newborn swine fed sulfate in water mixed with formula did not show signs of loose stools until levels reached 1,600 ppm and higher and displayed no adverse effects on growth or development. Evaluations of whether sulfate levels are harmful should also consider that (1) The osmotic laxative effect of sulfate is not known to cause significant dehydration or loss of salt, and (2) normal laxative effects of breast milk exceed those of sulfate at the levels that occur naturally in water supplies. Thus, an advisory for sulfate in water, especially as low as 500 ppm, seems unnecessary.

1175 TRANSLATING THE RESULTS OF RISK CHARACTERIZATION INTO HUMAN HEALTH CONDITIONS (HHC) IN USEPA'S COMPARATIVE RISK FRAMEWORK METHODOLOGY (CRFM).

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U.S. EPA's 1998 draft CRFM and Case Study (http://www.epa.gov/ncea/new.htm) combines elements of human health risk assessment and prevention effectiveness analysis to aid decisions about environmental health interventions posing disparate health risks. The CRFM compares the health consequences (e.g., cancer, microbial illness) and the financial costs associated with each intervention. The likelihood of human health effects and the general nature of the human health effects are estimated through evaluation of both experimental toxic endpoints and epidemiologic data. This effort focuses on one CRFM component, translating the health effects, as determined in the risk characterization step of the risk assessment paradigm, into incrementally-measurable HHC (e.g., a developmental effect, a clinical infection). The HHCs are the anatomic/physiologic properties that comprise the structure and functioning of the human body, defining the health-related quality of life. Differences in HHC's latency, duration and severity necessitate that they be represented using a common metric. A CRFM approach is to assess confidence in the accuracy of the translation into HHC based on the underlying data. In the Case Study, this translation allowed quantification of the impact on human health for two different drinking water treatment options: chlorination +/- pre-ozonation. Pre-ozonation greatly reduced the health impact of microbial illnesses, slightly reduced the health impact of both reproductive and developmental toxicity, and slightly increased the health impact of cancer-related effects, for an overall 68% reduction in combined health impact compared to chlorination alone.

1176 INDEPENDENT PEER REVIEW AND RISK VALUES DATABASE.

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Risk values developed by various organizations often differ due to scientific judgments, the mission of the organization, or because of the use of more recently published data. Toxicology Excellence for Risk Assessment (TERA) initiated an independent program to help validate the soundness of these risk values by making peer review available to all organizations. This process has resulted in the review of independently derived risk values for several dozen substances such as acrylonitrile, methylmercury, hexavalent chromium, Telone II, barium, arsenic, ethylene oxide, and soluble nickel salts (see http://www.tera.org/peer). A database of risk values, International Toxicity Estimates for Risk (ITER), provides a side-by-side comparison of values from government agencies and private organizations and makes the results of these peer review meetings widely available (see http://www.tera.org/iter on the Internet). To verify the utility of the program, we analyzed the ITER entries for 13 substances from the ATSDR/EPA 1997 list of top 20 priority substances. Of these, the noncancer risk values were the same for two chemicals between ATSDR and EPA, were different for 5 substances, and no value was published for the remainder. It is also important to provide a resource for compilation of risk values from multiple organizations to ensure identification of all available risk values. For example, we compared the cancer risk values for these same 13 priority substances and found that both EPA and Health Canada had published values for four substances, EPA but not Health Canada had a

risk value for four substances, and Health Canada but not EPA had a value for one substance. These comparisons demonstrate that the Peer Review program and ITER database are valuable tools for ensuring that risk assessors have ready access to the most current risk values.

1177 RESULTS OF QA/QC TESTING OF EPA BENCHMARK DOSE SOFTWARE VERSION 1.2.

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EPA is developing benchmark dose software (BMDS) to support cancer and non-cancer dose-response assessments. Following the recent public review of BMDS version 1.1b, EPA developed a Hill model for evaluating continuous data, and improved the user interface and Multistage, Polynomial and Power models. The BMDS system was subjected to an extensive QA/QC program, which included the independent verification of 7 dichotomous, 3 continuous and 3 developmental models. Each BMDS model was compared to an equivalent non-EPA model using data sets obtained from EPA cancer and non-cancer data bases. Many different configurations (combinations of options) for each of the EPA and non-EPA models were applied to 100 dichotomous or continuous dose-response data sets, resulting in well over 1,000 runs per model. Textual and graphical results of the model runs (e.g., maximum likelihood values, p-values, shape of the fitted curve) were collected and evaluated to determine strengths and weaknesses of each model. Preliminary results indicate that the BMDS dichotomous models calculate a BMD over 99% of the time, and fitted parameters such as benchmark dose and likelihood values from the BMDS Multistage model agree with values obtained from the THRESH model developed by Crump, Inc. The only deviations between the BMDS Multistage and THRESH were degrees of freedom differences for the chi-squared goodness-of-fit statistic resulting from a philosophical divergence on the appropriate approach for background estimates that reach the zero lower bound constraint. EPA will initiate a similar QA/QC analysis of several new BMDS models, including the Agency's new cancer model, a dichotomous Hill model and additional continuous (e.g., Hybrid) models in 2000.

1178 IMPROVING UNDERSTANDING OF WATER QUALITY AND HUMAN HEALTH ISSUES IN STUDENTS, TEACHERS, AND COMMUNITY MEMBERS IN RURAL OREGON.

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In 1996, a collaborative group at Oregon State University consisting of the Environmental Health Sciences Center, the Science and Math Investigative Learning Experiences (SMILE) program, and the Department of Public Health, was awarded a 4-year grant from the National Institutes of Environmental Health Sciences (NIEHS) to improve understanding of environmental health science by students, teachers, and community members in rural Oregon. The project makes use of the existing network of 75 teachers and over 700 minority and disadvantaged students in the pre-college SMILE program. Environmental health science activities are conducted at three main events: teacher development workshops, family science nights, and high school Challenge Weekends. Each year a different environmental health theme is addressed: chemical hazards in the home, indoor air quality, water quality, and food safety and human health. Year three of this grant focused on water quality and human health. Educational activities included visiting scientists laboratories, testing well-water for nitrates, and learning about risk assessment and major sources of water pollution This presentation describes the types of hands-on science activities conducted to educate teachers, students, and community members about water quality and human health.

1179 ENVIROHEALTH LINK SUMMER INSTITUTE AND WEB SITE-http://www.mpt.org/chl/home.html.

C. Mutryn. *Maryland Public Television, Owings Mills, MD.* Sponsor: M. A. Truch.

Maryland Public Television and the Johns Hopkins School of Hygiene and Public Health maintain a professional development program to provide middle and high school teachers with new scientific information and technology skills, so they can better incorporate environmental health science topics into their classroom curricula. The program keeps teachers up-to-date with current information on this science and offers strategies to transfer complex scientific information to their students in an understandable and usable way. The program consists of: 1) an annual week-long professional development Institute to provide "Master Teacher"-led environmental health lesson planning workshops, Internet and multimedia software workshops, lectures and panel discussions by Hopkins scientists, and hands-on laboratory and field experiences. 2) the resource-rich EnviroHealth Link web site, which (http://www.mpt.org/chl/home.html) provides teachers a place where they can get immediate answers to questions, updated environmental health information, Internet resources, and feedback from other teachers about environmental health-based classroom activities.

1180 HEALTH AND ENVIRONMENTAL RESOURCES AT THE UNIVERSITY OF WASHINGTON (HERE@UW).

J. F. Sharpe. *University of Washington, Seattle, WA.* Sponsor: D. L. Eaton.

HEALTH AND ENVIRONMENTAL RESOURCES AT THE UNIVERSITY OF WASHINTON (HERE@UW).Since 1994, the HERE@UW (Health and Environmental Resources for Educators at the University of Washington) programs has been working with K-12 teachers and students to make environmental health concepts a part of every child's early educational experience. This program includes an "Environmental Health for Educators" workshop that provides middle and high school teachers from all subject areas the content knowledge necessary to make environmental health (EH) a part of what they already do in the classroom. Participants learn about EH issues from community and university health professionals, share with us lesson plans they have developed and used in their classrooms, and earn continuing education credits for their participation. We have also developed two computer-based curricula. The first, Essentials of Cell Biology: Toxicology in Action, is an interactive CD-ROM that shows how cell biology connects with the principles of toxicology and risk. Resources in a virtual toxicology office allow the user to learn about cell biology and its direct link to human and environmental health. The second curriculum, Project Greenskate, is a web-based program that asks students to investigate the potential health concerns surrounding the development of a city park on a former industrial site contaminated with certain common environmental pollutants (lead, PCB, and TPHs). Using an engaging, game-like format, Greenskate introduces basic toxicology concepts such as dose-response, routes of exposure, thresholds for toxic effects, biotransformation, and risk assessment. HERE@UW has developed and is distributing "Tox in a Box," a resource kit that prepares EH professionals to visit K-12 classrooms by providing them with the materials and confidence they need to be a success. This presentation will emphasize how Tox-in-a-Box can help all SOT members become active in k-12 outreach.

1181 ENVIRONMENTAL AND RURAL HEALTH EDUCATION PARTNERSHIP.

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The long-term goal is to develop an engaging model for enhancing environmental and health science education of grade levels 6-8 in rural settings. Environmental health in the rural setting is emphasized because of public concern for increased numbers of environmentally health-related diseases in rural Texas (birth defects, lead poisoning, etc.). Further, the Center for Environmental and Rural Health (CERH) provides knowledge and research experiences for outreach. Middle school grade levels are chosen to focus the application on the prime developmental period for social skills of public school students. Rural schools are least likely to receive information on environmental health issues, but may need it most. Specific aims are: 1) to develop engaging multimedia learning materials tailored for rural middle school students (develop a health science curriculum transmitted to public schools via currently established and popular Internet Web sites); 2) to provide professional staff development programs (develop/execute short courses for teachers that integrate implementing Web-based environmental and health science curriculum units that describe distant education design options with current state of knowledge in environmental health, and that enhance teachers' awareness of rural health problems and potential solutions); and 3) to provide a human interface and online interaction to establish partnerships between public middle school students and scientists directly (faculty and graduate students visit and present scientific findings and excitement of doing research to students directly and on-line). These specific aims will improve the public understanding of health sciences and encourage a large number of

students to enter and remain in science academic tracks to produce the nation's needed workforce for future scientists and related workers in biomedical and health-related sciences.

1182 ENVIRONMENTAL CYBER SCHOOLHOUSE.

M. O. Dereski and L. Pietrantoni. *Wayne State University, Detroit, MI.*

"Health Quest" is the first lesson of the Environmental Cyber Schoolhouse Curriculum. The Cyber Schoolhouse is a World Wide Web-based series of interactive, multimedia environmental health lessons geared toward seventh, eighth, and ninth grade students. The goals of the lesson are to: increase student's understanding of how basic scientific research provides information necessary to identify risk factors and safeguard human health; introduce students to science related careers; allow for virtual as well as hands on experimentation in the classroom. The focal point of the lesson is the main character Maria, a young child who has a mildly elevated blood lead level (15μg/dL) that has been detected through routine screening at a local health fair. The students will collect information about lead poisoning and prevention by joining Maria at the health fair and at her subsequent visit to her pediatrician's office. The students will gather enough information to allow them to outline a protocol to decrease Maria's exposure to lead by providing a lead safe environment and subsequently lowering her blood lead level. Field testing of the site is currently being carried out with two urban schools, one suburban school, and two urban community youth centers programs. This projected is supported by Grant 5 25 ES09881-02.

1183 THE TOXRAP™ NETWORK: A SUCCESSFUL MODEL FOR BRINGING TOXICOLOGY TO SCHOOLS.

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In recent years the Society of Toxicology has placed increased emphasis on the need to communicate basic toxicological principles to the general public. The ToxRAP™ Network is an example of an outreach program that has successfully trained teachers to include toxicology concepts in their K-9 classrooms. This program, offered by the Environmental and Occupational Health Sciences Institute (EOHSI) and the Southwest Environmental Health Sciences Center (SWEHSC), used a train-the-trainer model to create local teacher expertise in environmental health sciences. Leadership Teams, made up of teachers and administrators, were brought to campus for a series of workshops focused on the award-winning ToxRAP™ curriculum series. As part of the training program, EOHSI and SWEHSC developed innovative methods for helping teachers understand scientific materials, develop regional implementation plans, and share program successes. The Teams returned to their school district or geographic area and trained other teachers to use ToxRAP™ curriculum. Twenty-seven teams from 11 states participated in the program. In turn, they trained approximately 750 teachers. All workshops were rated highly by participants. Formal and informal evaluations indicated that Leadership Teams successfully trained local educators to teach toxicology and related principles to their students. Several participating districts adopted the curriculum for district-wide implementation. Several projects demonstrating students' knowledge of environmental health risk assessment resulted, including a student-authored book about an environmental health investigation they conducted in their school. A train-the-trainer model is an effective method of integrating toxicology into K-9 curricula and helping students utilize scientific information to make decisions. This model can be used by universities and organizations such as local SOT chapters who wish to support environmental health sciences education for classroom teachers. (This project was supported by NIEHS, NIH [ES08221, ES05022 & ES06694].)

1184 SCHOOL LIFE SCIENCE LABORATORY: CLASS PROJECTS CENTERED ON THE EFFECTS OF TOXIC AGENTS ON AQUATIC MODEL ORGANISMS.

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Scientists and educators have collaborated in creating a set of experiment modules that provide students with intensive problem based instruction focusing on the scaffolding of critical thinking skills to understand basic science and environmental health science concepts. These concepts are addressed within the context of hands-on classroom experiments with aquatic organisms—fish, frogs, and lower life forms-to participate in experiments related to bioaccumulation of chemicals and the developmental and neurobehavioral effects of toxic agents such as lead and ethanol. A one-week intensive summer workshop prepares teachers to implement the modules in their curriculum by combining a short course in central concepts of environmental health with extensive laboratory experience. Center scientists lead the experiments in the modules and the presentation of educational strategies to promote collaborative learning, higher order thinking, and strategic learning. Teachers also acquire training in the integration of instructional technology in the science curriculum and the use of multimedia and Internet communications tools to provide contact between students, teachers, scientists, and educators.Full support during the school year to implement the modules is provided. The results have been dramatic; many teachers consider work with the modules to be the highlight of their year, parents and school staff acquire a better understanding of environmental health concepts and students understand that they are engaged in authentic scientific learning. (Supported by NIEHS grant ES-08271.)

1185 RISKS & CHOICES: TEACHING ENVIRONMENTAL HEALTH SCIENCE.

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Built as a collaborative effort between Miami University's Center for Chemical Education and the University of Cincinnati Medical Center's Department of Environmental Health, *Risks and Choices* is a four-year, multifaceted teacher enhancement project for middle and high school teachers. In Year One 40 master science teachers participated in an intensive course in environmental health science principles held at Miami University and taught collaboratively by environmental health scientists from the University of Cincinnati and instructional staff from the Center for Chemical Education. These participants developed classroom environmental health science lessons for their students based upon their experience. During the 1997-98 academic year, these Facilitators tested these materials with over 3000 students. The revised instructional materials serve as the basis of instructional units for subsequent implementation workshops for teachers, grades 5-12. These workshops are taught by Teacher Leaders selected from the original master teachers. The workshop content includes investigating environmental and genetic factors affecting the interaction of environmental toxins and human health, principles of toxicology, risk assessment and communication, and the issues of ethics and environmental justice that impinge on decisions concerning environmental health issues. As a result of these workshops, we have a group of teachers throughout the country who are experienced in integrating environmental health issues into their regular curriculum. The final phase of our present program includes a series of one and two-day Academies that focus on important *Risks & Choices* topics. Academies are also being conducted by our affiliates in Texas, Connecticut, and California. Academy Resource Guides are being prepared for distribution.

1186 EATING FOR YOUR HEALTH.

H. Losey and M. O. Dereski. *Wayne State University, Detroit, MI.*

As part of the "Chemicals in My World" curriculum series, the lesson "Eating for Your Health" has been developed to deal with the nutritional and exercise concerns of today's youth. The goals of this lesson intended for students in grades six through eight, are to introduce them to the influence of good nutrition and exercise on human health and disease. The following topics are included in the lesson: the definition of a calorie with a hands on calorimetry experiment; testing of various foods for their chemical components (protein, sugar, starch and vitamin C); a fat extraction experiment; and demonstration of the energy (calories) expended in various forms of exercise. The topics covered in this lesson have been aligned with the national science education standards. The Eating for Your Health lesson has been presented to several groups of students and teachers around the Metropolitan Detroit area, with positive reviews and feedback. This lesson is currently available for use by educators in their own classrooms. (This work is supported by NIEHS Grant P30 ES06639.)

1187 AN ELECTROPHILE RESPONSE ELEMENT (EpRE) MEDIATES INDUCTION OF THE γ-GLUTAMYLCYSTEINE SYNTHETASE REGULATORY SUBUNIT (GCSl) GENE.

A. M. Erickson, H. R. Moinova and R. T. Mulcahy. *University of Wisconsin, Madison, WI.* Sponsor: A. Elfarra.

γ-Glutamylcysteine synthetase, the rate-limiting enzyme in glutathione synthesis, consists of a catalytic (GCSh) and a regulatory (GCSl) subunit. Two cis-elements, an AP-1 site (-340:-334) and an Electrophile Response Element (EpRE) (-301:-290), have been implicated in the regulation of GCSl expression in response to pro-oxidants. The AP-1 element has been shown to regulate the basal expression of GCSl gene. However, regulation of GCSl inducible expression is complex (see Moinova and Mulcahy. (1998) JBC 273:14683-14689 and Galloway and McLellan. (1998) Biochem. J. 336:535-539). The function of the EpRE in GCSl inducibility was further investigated by transient transfection of mutant and synthetic constructs. Mutation of the AP-1 site results in reduced basal expression, while mutation of both the AP-1 and EpRE sites leads to the elimination of induction by both tert-butylhydroquinone (tBHQ) and b-naphthoflavone (b-NF), confirming our earlier report. While deletion of the 3' sequences flanking the EpRE reduces both basal and inducible expression, retention of the core EpRE sequence still supports induction by tBHQ. Further analysis of potential cis-elements near the EpRE and AP-1 site is currently under investigation. Nevertheless, current studies support a functional role for the EpRE in mediating inducible gene expression by tBHQ and b-NF. (Funded by grants ES09749 and CA57549.)

1188 MOUSE GLUTAMATE-CYSTEINE LIGASE REGULATORY SUBUNIT: GENE STRUCTURE AND REGULATION BY OXIDATIVE STRESS.

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The abundant cellular thiol, glutathione (GSH), maintains cellular redox homeostasis. Glutamate is first ligated to cysteine to form γ-glutamylcysteine by glutamate-cysteine ligase (GCL), the first and rate-limiting step in *de novo* GSH synthesis. GCL is an heterodimer composed of a 70-kD structural subunit (GCLS) having the catalytic activity and a 30-kD regulatory subunit (GCLR) that modifies the Km for the GCL substrate. We have cloned and characterized the structure of the mouse *Gclr* gene (spanning ~22 kb). An 8-fold induction of GCLR mRNA was observed after *tert*-butylhydroquinone (tBHQ) cell treatment. Nuclear run-on assays showed that GCLR mRNA accumulation is due to a higher rate of *Gclr* transcription (6- to 8-fold), compared to the 2- to 3-fold increase in *Gcls* transcription rate by tBHQ. RNase-protection analysis revealed two major and several minor transcription start sites within the proximal 300-bp adjacent to the start of translation. Transient transfection analysis with luciferase reporter constructs containing progressive promoter deletions (5 to 0.3 kb) show high basal and 2-fold-inducible expression. Nonetheless, a construct containing 0.3 kb of promoter that omits the 5' distal transcription start site shows a striking decrease in basal activity and no tBHQ-inducibility. In contrast, a construct in which the proximal transcription start site has been ablated gave low basal activity with no effect on tBHQ-inducibility. A putative electrophile response element (EPRE) is located within 0.3 kb of the 5'-most transcription start site. Site-directed mutagenesis demonstrated that this EPRE is not required, however, for tBHQ-induction. Robust expression of the *Gclr* gene is thus up-regulated by oxidative stress, at least in part, by *cis*-acting elements within 0.5 kb of the proximal promoter. (Supported by NIH Grant R01 AG09235.)

1189 GENE EXPRESSION ANALYSIS OF MOUSE LIVER AND MOUSE HEPATOCYTES FOLLOWING EXPOSURE TO OXIDATIVE STRESS AGENTS MENADIONE AND DIQUAT.

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Transcription profiling following compound exposure may be predictive for specific toxicity processes. Changes in gene expression were determined in livers from CD-1 mice given single (s.c.) dose treatments of menadione (40, 100 mg/kg) or diquat dibromide (50 mg/kg), two mechanistically related oxidative stress compounds. Animals were killed at 6,12, 24 and 48h following dosing. In addition, primary cultures of hepatocytes from untreated mice were exposed to 40μM menadione or 50μM diquat for 6 and 24h *in vitro*. The pattern of gene expression was compared between treatment groups and controls. Affymetrix GeneChip analysis was used to monitor the relative abundance of approximately 6,500 murine genes and ESTs. Approx. 3% to 6% of sequences on the GeneChip exhibited 2-fold or greater change in expression in treated groups compared to controls for both *in vivo* and *in vitro* studies. 32-41% and 24-31% of sequences showing 2 fold or greater change in expression were common to both chemical treatments *in vivo* after 6h and 24h respectively. Similarly, 32-51% and 13-37% of genes showing 2 fold or greater change in expression were common to both chemical treatments *in vitro* after 6h and 24h respectively. These results suggest a substantial percentage of genes are similarly affected following exposure to two related redox cyclers. Up-regulation of acute phase proteins and down-regulation of metabolic enzymes were found to be a consistent feature of *in vivo* toxicity. Analysis of *in vitro* samples revealed a markedly different pattern of gene expression, although there was a high concordance between the two oxidative stress compounds. This probably reflects differences in complexities of toxic responses *in vivo* versus *in vitro*.

1190 1,2-DICHLOROBENZENE ACTIVATES THE NUCLEAR TRANSLOCATION OF ACTIVATOR PROTEIN-1, NUCLEAR FACTOR-κ B AND ELECTROPHILE RESPONSIVE ELEMENT IN HEPATOCYTES ISOLATED FROM MALE FISCHER 344 RATS.

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1,2-Dichlorobenzene (1,2-DCB), an industrial solvent, is a known hepatotoxicant. Work done by our group and others suggests that two oxidative events that mediate liver injury occur following 1,2-DCB treatment. The first oxidative phase is caused by the bioactivation of 1,2-DCB to reactive intermediates, while activation of inflammatory cells (i.e. Kupffer cells) mediates a second oxidative stress. To investigate the molecular and cellular processes of hepatocellular oxidative stress, hepatocytes were isolated from male Fischer-344 rats, and examined for glutathione disulfide (GSSG), a marker of oxidative stress, and enhanced nuclear translocation of activator protein-1 (AP-1), nuclear factor κB (NF-κB) and electrophile responsive element (EpRE) transcription factors following incubation with 1,2-DCB (3.55 or 7.12 nmol). Oxidative stress occurred in hepatocytes incubated with 1,2-DCB as concentrations of GSSG were maximally elevated (15% above control) by 1 hr, while glutathione concentrations progressively decreased to 78% of control by 6.5 hr. The activities of AP-1 and NF-κB were increased by as much as 3-fold by 6 hr of 1,2-DCB treatment as compared to control. Nuclear translocation of EpRE was also enhanced by 3-fold but occurred earlier (2 hr) following 1,2-DCB treatment. Moreover, jun N-terminal kinase was activated in 1,2-DCB treated hepatocytes by 0.5 hr and was maintained for up to 2 hr. These data suggest that 1,2-DCB induced oxidative stress triggers a cascade of molecular processes that promote the nuclear translocation of transcription factors involved in regulating the expression of oxidant sensitive genes in hepatocytes. (Supported by Center Grant P30-ES06694 and an AFPE Fellowship.)

1191 OXIDATIVE INJURY MODULATES EXTRACELLULAR MATRIX-REGULATED NFκB BINDING ACTIVITY IN VASCULAR SMOOTH MUSCLE CELLS.

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Repeated exposure of rats to allylamine induces proliferative (i.e. atherogenic) phenotypes in vascular smooth muscle cells (vSMCs). This atherogenic response is mediated by permanent changes in signaling pathways associated with integrin-coupled extracellular matrix interactions. The present study investigated the relationship between increased proliferative capacity and extracellular matrix signaling in vSMCs derived from rats subjected *in vivo* to an atherogenic allylamine regimen. Because extracellular matrix signaling is coupled to integrin-mediated activation of NF-κB binding, electrophoretic mobility shift assays and measurements of cell proliferation were conducted to compare cells seeded on plastic, collagen, fibronectin, and laminin. Allylamine cells displayed a proliferative advantage when seeded on plastic, fibronectin and laminin, but not when seeded on collagen. Five major NF-κB complexes were resolved on 7% non-denaturing polyacrylamide gels. The predominance of individual complexes was substrate-dependent with complexes 2, 3, 4 and 5 being prominently induced on plastic, laminin and fibronectin, and complexes 3 and 5 on collagen. Allylamine cells exhibited increased NF-κB binding relative to controls when seeded on plastic, laminin and fibronectin, but not on collagen, indicating that NF-κB binding activity correlates with the occurrence of proliferative phenotypes in vSMCs. These results implicate integrin-coupled NF-κB signaling in the induction of athero-

genic phenotypes by allylamine. (Supported in part by NIH Grant ES 09106 and a TAMU Interdisciplinary Grant.)

1192 ACTIVATION OF HEPATIC NF-κB BY POLYCHLORINATED BIPHENYLS (PCBS) *IN VIVO* AND IN CULTURED RAT HEPATOCYTES.

Z. Lu, B. T. Spear, L. W. Robertson and H. P. Glauert. *University of Kentucky, Lexington, KY.*

Polychlorinated biphenyls (PCBs) are environmental pollutants that, due to their persistence and biomagnification, raise concerns about the health consequences of long-term exposure. The mechanism of the promoting activity of PCBs has not yet been determined. Previous studies show that oxidative stress occurs during metabolism of PCBs, with the formation of free radicals and oxidative DNA damage, which may contribute to their promoting activity. In this study, we examined whether oxidative stress-sensitive transcription factors NF-κB or AP-1 would be induced by PCBs *in vivo* or in primary hepatocyte culture. Male Sprague-Dawley rats were injected i.p. with corn oil, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153, 30, 150 or 300 umol/kg), 3,3',4,4'-tetrachloro-biphenyl (PCB-77, 30, 150 or 300 umol/kg) or both PCBs (30 or 150 umol/kg each). Rats were killed 2, 6, or 24 hours, or 2, 6, and 10 days after the PCB injection. Electrophoretic mobility shift assays were performed to determine the DNA binding activities of NF-κB and AP-1. The highest NF-κB DNA binding activity was observed in rats receiving higher doses of PCB-153 (150 and 300 umol/kg), with a peak occurring 2 days after injection. NF-κB activity was also increased in rats receiving both PCBs to a lesser extent, but no effect was seen in rats treated with PCB-77. Primary rat hepatocytes were cultured on collagen gels in serum-free L-15 medium with or without PCBs. NF-κB binding activity in hepatocytes culture was increased after a 48 hour exposure to PCB-153 (20 μM). These results show that hepatic NF-κB binding activity can be activated by specific PCBs *in vivo* and in cultured rat hepatocyte culture, indicating hepatic oxidative stress induced by PCBs. (Supported by ES 07380.)

1193 BCL-X1 AND BCL-2 EXPRESSION IN RAT VESTIBULAR AND AUDITORY BRAINSTEM NUCLEI FOLLOWING *IN VIVO* EXPOSURE TO *m*-DINITROBENZENE.

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m-Dinitrobenzene (DNB) induces dose-dependent gliovascular lesions in auditory and vestibular brainstem nuclei with secondary damage to neurons. This study investigates Bcl-2 and Bcl-X_L expression in rat brainstem nuclei during DNB-induced neurotoxicity *in vivo*. Male Fisher 344 rats were given either 7 mg/kg (mild lesion) or 10 mg/kg (severe lesion) DNB in DMSO twice daily via intraperitoneal injection for 1-3 days and sacrificed at 24, 48, 54 and 72 h after the first dose. Immunohistochemistry and western immunoblotting were used to examine expression of Bcl-X_L and Bcl-2 in affected regions such as inferior colliculi, deep cerebellar roof, vestibular, and cochlear nuclei. Regions resistant to DNB-induced neurotoxicity, such as cerebellum and hippocampus, were also examined. Positive staining for Bcl-X_L and Bcl-2 was noted in neuronal somata, dendrites and proximal axons of brainstem neurons in rats given 60 mg/kg DNB at 10 mg/kg twice daily for three days. No expression of Bcl-X_L and Bcl-2 was observed in neurons and astrocytes of control brains. Western blotting revealed moderate basal expression of Bcl-X_L and Bcl-2 in the inferior colliculi, deep cerebellar roof and cochlear nuclei of all control and DNB-exposed animals. A time-dependent increase in Bcl-X_L and Bcl-2 expression was observed in cerebellum and hippocampus in animals exposed to 10 mg/kg DNB, but not in controls at any timepoint. DNB-related induction of both proteins began at 24 h and was maximal at 72 h. Increased levels of Bcl-X_L and Bcl-2 were observed by immunohistochemical analysis in all brainstem nuclei examined from animals exposed to 7 mg/kg DNB twice daily for three days. No increase in expression of Bcl-X_L and Bcl-2 was observed by western blot analysis with the 7 mg/kg DNB dosing regimen. These results suggest that differential expression of Bcl-X_L and Bcl-2 correlates with lack of susceptibility to DNB neurotoxicity. (This research is supported by PHS-NIH Grants ES08846 & ES06103.)

1194 DIFFERENTIAL EXPRESSION AND ACTIVITY OF *Nrf2* IN LUNGS OF HYPEROXIA-SUSCEPTIBLE AND -RESISTANT MICE.

H. Cho, S. P. M. Reddy, L. Zhang, A. E. Jedlicka and S. R. Kleeberger. *Johns Hopkins University, Baltimore, MD.*

Acute respiratory distress syndrome (ARDS) is a major lung disease mediated by reactive oxygen species. An *in vivo* model of acute lung injury with similar features of ARDS has been produced by administration of hyperoxia (>95% oxygen) to animals. Among inbred mouse strains mice, C57BL/6J (B6) have greater sensitivity to hyperoxia than other inbred strains, including C3H/HeJ (C3). We recently identified significant hyperoxia-susceptibility quantitative trait locus (QTL) on mouse chromosome 2. A strong candidate gene within this QTL is *Nrf2*, an essential transcriptional regulator of antioxidant enzymes that play key roles in protecting cells against carcinogenicity and oxidative stress. To test the hypothesis that *Nrf2* confers differential susceptibility to oxygen toxicity, *Nrf2* mRNA expression (by reverse transcriptase-polymerase chain reaction) and DNA binding activity (by gel shift/supershift analyses) were evaluated in the lungs of C3 and B6 mice exposed to hyperoxia. Exposure markedly and similarly induced *Nrf2* mRNA expression at 90 min and 6 hr in both strains, compared to strain-matched air-controls. The mRNA levels returned to basal levels at 24 hr in both strains. A second increase in *Nrf2* mRNA expression occurred after 48 hr in C3 mice; in B6 mice, the mRNA level did not increase again until after 72 hr. The basal NRF-2 activity was greater in B6 mice compared to C3. After 90 min exposure, NRF-2 activity increased in C3 mice proportional to gene expression and remained elevated until 48 hr. In contrast, NRF-2 activity was markedly decreased in B6 mice by 6 hr and continued to decrease during the exposure. These results indicate that there is a significant strain-specific variation in *Nrf2* expression and activity after hyperoxia exposure. The results also suggest the early increase in NRF-2 activity conferred greater protection against oxidant injury in resistant C3 mice, while decreased NRF-2 activity may enhance susceptibility in B6 mice. (Support: ES 09606, HL 57142.)

1195 ACTIVATION OF ACTIVATOR PROTEIN-1 BY REACTIVE OXYGEN SPECIES ASSOCIATED WITH ASBESTOS.

V. Vallyathan, M. Ding, X. Shi and V. Castranova. *NIOSH, Morgantown, WV.* Sponsor: D. Jones.

Inhalation of asbestos causes alterations in cell signaling cascades, gene expression, cell injury and cell proliferation which may lead to pulmonary fibrosis, lung cancer, or mesothelioma. Asbestos-mediated free radical reactions are believed to trigger a number of cellular and molecular events that may promote fibrogenesis and carcinogenesis. Because activator protein 1 (AP-1) plays an important role in pre-neoplastic-to-neoplastic transformation, tumor promotion and metastasis, we studied the possible activation of AP-1 *in vitro* in cultured JB6 cells and *in vivo* using transgenic mice after exposure to crocidolite asbestos. *In vitro* exposure to asbestos, caused a dose- and time-dependent activation of AP-1 in cultured JB6 cells. Exposure of mice to crocidolite asbestos caused a significant (22-fold) activation of AP-1 in bronchiolar tissue compared to a moderate 10-fold increase in the lung tissue. The induction of AP-1 in asbestos exposure appears to be mediated through the phosphorylation of mitogen-activated protein kinases, Erk 1 and Erk2. Hydroxyl radical scavengers inhibited asbestos-induced AP-1 activation. These data support the hypothesis that oxygen radical mechanisms may be associated with pulmonary fibrosis and carcinogenesis.

1196 METHODS FOR MEASURING EXPRESSION OF IL-1 ALPHA, NITRIC OXIDE SYNTHASE, AND NITRIC OXIDE IN F-344 RAT SKIN IN RESPONSE TO DERMAL EXPOSURES TO FUELS OR SOLVENTS.

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Organic chemicals such as jet fuels and solvents are recognized to cause skin irritation after dermal exposures. The molecular responses to these chemicals that result in acute irritation are not understood well enough to allow the establishment and choice of safe exposure limits. We conducted studies to determine the feasibility of measuring various inflammatory factors, including IL-1 alpha, nitric oxide synthase, and nitric oxide. Male F-344 rats were exposed to organic chemicals for one hour using Hill Top Chambers®. Three hours after the exposures treated and control skin samples (1 to 1.5g) were collected. Light microscopy evaluation was performed on formalin-fixed and

paraffin embedded skin sections using anti-rat IL-1 alpha and anti-rabbit nitric oxide synthase polyclonal antibodies. Minced skin was placed in liquid nitrogen and pulverized with a stainless steel mortar and pestle, and then homogenized in Tris buffer solution using an ultrasonic homogenizer. After centrifugation, the supernatant was utilized for measurement of IL-1 alpha using commercial ELISA kit. Spectrophotometry was used to measure nitric oxide production using the Griess reagent. Treated skin showed nitric oxide levels increased 1.5- to 2-fold. Immunohistochemical staining indicated increased (two-fold) expression of IL-1 alpha and nitric oxide synthase compared to control. These methods will be appropriate to determine the time profile of irritant changes with solvent and fuel exposures. (Supported by CDC/NIOSH RO1 OH03654-01 and AFOSR 92HE05COR.)

1197 INDUCTION OF TRANSFORMING GROWTH FACTOR-BETA1 IN SPLENOCYTES OF ANILINE-TREATED RATS.

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Fibrosis appears to be an important preneoplastic event in the splenic toxicity of aniline. We have shown that aniline exposure in rats causes oxidative stress in the spleen and hypothesize that this oxidative stress may cause the induction of fibrogenic cytokines leading to splenic fibrosis. To demonstrate the role of cytokines in the aniline-induced splenic fibrosis, male SD rats were given 1 mmol/kg/day aniline hydrochloride (by gavage in 0.5 ml water) for 7 days and euthanized 24 hr following the last dose. Aniline exposure caused significant increases in spleen weights (97%), splenocyte cell counts (25%) and malondialdehyde-protein adducts (56%) as compared to controls. Transforming growth factor-beta1 (TGF-b1), which is considered one of the most fibrogenic cytokines, was quantitated in the splenic cells and culture supernatants by an ELISA. Total TGF-b1 content in the freshly isolated splenocytes from aniline-treated rats showed a 30% increase ($p<0.01$) as compared to the controls. The culture supernatants (from splenocytes cultured for 24 hrs) from aniline-treated rats showed a 60% increase in total TGF-b1 ($p<0.01$) as compared to controls. Our results show that aniline exposure causes induction of TGF-b1 in splenocytes and support the role of cytokines in the aniline-induced splenic fibrosis.

1198 HYPEROXIA DIMINISHES GLUTATHIONE REDUCTASE (GR) ACTIVITIES IN MURINE LUNG AND LIVER MITOCHONDRIA BUT NOT IN NUCLEI.

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Underdeveloped antioxidant systems in premature infants contribute to many of the diseases that are common in these individuals. Glutathione (GSH) serves in reduction of oxidants, with concomitant formation of glutathione disulfide (GSSG), which is reduced back to GSH by GR. Plasma GSH levels are low and GSSG levels are high in premature infants, suggesting deficiencies in GSH synthesis and/or GR activities. In the present studies, we exposed C57/BL6 mice to FiO_2>95% for 0, 24, 48 and 72h and prepared liver and lung subcellular fractions by differential centrifugation, purifying the nuclei using a discontinuous sucrose gradient. At all time points, GR activities were higher in nuclei than in either cytosol or mitochondria. Hyperoxia did not affect nuclear GR activities prior to onset of lung injury. In room air (RA), nuclear GR activities were 16.7 (0.9) [mU/mg protein (SEM)] in livers and 80.1 (3.2) in lungs. Mitochondrial GR activities decreased as a function of time in hyperoxia, from 10.5 (0.6) in RA to 6.6 (0.7) at 48h in livers and from 14.7 (0.8) to 11.2 (0.4) in lungs ($p<0.05$ in both). The decreases in mitochondrial GR activities in the lungs and livers of animals exposed to hyperoxia preceded increases in lung weights, suggesting that diminished mitochondrial GR activities may have contributed to the development of hyperoxic lung injury. Therapeutic strategies based on augmenting GR activities in lung and/or liver mitochondria to maintain highly reduced GSH/GSSG ratios in these compartments may attenuate or delay the development of hyperoxic lung injury. The consistently high nuclear GR activities suggest that GR is essential for normal functioning in this critical cellular compartment, although the exact roles are yet to be defined. (Supported in part by GM44263.)

1199 ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION AND LOCALIZATION IN THE RAT UTERUS AFTER ADMINISTRATION OF THE XENO-ESTROGENS BISPHENOL A, P-*TERT*-OCTYLPHENOL AND *O,P'*-DDT.

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The potential impact of xenoestrogens on human health is currently under debate, also due to the lack of data. To analyze molecular mechanisms of the action of xenoestrogens we have investigated the expression and localization of the estrogen receptor (ER) and the progesterone receptor (PR), after administration of bisphenol A (BPA), p-tert-octylphenol (OCT) and o,p'-DDT (DDT) in comparison to ethinylestradiol (EE) in the uterus of ovariectomized female DA/HAN rats. The animals were treated for 3 days p.o. with the xenoestrogens BPA (5, 50 and 200 mg/kg/day), OCT (5, 50 and 200 mg/kg/day), DDT (20, 100 and 500 mg/kg/d) and EE (0.1 mg/kg/day). Expression and localization of ER and PR protein was analyzed using immunohistochemistry. The wet weight of the uterus was dose dependently stimulated after administration of DDT (100 and 500 mg/kg/day) and after administration of 200 mg/kg/day OCT. A slight but significant stimulation was detectable after administration of 200 mg/kg/day BPA. Immunohistochemical analysis revealed estrogen like reactions in ER and PR expression and localization after dose dependent administration of OCT and DDT at doses 1000 to 10000 times higher than EE. No changes could be detected after administration of BPA at any dose administered. A comparison of these data to the uterotrophic response revealed that estrogen like changes in ER and PR expression and localisation after administration of OCT and DDT occurred at doses where no uterotrophic response was detectable. Interestingly, the tiny increase of uterine wet weight after administration of BPA does not correlate with an alteration of the expression pattern of ER and PR in the uterus. In summary, our data provide evidence that in regard to ER and PR expression and localization OCT and DDT act in the uterus in a typical estrogen like manner but with a low potency. The uterotrophic effect of BPA seems to be very tiny even if compared to OCT and DDT. Further investigations to elucidate potential alternative molecular mechanisms, e.g., (anti-) androgen-like action, are ongoing.

1200 THE EFFECTS OF LOW AND HIGH DOSE PRENATAL EXPOSURE TO BISPHENOL A ON FEMALE RAT OFFSPRING.

X. Wu and I. Chahoud. *Institute of Clinical Pharmacology and Toxicology, Berlin, Germany.* Sponsor: D. Acosta, Jr.

Bisphenol A (BPA) is a monomer of polycarbonate plastics used in many consumer products. Many studies have shown that BPA has estrogenic activity both *in vitro* and *in vivo* experimental test systems. It has been reported that a low dose of BPA may affect male offspring in mice differently from a high dose. As effects on female offspring have been neglected, the aim of the present study was to investigate the effects of high and low doses of BPA on the reproductive function in female offspring after prenatal exposure. Pregnant Sprague Dawley rats were randomly assigned to 4 groups (20 rats per group), housed singly, and maintained under standard conditions. Two groups were treated by gavage with doses of 0.1 and 50 mg Bisphenol A/kg b.w. on day 6-21 post conception. The third group received 0.2 mg/kg b.w. of 17-alpha-estradiol as positive control, and the control group was given the vehicle on the same days. In the female offspring the following endpoints were investigated: mean age at vaginal opening, length of estrous stage, organ weight, histological examination, sexual hormone level, and expression of c-fos and estrogen receptor genes in uterus. The results show that the mean age at vaginal opening (in days) was higher in the low dose group of BPA (49.4±2.7) and the estradiol treatment group (46.2±1.7) compared to the control (44.0±4.0); in contrary, it was lower in the high dose group of BPA (41.8±2.9 day). The length of the estrous stage was longer than 1 day only in 17% of the control females. In all estradiol females the length fasted more than 1 day. This effect was more pronounced in the lower dose of BPA (74%) than in the high dose (60%). Relative uterine weight was measured in estrous (ES) and diestrous (DS) stage. It decreased in the estradiol group in both stages (ES=0.160±0.024, DS=0.118±0.003) when compared to the control group (ES=0.256±0.055, DS=0.192±0.024). Such changes could not be confirmed in BPA treatment groups. The gene expression, given as median, of the estrogen receptor alpha was up-regulated in the high BPA dose level in ES and DS (ES=81.7%, control: 54.0%; DS=117.7%, control: 42.7%). In the estradiol group the effect occurred only in DS (66.4%). In the low dose of BPA this effect was not observed. Generally, it can be concluded that different doses of BPA may induce different effects in different tissues. Additionally, effects observed in one dose cannot always be used to be extrapolated to others. (Support: BMBF.)

1201 AN ESTROGEN EQUIVALENTS COMPARISON OF DAILY DOSES OF BISPHENOL A FROM POLYCARBONATE BABY BOTTLES VS. NATURALLY-OCCURRING COMPOUNDS.

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It has recently been suggested that potential migration of residual bisphenol-A (BPA) from polycarbonate baby bottles may pose an endocrine disruptive health threat to infants. Although high doses of BPA in animals can cause estrogenic effects, such as increased uterine wet weight, conflicting evidence exists as to whether such effects occur in animals at the much lower doses that might be experienced by bottle-fed infants. To date, there have been no studies to determine whether estrogenic effects might occur in infants exposed to BPA through product use. In order to accurately assess whether such effects might occur, it is important to account for the daily estrogenic dose associated with the presence of natural estrogens and other "estrogen-like" hormones in breast milk and cow's milk. In addition, most vegetable-based baby foods contain high levels of phytoestrogens that are more potent as estrogens than BPA. We present a comparative analysis of the daily infant estrogenic dose associated with the presence of BPA in consumed liquids (breast and cow's milk, juices, etc.) vs. the dose associated with the consumption of naturally occurring estrogenic compounds in an infant's diet. Data from recent extraction studies conducted with polycarbonate bottles were used to derive estimates of BPA concentrations that might exist in liquids that are contained in the bottles under a variety of food preparation conditions. Measured concentrations of naturally occurring estrogenic compounds in baby foods were taken from the primary literature. Published estimates of the volume and composition of the daily infant diet were used to derive plausible estimates of daily doses of BPA and naturally occurring estrogenic compounds for a variety of conditions. These doses were then normalized to "estrogenic equivalent" doses using published estimates of the relative estrogenic potency of BPA and the naturally occurring compounds. Our findings indicate that the maximally plausible estrogenic dose of BPA that might be experienced by an infant is several hundred times lower than the estrogenic equivalence of isoflavones consumed with soy-based infant formulas. Moreover, this dose of BPA would have less than 2 percent of the estrogen equivalence of naturally occurring estradiol in human breast milk. Based on these findings, we conclude that migration of BPA from polycarbonate bottles poses no endocrine disruptive health risk to infants.

1202 THE EFFECT OF SAMPLING DESIGN ON THE OUTCOME OF ENDOCRINE, REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES.

D. B. Janszen, B. A. Elswick and F. Welsch. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

A common experimental design in endocrine and reproductive toxicity evaluations is to expose the pregnant dam to a test chemical, and then to measure the response of interest in littermates at subsequent points in time. Litter sizes may be standardized soon after birth, or only a single randomly selected offspring per sex from each litter may be examined at each time point or become a parental animal for the next generation. In a study of the weak xenoestrogen bisphenol A (BPA) [see preceding abstract], possible treatment-related effects on ventral prostate (VP) fresh weights were found in rat offspring at six months of age using a study design in which one or two male pups per litter were randomly selected. However, in subsequent BPA and di-n-butyl phthalate studies, where VP weights of entire litter complements were examined, large intralitter variability of this specific end point became apparent. These data led us to believe that the earlier VP BPA results were likely due to selecting one or two males per litter where intralitter variability was large. This hypothesis was tested by simulating the effects of sampling design (one, two, or three male pups randomly selected from the full litter complement). These simulations used data from actual experiments as well as data created for the simulation. After 1000 simulations, the measure of the combined iterations was the percentage of correctly predicted results, defined as to whether there was a treatment effect at the significance level of $p<0.05$. False positive (Type I) or false negative (Type II) interpretations about treatment effects may be made more than 50% of the time when only one pup per litter is randomly selected. These statistical power considerations raise concern about selection of one pup per litter for highly variable end points.

1203 EFFECTS OF PERINATAL EXPOSURE TO LOW DOSES OF BISPHENOL A IN MALE OFFSPRING OF SPRAGUE-DAWLEY RATS.

B. A. Elswick, D. B. Janszen, J. C. Gould, D. B. Stedman and F. Welsch. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

Bisphenol A (BPA) is a constituent of epxoy resins and polycarbonate plastics used in the packaging of canned foods and beverages. Leaching of BPA into the contents may result in ingestion of small quantities. BPA is a relatively weak estrogen receptor agonist and is suspected of perturbing endocrine homeostasis. Here we describe reproductive and developmental effects of BPA on male F1 offspring from birth to adulthood. Two replicate blocks of 8 dams/treatment group (resulting in an overall sample size of 13-16 pregnancies/group) consumed drinking water with 0, 0.005, 0.05, 0.5, 5 or 50 mg BPA/L from gestation day 2 through postnatal day (PND) 21. Estimated daily intakes ranged from ~0.001 to ~10 mg/kg/day. BPA treatment neither affected anogenital distance nor body weights on PND 2, or body weight gains up to PND 21. Additionally, BPA did not affect preputial separation, a developmental landmark. On PND 21, 41, and 177, one or two randomly selected male pups were necropsied. BPA treatment did not alter either fresh organ weights or hormone levels on PND 21 and 41. Additionally, Western blot analysis for androgen receptor in ventral prostates (VP) from PND 41 males did not display BPA related changes. At PND 177, BPA exposed rats did not display significant differences in hormone levels, sperm counts, or immunohistochemical VP AR levels. No significant alterations emerged when fresh organ weights were compared to concurrent controls except for a significant increase in VP weights in the 0.05, 5 and 50 mg BPA/L dosing groups. The apparent weight increase was not dose-related and occurred without concomitant histological changes. Large intralitter variability of VP fresh weights may be a confounding factor in these results. In a mating study, the males displayed no detectable changes in fertility. Aside from the equivocal VP effect, the data indicate that under the indirect pre- and postnatal BPA exposure conditions (drinking water of the dam and lactation) the differentiation and function of the reproductive system in male rats appeared to be unimpaired.

1204 EFFECTS OF PERINATAL EXPOSURE TO LOW DOSES OF BISPHENOL A ON FEMALE OFFSPRING OF SPRAGUE-DAWLEY RATS.

F. Welsch, B. A. Elswick, and D. B. Stedman. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

Bisphenol A (BPA) is a constituent of polycarbonate materials used in packaging canned foods and beverages. Leaching into the contents may result in human ingestion of small quantities of BPA. BPA is a weak estrogen agonist suspected of being able to perturb endocrine homeostasis. The study design consisted of two replicate blocks of 8 dams/treatment group (resulting in an overall sample size of 13-16 pregnancies per treatment). Dams consumed drinking water with 0, 0.005, 0.05, 0.5, 5 or 50 mg BPA/L from gestation day 2 through postnatal day (PND) 21 with estimated daily intakes ranging from ~0.001 to 10 mg/kg/day. Diethylstilbestrol (DES; 0.05 mg/L) was used as an estrogenic reference chemical. The present report describes the reproductive development and function of female F1 offspring randomly selected from each litter for specific end points in comprehensive postnatal assessments. The observations extended from PND 2 to ~10 months of age. There were no differences in anogenital distance and body weights on PND 2 among BPA treatments compared to control, nor were there any differences in body weight gains up to PND 21. However, there were significant reductions in body weights from age-matched concurrent controls in DES exposed offspring. This body weight reduction was later recovered in the adult. DES females were younger than control offspring when achieving the developmental landmarks of vaginal opening and first estrus, but the BPA-treated rats were unaffected. Estrous cyclicity up to ~7 months and ovarian follicle counts at 4 months of age were not different between control and any of the DES or BPA treated females. Neither fertility and fecundity in continuous breeding evaluations nor lordosis behavior were affected in any treatment groups. No meaningful differences were found in the organ weights at various ages. Overall, the data indicate that under the indirect pre- and postnatal BPA exposure conditions (drinking water of the dam and lactation) the differentiation and function of the reproductive system in female rats appeared to be unimpaired.

1205 ENDOCRINE DISRUPTION ACTIVITY OF ENDOSULFAL IN YEAST TRANSFORMANTS AND IN MICE.

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Recently, the concern for chemicals suspected as one having steroid-like activity is increased higher than ever. Endosulfan, an organochlorine pesticide of the cyclodiene group, widely used for control of insects in crops, is known as a mutagen and reproductive toxicant. Human health risk can be affected in the case of ingestion of livestock product or crops contaminated with this hazardous chemical.We studied the endocrine disruption activity of endosulfan in yeast transformants contained the human estrogen, androgen or progesterone receptor along with the appropriate steroid responsive elements upstream of the β-galactosidase activity. We also compared the endocrine disruption activities in yeast transformants to those in mice to know if the yeast transformants are reliable tool for screening the endocrine disrupters.Endosulfan induced increase of β-galactosidase activity 2-3times more than control in estrogen receptor gene transformed yeast at the doses of more than $2.5x10^{-4}$M and androgen receptor gene transformed yeast at the doses of more than $2.5x10^{-5}$M. In progesterone receptor gene transformed yeast, β-galactosidase activity was increased 5-6 times more than control at the doses of more than $5x10^{-6}$M. The relative weight of adrenal gland was increased and estrus cycle was delayed in female mice treated with endosulfan(10mg/kg B.W.) via gavage once per day for 14 days. The contents of estrogen and progesterone in serum were decreased significantly in endosulfan-treated female mice at pre-estrous stage. The sperm motility was decreased significantly in male mice exposed orally with 10mg/kg B.W. of endosulfan once per day for 48 days.These results suggested that endosulfan could react with progesterone receptor moderately and with estrogen and androgen receptor weakly and be an endocrine disrupter in both of yeast transformants and mice. In addition, yeast transformants contained the human estrogen, androgen and progesterone receptor combinated with the steroid responsive elements can be simple and reliable screening tools for the investigation of endocrine disrupters.

1206 LOCALIZATION OF GLUTAMATE CYSTEINE LIGASE (GLCL) SUBUNIT MRNAS WITHIN THE RAT OVARY.

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GLCL, the rate-limiting enzyme in glutathione (GSH) synthesis (also known as gamma-glutamylcysteine synthetase), is expressed in most tissues, including the ovary. We have previously observed that ovarian reduced GSH and GLCL catalytic (GLCL-c) and regulatory (GLCL-r) subunit mRNA levels fluctuate significantly during the rat estrous cycle. Such fluctuations suggest that ovarian GSH synthesis may be hormonally regulated and that GSH may play a role in normal follicular development. In order to begin to delineate the role of GSH in follicular development we used in situ hybridization to study GLCL-c and GLCL-r mRNA expression in the rat ovary during the 4-day estrous cycle. Three rats were sacrificed on each day of the cycle, ovaries were removed, and sections were hybridized with ^{35}S-labeled antisense riboprobes for GLCL-c and GLCL-r. Sense riboprobes served as negative controls. Mouse kidney sections were run as positive controls with each experiment. Visualization was done by autoradiography and dark-field microscopy. We observed high GLCL-r expression in the granulosa cells and oocytes of healthy growing and antral follicles, but not in primary or atretic follicles or in other cell types. In contrast, GLCL-c was expressed rather homogeneously throughout the ovaries. High GLCL-r expression in growing follicles, but not in atretic follicles, supports the hypothesis that GSH plays a role in protecting developing follicles from reactive oxygen species which are produced during normal follicular development. (Supported by NIEHS Center for Ecogenetics and Environmental Health [1 P30 ES07033-03] and by US EPA #R-825358-01-0 [to E.F.].)

1207 *IN UTERO* ANTI-ANDROGEN (FLUTAMIDE) EXPOSURE INCREASES LEYDIG CELL ACTIVITY IN ADULT RATS.

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In the testis, the main function of Leydig cells is to produce steroids under the control of gonadotropins. Studies have clearly shown that androgens are absolutely required to maintain normal testicular function. In the present study we assessed by semi quantitative RT-PCR whether prenatal exposure to the anti-androgen flutamide affects post natal Leydig cell functions. Pregnant Sprague Dawley rats were treated with flutamide (at 0, 2, 10, 50 mg/kg/day, from day 6 up to delivery). At post-natal day 90 (PND 90), male offspring were sacrificed, blood samples were taken for testosterone and LH levels and testes were collected for extraction of total mRNA. Exposure to flutamide during the fetal period resulted in an increase of testosterone production by Leydig cells as evidenced by higher testosterone levels in mature males (PND 90) from all treatment groups. This effect was maximal at 10 mg/kg/day (137% of control). In an attempt to explain this increase and to determine the level of action, we studied the expression of genes implicated in the production of testosterone by Leydig cells. The expression of CYP Side Chain Cleavage (CYP SCC) and CYP 17alpha hydroxylase (CYP 17alpha) messenger were only slightly increased (about 10%) at 10 and 50 mg/kg/day. In contrast, the level of the Steroidogenic Acute Regulatory protein (stAR) mRNA was increased in a dose-related manner (30% and 56% at respectively 10 and 50mg/kg/day). At 2 mg/kg/day, no effects were observed on CYP SCC, CYP 17alpha and StAR gene expression. In conclusion, it appears that Leydig cell activity in the adult testis is affected after a fetal androgen disruption. Assessment of the expression of these genes at different critical periods of Leydig cell maturation is in progress to better define the impact of prenatal exposure to an anti-androgen.

1208 LONG-TERM GENISTEIN EXPOSURE HAS MINIMAL EFFECTS ON SEX BEHAVIOR IN FEMALE RATS.

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The estrogen mimic genistein occurs naturally in several plants including soybeans; human exposure is via consumption of soy-based products (e.g., tofu and soy milk), dietary supplements, or infant formula. Adult human exposure estimates range from 0.1 mg/kg/day for the typical Western diet to 1.4 mg/kg/day for a typical Asian diet. There are little data on the developmental effects of genistein exposure. Here, pregnant rats consumed soy-free diets containing 0, 5, 100, or 500 ppm genistein, approximately equivalent to 0, 0.4, 8, and 40 mg/kg/day beginning on postnatal day (PND) 42 (prior to mating). Their offspring continued on the same diets throughout this study. F1 females were tested for sex behavior between PNDs 65-95 by rating their lordosis response on a scale of 0-3 following handling. Intact animals (n=40, 10 per dose) were tested for 5 consecutive days and ovariectomized animals (n=40, 10 per dose) received estradiol and progesterone before a single day of testing. Mean lordosis ratings (LR) and lordosis quotients (LQ) were calculated and analyzed using 1 way ANOVA with $p<0.05$ required for significance. The ranges for mean LR were 1.54 to 2.19 in intact animals in estrus (i.e., their highest score) and 0.64 to 1.39 in hormone-primed ovariectomized animals. The ranges for LQ were 7.00 to 9.00 in intact females and 3.44 to 6.71 in ovariectomized. There were no significant genistein-related effects. These results suggest that long-term genistein exposure in rats, at doses approximating human exposures, does not cause gross alterations in female sex behavior. There may be, however, genistein-induced alterations not observable in this type of sex behavior assessment. Additional studies are underway to identify changes in other behaviors that may be altered after multiple generations of genistein exposure. (Supported by Interagency Agreement #224-93-0001 between the FDA and the NIEHS.)

1209 EVALUATION OF THE EDSTAC MALE PUBERTAL ASSAY IN CD RATS USING TESTOSTERONE, STEROID BIOSYNTHESIS INHIBITORS, DOPAMINERGIC (DA) AGENTS AND THYROID INHIBITORS.

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The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) has designated the male pubertal assay as an alternate Tier I screening test to detect potential endocrine disrupting (ED) chemicals. We began evaluating the ability of this assay to detect EDs acting through a variety of mechanisms. Weanling male rat pups were dosed for 30 d by gavage with vehicle (0.5% methocel) or the following test compounds (mg/kg/d): testosterone (T, 0.1, or 0.4), ketoconazole (KETO, 24), finasteride (FIN, 20 or 80), testolactone (TL, 220), fadrozole (FAD, 0.6 or 6.0), haloperidol (HALO, 2 or 4), bromocryptine (BRC, 10 or 50), phenobarbital (PB, 50 or 100) or 6-propylthiouracil (PTU, 240). In vehicle-treated males, age at pubertal onset, as evidenced by preputial separation (PPS), was 44.4±2.0 d. Only TL, PTU, and high dose BRC significantly delayed the age at PPS (α=0.05). Considerable interanimal variability within the control population (± 2 d) suggests that age at PPS may be a relatively insensitive endpoint for assessing

endocrine function. Conversely, significant reductions in body weight (~10% at PPS) did not alter age at PPS spuriously. Additional endpoints enhanced assay sensitivity. Epididymal weights were significantly reduced by KETO, a broad spectrum steroid biosynthesis inhibitor, FIN, a 5α-reductase inhibitor, and TL, an aromatase inhibitor. TL's effects were consistent with antiandrogenic activity by this compound. FAD, a more specific aromatase inhibitor, did not alter any assay parameters. PTU, a thyroid inhibitor, and HALO, a DA receptor antagonist, altered thyroid weight. PTU also altered thyroid histology and hormone levels. Thus, this assay was capable of detecting EDs operating through a variety of mechanisms; however, it failed to detect T or FAD. Hypothetically, higher doses of T should be detectable with this assay, but its capacity to detect aromatase inhibitors remains in question.

1210 *IN UTERO* FLUTAMIDE AND DIETHYSTILBESTROL TREATMENTS DECREASE OSP/CLAUDIN11 EXPRESSION IN ADULT TESTIS.

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In the testis, OSP (Oligodendrocyte Specific Protein)/Claudin 11 is expressed specifically by Sertoli cells and is involved in the formation of the hemato-testicular barrier. In the present study we assessed by semi-quantitative RT-PCR whether the prenatal exposure to endocrine modulators such as anti-androgen flutamide or estrogen diethylstilbestrol affect the postnatal OSP/Claudin 11 expression. Pregnant Sprague Dawley rats were treated with flutamide (0, 2, 10 mg/kg/day) or with DES (0, 1, 5, 10 μg/kg/day) from day 6 to delivery. After sacrifice, testes were either processed for RNA extraction immediately (PND 90) or enzymatically digested to obtain Sertoli cells (PND 15) from which RNA was extracted after culture. These two different experimental protocols were used since at PND 15 but not at PND 90, Sertoli cells can be easily separated and purified from other testicular cell types. Treatment of dams by all doses studied of either flutamide or DES was associated with a decrease in OSP mRNA level (with a maximum of about 80% and 50% for all concentrations of flutamide and DES, respectively) in whole testes from PND 90 offspring. In order to assure that a decrease in OSP expression was not simply due to the treatment-associated reduction of the Sertoli cell number at PND 15, Sertoli cells originating from either control or treated groups (flutamide or DES) were cultured at the same density. In this experimental setup, as with PND 90, we also observed a decrease in OSP/Claudin 11 expression. Thus, a decrease (45%) in OSP/Claudin 11 mRNA was observed in rats born by mothers treated with 10 mg/kg/day of flutamide. Pre-natal treatment with DES (5 and 10 μg/kg/day) decreased OSP/Claudin 11 expression about 30% and 50%, respectively. In conclusion, OSP/Claudin 11 mRNA expression in adult testis is reduced in animals exposed *in utero* to flutamide or to DES. Since OSP/Claudin 11 is the major constituent of tight junction strands of Sertoli cells, it is possible that adult testicular compartmentalization is affected by the decrease in OSP/Claudin 11 in rats exposed to flutamide or DES during the fetal life.

1211 COMPARATIVE ENDOCRINOLOGY STUDY OF DEHYDROEPIANDROSTERONE (DHEA) AND A FLUORINATED ANALOGUE IN BEAGLE DOGS.

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DHEA and its sulfated metabolite, DHEA-S, are major endogenous adrenal steroids. DHEA is under development as a potential cancer chemopreventive agent. However, due to DHEA's endocrine effects, a fluorinated analogue (fluasterone; fl-DHEA) was synthesized as a potential alternate agent. This study evaluated the endocrine effects of DHEA and fl-DHEA in adult male and female Beagle dogs following 4 weeks of daily oral (capsule) treatment. Dose levels studied were 0, 10 and 100 mg/kg/day for each drug (3 dogs/sex/group). Neither administration of DHEA nor fl-DHEA resulted in mortalities, clinical signs of toxicity, histologic lesions or changes in body weights, food consumption or organ weights. Administration of DHEA resulted in dose-dependent increases in plasma DHEA and DHEA-S 1 hr after dosing, with the highest values in the males. fl-DHEA produced dose-dependent increases in plasma DHEA and fl-DHEA levels at 1 hr. Female dogs treated at 100 mg/kg/day DHEA had significant increases in plasma dihydrotestosterone and androstenedione, and nonsignificant increases in mammary tissue levels of DHEA and estradiol. These changes were not seen in DHEA-treated males or in the fl-DHEA dose groups, and indicate that DHEA can be converted to androgenic steroids by female dogs. Administration of either 10 or 100 mg/kg/day fl-DHEA to male and female dogs did not result in treatment-related effects. (Supported by NCI Contract No. NO1-CN-75127-MAO.)

1212 A MORPHOMETRICAL ANALYSIS OF EFFECTS OF FEMALE SEX HORMONES ON HAMSTER PANCREATIC ISLETS.

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It is known that sex hormones, including estrogen, can stimulate the release of insulin. Therefore, the modulating effects of female sex hormones, ethinylestradiol (EE) and levonorgestrel (LN), on the hamster pancreatic islets were investigated by morphometrical analysis. A total of 30, 9-week-old female Syrian hamsters were used in this experiment. Animals in groups 1-3 (each consisting of 10 hamsters) were respectively fed diet supplemented with 1 ppm EE (group 1) and 10 ppm LN (group 2), and without supplementation (group 3) for 26 weeks. At the end of the experiment, all animals were sacrificed and the pancreatic tissues were collected and processed for histological examination. Areas and diameters of the pancreatic islets were significantly increased, and their roundness significantly decreased by the EE or LN treatment. Thus histopathologically, the EE and LN groups showed telangiectasia and irregularity in form of the islets. Positive rates for insulin producing cells in the islet were significantly higher in the EE and LN groups than in the control group, although those for glucagon and somatostatin were lowered. Cell proliferative activity in both islets and acini was significantly increased in the EE and LN groups as compared to the control values. The results in the present experiment demonstrate that synthetic estrogen and progestins can affect hamster pancreatic islets when administrated in the diet.

1213 DETECTION OF DOPAMINERGIC MODULATORS IN A TIER I SCREENING BATTERY FOR IDENTIFYING ENDOCRINE-ACTIVE COMPOUNDS (EACs).

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Apomorphine (APO; D_2 receptor agonist), haloperidol (HAL; D_2 receptor antagonist), and reserpine (RES; dopamine depletor) have been examined in a Tier I screening battery, which has been designed to detect endocrine-active compounds (EACs). The Tier I battery incorporates two short-term *in vivo* tests (5-day ovariectomized female battery and 15-day intact male battery using Sprague-Dawley rats) and an *in vitro* yeast transactivation system (YTS). In the *in vivo* female battery, both HAL and RES increased serum PRL concentrations as expected. Increases in serum PRL levels are enhanced when daily dosages are administered via multiple-daily dosing of the test compound, which results in higher sustained blood levels of the test compounds. APO failed to decrease serum PRL concentrations in the female battery. In the *in vivo* male battery, HAL increased serum PRL concentrations as expected. However, APO and RES failed to effect serum PRL concentrations. In summary, HAL-like compounds would be identified in the Tier I male and female battery primarily via increased serum PRL concentrations. RES-like compounds would be identified in the Tier I male battery via decreased gonadotropins and steroids and possibly in the Tier I female battery by a minimal increase in serum PRL concentrations. Compounds which produce a marginal increase in serum PRL when administered using single daily dosing can also be confirmed in an *in vivo* female battery with multiple dosing since this regiment increases the magnitude of the PRL increase. APO, a D_2 receptor agonist, was not detected in the *in vivo* male or female batteries, but in both instances the top dosage produced minimal decreases in body weight (99-96% of control). Hence, the proposed Tier I battery needs to be further evaluated with higher dosages of APO and other D_2 receptor agonists to determine whether it is capable of detecting such agents.

1214 EFFECTS OF AFLATOXIN-B_1 (AFB_1) AND ITS METABOLITES AFLATOXICOL (AFLO) AND AFLATOXIN M_1 (AFM_1) ON THE RODENT UTERUS.

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Reproductive associated problems in bovine and avian species have been attributed to dietary AFB_1 exposure. AFB_1-induced hypovitaminosis - A,

and/or structural similarities of AFB_1 to estradiol-17β are thought to be possible mechanism (s) by which reproductive problems in these species develop. Evaluating the role of AFB_1 in rodent reproduction in terms of its estrogenic and antiestrogenic potential on uterine growth *in vivo*; and its binding to uterine estrogenic receptors seems prudent. Immature (21 day old) CD rats were treated with increasing doses of AFB_1 (1.0 μg/kg-4.0 mg/kg) alone or in combination with estradiol -17β (fixed dose 0.016 μg/kg) subcutaneously for three days. All rats were sacrificed 24 hrs. following the last injection, the uteri excised, cleared free of fat and other tissues, and weighed. Kidney, liver, ovarian, and adrenal weights were also recorded. Uterine weights increased in a dose - dependent fashion with estradiol-17β alone but not with AFB_1 alone, even when administered at doses as high as 4 mg/kg. AFB_1 in combination with estradiol-17β had little or no effect on estrogen-stimulated increase in uterine weights. Kidney weights appeared to be increased at high doses "2.0 and 4.0 mg/kg" of AFB_1, in the presence of estradiol - 17β. Body weight gains were decreased significantly in rats treated with high levels of AFB_1(1.0, 2.0, and 4.0 mg/kg). Utilizing *in vitro* methods (Korach et al. J. Biol.Chem. 254: 8963-8968, 1979) Aflatoxicol (AFL0), but not AFB_1 or AFM_1 appeared to compete, with diethylstilbestrol (DES) for uterine estrogenic receptors. Further studies are required to delineate AFB_1- induced reproductive associated problems in bovine and avian species.

1215 THE HERSHBERGER ASSAY FOR (ANTI)- ANDROGENS: AN INTERLABORATORY COMPARISON.

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The rat 7-day Hershberger assay has been recommended by the US EPA's Endocrine Disruptor Screening Program to identify substances with androgenic and anti-androgenic activity. Here we assess the ability of two laboratories to independently use this assay to correctly identify chemicals with known (anti)-androgen activity. Immature 28-34 day old rats castrated at day 21 (10 rats/group) were dosed by gavage with vehicle (corn oil), flutamide (100 mg/kg/day), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'-DDE; 100 mg/kg/day) or dibutylphthalate (DBP; 1000 mg/kg/day) with (to assess antiandrogenic activity) and without (to assess androgenic activity) a subcutaneous injection of testosterone propionate (TP; 50 μg/day) for seven consecutive days. In both laboratories, TP injections alone increased ventral prostate (VP; ~ 4-fold), seminal vesicle (SV; ~5-fold) and levator ani/bulbocavernosus (LABC; ~ 2-fold) weights compared to vehicle treated controls. The potent androgen receptor antagonist flutamide completely blocked the androgenic effects of TP in all tissues, while the weak androgen receptor antagonist p,p'-DDE reduced VP, SV and LABC weights in TP injected rats by approximately 50 percent; similar results were obtained in both laboratories. Neither laboratory was able to detect the non-receptor mediated antiandrogenic effects reported for DBP. Neither flutamide, p,p'-DDE nor DBP increased VP, SV or LABC weights over vehicle treated controls suggesting that these chemicals lack androgenic activity. Taken together, the results suggest that the Hershberger assay is effective to assess (anti)-androgenic activity of substances acting through the androgen receptor and is repeatable among independent laboratories.

1216 EVALUATION OF A TIER I SCREENING BATTERY FOR DETECTING ENDOCRINE-ACTIVE COMPOUNDS (EACS) USING THE POSITIVE CONTROLS TESTOSTERONE, COUMESTROL, PROGESTERONE, AND RU486.

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Coumestrol (COUM; estrogen receptor (ER) agonist), testosterone (TEST; androgen receptor (AR) agonist), progesterone (PROG; progesterone receptor (PR) agonist), and mifepristone (RU486; PR antagonist) have been examined in a Tier I screening battery designed to detect EACs. The Tier I battery incorporates two short-term *in vivo* tests using CD rats (5-day ovariectomized female and 15-day intact male batteries) and an *in vitro* yeast transactivation system (YTS). In the female battery, TEST increased uterine weight, uterine stromal cell proliferation (CP), and altered serum hormone concentrations; in the male battery, TEST increased accessory sex gland weights, altered serum hormone concentrations, and produced microscopic changes of the testis; and, in the YTS, TEST activated gene transcription in the yeast containing the AR or PR. In the female battery, COUM increased uterine weight, uterine stromal CP, uterine epithelial cell height, and increased serum PRL; in the male battery, COUM altered serum hormone concentrations; and, in the YTS, COUM activated gene transcription in the yeast containing the ER. In the female battery, PROG increased uterine weight, uterine stromal CP, uterine epithelial cell height, and altered serum hormone concentrations; in the male battery, PROG decreased epididymis and accessory sex gland weights, altered serum hormone concentrations, and produced microscopic changes of the testis; and, in the YTS, PROG activated gene transcription in the yeast containing the AR or PR. In the female battery, RU486 increased uterine weight and decreased uterine stromal CP; in the male battery, RU486 decreased epididymis and accessory sex gland weights, and increased serum FSH and LH; and, in the YTS, RU486 activated gene transcription in the yeast containing the ER, AR, or PR. Using the responses obtained for the Tier I battery, a distinct "fingerprint" was produced for each type of endocrine activity against which compounds with unknown activity can be compared.

1217 PHYSIOLOGICALLY BASED MODELING OF THE RAT ESTRUS CYCLE AND HUMAN MENSTRUAL CYCLE INCLUDING A LINK TO BREAST CANCER INCIDENCE RATES.

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Physiologically based models for both the human menstrual cycle and rat estrus cycle have been developed, and are presented as autonomous hormonal circuits in which no forced triggers, like fixed cycle length or day of ovulation, are incorporated. These models incorporate six organ systems belonging to the endocrine system: the brain/hypothalamus, the hypophysial portal system, the pituitary gland, the ovaries, the liver and the gastro-intestinal tract. Serum hormone levels in cycling women (estrogen, progesterone, LH, FSH, Inhibin) and in female rats (estrogen, progesterone, LH, FSH, prolactin) are used for model optimization. Developing such models has several advantages. Physiologically based models which describe the production of and interaction between the numerous hormones involved in the menstrual and estrus cycle are useful in studying the effects of exogenous compounds or endocrine disrupters on hormonal equilibrium. Furthermore, even though endogenous estrogen levels are already related with e.g. breast cancer in women, the response to an exogenous impact is still very much unknown. By linking the changes in simulated estrogen levels with changes in parameters used in cancer models (e.g., two-stage model), an exploration of changes in dose-response curve shapes, as a result of the exposure, is feasible.

1218 DISTURBANCE OF THYROID HORMONE HOMEOSTASIS FOLLOWING CONTINUOUS INTRAVENOUS INFUSION OF PROBENECID IN RATS.

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The goitrogenic potential of probenecid, a widely used renal tubular transport blocking agent, was investigated following continuous intravenous infusion. Groups of 10 rats/sex were administered the vehicle, or 5, 10, and 20 mg/kg/hr for 2 weeks (dose-response phase). In addition, 5 males received 20 mg/kg/hr for 3, 7, or 14 days (time-course phase). **Dose-Response Phase:** Probenecid plasma concentrations were below the limit of quantification at 5 mg/kg/hr, and within the intended therapeutic range (30-150 μg/mL) at 10 mg/kg/hr. At 20 mg/kg/hr concentrations ranged from 279 to 658 μg/mL. Serum levels of thyroxine (T_4) and triiodothyronine (T_3) were significantly lower in both sexes at 10 mg/kg/hr (T_4: 25% to 50% of controls; T_3: 84%) and 20 mg/kg/hr (T_4: 27% to 51%; T_3: 82%). Increases in absolute (16% to 21%) and relative (20% to 22%) liver weight, and an increased incidence of minimal-to-mild follicular cell hypertrophy in males were noted at 10 and/or 20 mg/kg/hr. **Time-Course Phase:** Levels of T_3 (24% to 32% of controls) and reverse T_3 (58% to 73%) decreased on days 3-14 and 7-14, respectively. T_3 was minimally affected on Day 14 (83%). Levels of thyroid stimulating hormone (TSH) increased 53% on Day 7, and were paralleled by an increase in absolute (91%) and relative (75%) thyroid weights, and by minimal-to-mild follicular cell hypertrophy (Day 7-14). Hepatic microsomal UDP-glucuronosyltransferase activity increased 75% to 96% on Day 3 and 14, and was inversely correlated with serum T_4 concentrations (r^2:0.47-0.69; $P<0.01$-0.05). Significant increases were also noted in CYP450, EROD, PROD, and erythromycin n-demethylase. These results suggest that thyroid changes associated with probenecid result from disturbances of the hypothalamic-pituitary-thyroid (HPT) axis consequent to increased biliary clearance of T_4-UDPGT. However, direct effects on the HPT axis cannot be excluded based on TSH and T_4 profiles, and structural similarities between probenecid and known inhibitors of thyroxine peroxidase.

1219 ATRAZINE DISRUPTION OF THE HYPOTHALAMIC-PITUITARY AXIS: CONSEQUENCES FOR REPRODUCTIVE FUNCTION.

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The triazine herbicide atrazine (ATR) has been reported capable of disrupting endocrine processes that govern reproductive function in rodents. Prior studies have pointed to an anti-estrogenic profile for ATR, and it was observed that high dose ATR treatment to ovariectomized (ovex), estrogen-primed Sprague Dawley (SD) rats led to an attenuated LH surge response. In this study the effects of ATR on the hypothalamic-pituitary axis in the presence and absence of estrogen were examined. Ovex SD rats were administered a control vehicle (carboxymethylcellulose; CMC) or ATR (300 mg/kg BW via gavage) for 6 days. On days 3-6 subgroups of the vehicle and ATR-treated animals received either peanut oil (100 µl s.c.) or beta-estradiol-3-benzoate (EB; 15 µg/100 µl oil s.c.). Trunk plasma and hypothalamus were collected from each animal between 14.00-15.00 hours on day 7. A significant main effect of EB was detected in the reduction of plasma gonadotropins and body weights, and in the augmentation of hypothalamic GnRH content. ATR exhibited an overall effect in the increase of plasma FSH and in the decrease of animal body weights. In addition, a trend was found toward a decrement in plasma LH and an augmentation of hypothalamic GnRH content. Interactions between EB and ATR were undetectable in any of the parameters measured, although an interaction did approach significance with plasma FSH. These results suggest that ATR does not likely alter estrogen regulation of the reproductive neuroendocrine axis, but that it can disrupt the normal processes taking place at the hypothalamic-pituitary level. It is possible that ATR interferes with the synthesis/release of GnRH or the activity of this peptide with its receptor. (Supported in part by Novartis Crop Protection.)

1220 MODULATION OF SERUM TESTOSTERONE AND PROSTATE ESTROGEN RECEPTORS α AND β BY DIETARY GENISTEIN.

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Genistein, a naturally occurring isoflavonoid, is primarily found in soybeans. It exerts its estrogenic/antiestrogenic effects primarily through binding to estrogen receptors α and β (ERα and β), and thereby may affect the growth and development of prostate. In addition, genistein has been reported to affect enzymes involved in steroid synthesis and metabolism. Here we report genistein's effects on ERα and β in prostate and on serum testosterone. Genistein was fed to F_0 male and female CD rats 28 days before mating at 0, 5, 100, 500 ppm. Exposure of F_1 pups continued throughout lactation. At weaning, the male pups from genistein dosed diet were either switched to control diet (designated as G/C, n=12) or continued to be on the same dose of dietary genistein (designated as G/G, n=12). At sacrifice (PND140), ventral prostate (VP) and dorsolateral prostate (DLP) were removed for histopathological evaluation (n=6-8 per treatment group) and measurement of ERα & β levels by Western blot (n=6 per treatment group). Radioimmunoassay analysis indicated that serum testosterone (T) and dihydrotestosterone (DHT) levels exhibit an increasing linear trend ($p \leq 0.05$) in G/G treated groups, but not in G/C groups. Serum T levels differed significantly from controls in the 500 ppm G/G group. Patterns of ER proteins, as detected by Western blot analysis, varied depending upon receptor type, tissue (DLP or VP), and dosing regimen. ERα was increased in DLP at 5 ppm (~50% over control) and in VP at 5 and 100 ppm (~25 and 31% over control, respectively) with the G/C treatment. In G/G groups, ERα was decreased in DLP at 100 ppm (~59% of control). ERβ was generally decreased in the DLP at all doses (46-89% of control) in both G/C and G/G groups. There were mixed results in the expression of ERβ in VP. These preliminary results suggest that genistein may alter serum T levels and act in dose- and tissue-dependent manner in modulating the expression of ERα and β. While the mechanisms whereby genistein alters hormone and receptor levels remain to be elucidated, the observed effects may have important implications in male reproductive tract function and susceptibility to or prevention of prostatic carcinogenesis.

1221 EFFECT OF 3,3',4,4',5,5'-HEXACHLOROBIPHENYL (PCB 169) ON FREE AND TOTAL THYROXINE IN RATS.

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Hypothyroxenemia in rats has been well documented as a result of either developmental or adult exposure to polychlorinated biphenyls (PCBs). PCBs may cause these effects through induction of catabolic enzymes or interference with plasma transport proteins. Transthyretine is the major thyroid hormone transport protein in rats. This preliminary study investigates the effects of a single oral dose of PCB 169 (concentration of 1 mg/kg given at 0.5, 1, 2, 4, 8, 24, or 48 prior to sampling), in 27 d old female Hooded Long-Evans rats. We hypothesized that displacement of thyroxine (T4) from transthyretine by PCB169 would result in an increase in the free concentration of T4. Serum total and free thyroxine concentrations (TT4 and FT4, respectively) were determined by radioimmunoassay kits. No significant increase in TT4 or FT4 concentrations was seen at any time point as a result of PCB 169 exposure. However, both TT4 and FT4 decreased about 20% after 24 to 48 hours. Based on these results it would appear that PCB 169 does not disrupt the free/bound ratio of thyroxine in young rats. The decrease in FT4 and TT4 at the later time periods is probably due to induction of catabolic enzymes in the liver. The lack of effect on FT4 during the early time points may result from either a minimal role for transthyretine binding in the PCB mediated decreases in T4 or to binding of T4 to alternative transport proteins. Young rats are known to have significant concentrations of thyroid binding globulin (TBG) up to eight weeks of age and PCBs do not displace T4 from TBG. Future studies will investigate this issue with older rats and other PCB congeners. (This abstract does not necessarily reflect the policy of the US Environmental Protection Agency.)

1222 EFFECT OF NONYLPHENOL ON SERUM TESTOSTERONE LEVELS AND TESTICULAR STEROIDOGENIC ENZYME ACTIVITY IN NEONATAL AND PUBERTAL RATS.

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Nonylphenol is an environmentally persistent degradative product of alkylphenol polyethoxylates, which are widely used in a variety of household and commercial applications. Perinatal administration of nonylphenol and other alkylphenols has suggested that these compounds may interfere with male reproductive tract development. Therefore, these studies examined the effects of nonylphenol on serum testosterone levels and testicular steroidogenic enzyme activities at critical developmental stages. Male rats were dietarily exposed to various doses of nonylphenol (0, 25, 200 and 750 ppm) throughout gestation and until sacrifice at either postnatal day 2 (PND2) or PND50. At PND2, serum testosterone levels were significantly decreased in all groups exposed to nonylphenol. The activity of 17 alpha-hydroxylase/C17-20 lyase (P450c17) in PND2 testicular homogenates was not affected by nonylphenol treatment. However, the addition of nonylphenol (0.1-100 micromolar) to the metabolic incubation resulted in a dose-dependent decrease in enzyme activity. In PND50 rats, nonylphenol treatment did not affect serum testosterone levels. The dorsal prostate weight was increased in the 200 and 750 ppm dose groups; no effects were observed in other male reproductive organs. Nonylphenol treatment did not affect P450c17 activity in microsomes prepared from testes of PND50 rats. However, the presence of 100 micromolar nonylphenol in the incubation resulted in a significant decrease in enzyme activity. These results suggest that nonylphenol exposure results in decreased serum testosterone levels in neonatal rats, and that inhibition of steroidogenic enzymes may be a contributing factor.

1223 THYROID HORMONE DISRUPTIVE EFFECTS OF BROMINATED DIPHENYL ETHERS FOLLOWING DEVELOPMENTAL EXPOSURE.

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Our previous work has demonstrated that short-term *in vivo* DE-71 (mostly tetra- and penta-bromodiphenyl ethers, a commercial polybrominated diphenyl ether mixture) exposure induces hypothyroxenemia via induction of uridinediphosphate-glucuronosyltransferases (UDPGTs). In this study, we examined thyroid hormones and thyroxine glucuronidation in rats following perinatal maternal exposure to DE-71. Primiparous rats were orally administered DE-71 (0, 1, 10 and 30mg/kg/day) in corn oil from gestation day (GD) 6 to postnatal day (PND) 21. Serum and liver samples obtained from dams

(GD21 and PND22), fetuses (GD21) and offspring (PND4, 14, 36 and 90) were analyzed for circulating total serum thyroxine (T4), triiodothyronine (T3), thyroid-stimulating hormone (TSH) or hepatic microsomal ethoxy- and pentoxy-resorufin-O-deethylase (EROD and PROD) and UDPGT activities. Serum T4 was reduced in a dose dependent manner in fetuses on GD21 (maximal 41%) and offspring on PND4 and PND14 (25, 50, and 70% maximal in the 1, 10, and 30 mg/kg/day groups, respect-ively), but recovered to control levels by PND36 and 90. Reduction in serum T4 was also noted in GD21 dams (44% at highest dose), as well as PND22 dams (49% at highest dose). There was no significant effect of DE-71 on T3 concentrations at any time in the dams or the offspring. Increased liver/body weight ratios were consistent with induction of EROD (100-fold) and PROD (20-30 fold) in offspring. These data support the conclusion that DE71, a commercial mixture of brominated diphenyl ethers, is an endocrine disruptor in rats during development. (This abstract does not necessarily reflect the policy of the US Environmental Protection Agency.)

1224 PURE POLYCHLORINATED BIPHENYL (PCB) METABOLITES CROSS THE PLACENTA IN THE RAT AND MAY REDUCE FETAL THYROID HORMONE LEVELS.

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This study was designed to determine the maternal to fetal transport of the polychlorinated biphenyl (PCB) PCB-metabolite 4-OH-2,3,3',4',5-pentaCB (PCB-OH) and the effects on maternal and fetal thyroid hormone homeostasis. Pregnant Wistar rats were orally exposed to 0 or 5 mg PCB-OH per kg body weight per day from gestation day (GD) 10 to 16. A separate group of rats received 4.2 mg/kg bw/day of a radiolabelled [14C] PCB-OH from GD10-16. On GD17 and GD20 maternal and fetal blood and a wide range of tissues were collected. Tracer analyses showed that most (approx. 88%) of the total administered PCB-OH was excreted in the faeces; maternal and fetal organs contained only 5% and 2.3%, respectively. Fetal brain contained 2-4 times more PCB-OH compared to maternal brain. Maternal plasma T3 and T4 levels were unaffected. Fetal T4 levels were decreased dose-dependently (maximal decrease of 91% on GD20, in high dose group). Fetal plasma TSH levels were significantly increased by 124% (5 mg/kg). No effects on maternal or fetal liver T4 UDP-glucuronyltransferase activity were detected. The PCB-OH in plasma was bound to fetal transthyretin. These data demonstrate that pure PCB-metabolites are able to cross the placenta and reduce fetal thyroid hormone levels by binding to transthyretin. (This study was supported by the commission of the European Communities [grant ENV-CT96-0170].)

1225 COMPARISON OF THE ORAL (GAVAGE) AND INTRAPERITONEAL ROUTES OF ADMINISTRATION FOR IDENTIFYING ENDOCRINE-ACTIVE COMPOUNDS (EACS) USING AN *IN VIVO* MALE BATTERY, AND EVALUATION OF IMMUNE SYSTEM ENDPOINTS.

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An *in vivo* screening battery using intact adult male rats has been evaluated for its ability to detect four EACs using two different routes of administration, oral gavage and intraperitoneal (IP) injection. The test compounds included the antiandrogens flutamide (FLUT) and *p,p'*-DDE (DDE), the steroid biosynthesis inhibitor ketoconazole (KETO), and the thyroid toxicant phenobarbital (PB). Male rats were dosed for 14 days via oral or IP administration and euthanized on the morning of test day 15. The endpoints evaluated included: final body and organ weights, serum hormone concentrations, histopathology of the testis and thyroid gland, and evaluation of immune system endpoints (humoral immune function, spleen and thymus weights, and spleen cell number). After IP compound administration, all four test compounds were identified as expected based on their specific mode of action. FLUT and DDE decreased the weights for the androgen-dependent tissues (ASG, epididymis, prostate, and seminal vesicles), caused hormonal alterations (increased serum T, E2, DHT, FSH, and LH), and caused histopathologic changes of the testis (Leydig cell hyperplasia). KETO decreased the weights for the androgen-dependent tissues (ASG, epididymis, prostate, and seminal vesicles) and caused hormonal alterations (decreased serum T and DHT; increased serum FSH, and LH). PB did not effect organ weights; however, serum hormone levels were effected (decreased serum T, DHT, PRL, FSH, LH, T_3, T_4; increased serum TSH and E2) and thyroid follicular cell hypertrophy was observed. After oral compound administration, FLUT and PB displayed the same pattern of altered endocrine and reproductive responses that were observed by the IP route. Also, orally administered FLUT and PB did not alter the primary humoral immune response to SRBC. PB increased spleen weight at the highest dose. DDE and KETO are currently being evaluated by the oral route.

1226 EVALUATION OF OESTROGENIC POTENTIAL OF BISPHENOL A IN THE UTEROTROPHIC ASSAY BY ORAL AND PARENTERAL ROUTE AT EXTENDED DOSE RANGES.

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The objective of this study was to evaluate the oestrogenic potential of bisphenol A (BPA) at both conventional doses and (ultra) low doses by different routes of administration using the current short-term *in vivo* model for oestrogenicity, the uterotrophic assay. Groups of 10 immature Wistar strain derived female rats were given doses of 0, 0.002, 0.02, 0.2, 1, 10, 100, 200, and 800mg/kg/d orally or 0, 0.002, 0.02, 0.2, 1, 10, 100, and 800mg/kg/d subcutaneously (s.c.) for 3 consecutive days. 17beta-oestradiol at a dose of 0.4mg/kg/d served as a positive control. In addition to determining wet and dry uterus weights, histopathology was carried out for elucidating more discreet oestrogenic responses. These detailed investigations revealed indications of oestrogenicity at oral doses of 200 and 800mg/kg/d, and at s.c. doses of 10, 100 and 800mg/kg/d. The responses were dose-related, with no indication of oestrogenic activity at lower doses. Differences in kinetics and bioavailability of the parent compound (Pottenger et al., The Toxicologist 1998; 42 (1S): 282) provide an explanation for the different response thresholds seen between the two routes. In our uterotrophic assays the oestrogenic potential of BPA revealed a monotonic dose response with thresholds above the NOELs of toxicity that have been previously established in conventional studies. The absence of an oestrogenic response at (ultra) low doses corroborate the absence of reproductive toxicity (including reproductive organ weight effects) of BPA at these dose levels (Cagen et al., Toxicol Sci 1999; 50: 36-44; and Dimond et al., The Toxicologist 1999; 48: 45-46). These results do not support the hypothesis of a non-monotonic dose response of BPA-related oestrogenic potential *in vivo*. (Studies were sponsored by The Bisphenol A Global Industry Group.)

1227 DEMONSTRATION OF DOSE-RESPONSE OF THE 3-DAY UTEROTROPHIC ASSAY IN IMMATURE CRL:CD(SD)IGS BR RATS WITH ETHINYL ESTRADIOL AND COMPARISONS OF ROUTES OF ADMINISTRATION.

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The purpose of this study was to validate and demonstrate the dose-responsiveness of the rodent uterotrophic assay with ethinyl estradiol, in 18 day old rats. Recent attention is focused on assay conduct and validation for regulatory purposes. In the current study, ethinyl estradiol (EE) was administered to weaned, immature female rats on postnatal days (PND) 18, 19, and 20 at concentrations of 0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 10 µg/kg. Two additional groups of rats were treated with 0.1 or 1.0 mg/kg of the estrogen antagonist, ICI-182,780 (ICI) immediately followed by administration of 3.0 µg EE/kg. Untreated control groups were also included in the study design. Administration of EE or ICI was by oral gavage (PO) or by subcutaneous injection (SC). There were no clinical signs of toxicity or alterations in body weights or food consumption detected. On PND 21 the animals were sacrificed. The uterus, ovaries, and vagina were examined, and the uteri were weighed with and without fluid. Administration by PO or SC at concentrations of 3.0 and 10.0 µg EE/kg produced significant increases (2 to 4.4 fold) in absolute uterine wet weight and increased uterine fluid imbibition. Administration of 1.0 µg EE/kg (by SC) also produced a statistically significant increase in uterine wet weight (160% of control). By contrast, concentrations of 0.01 to 0.3 µg EE/kg (by SC) or 0.01 to 1.0 µg EE/kg (by PO) produced no alterations on any of the parameters measured. ICI (0.1 or 1.0 mg/kg) did not antagonize the uterotrophic effects of 3.0 µg/kg EE when administered PO, but produced an approximate 30% reduction in the estrogenic increase in uterine weight and the EE-induced uterine fluid imbibition was ablated when administered SC. These results support previous reports

that the SC is a more sensitive route than PO and demonstrate the feasibility of using weaned 18 day old rats.

1228 THE *IN VIVO* AND *IN VITRO* ESTROGENICITY OF PENTACHLOROPHENOL AND ACETAMINOPHEN.

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The identification of increasing numbers of xenoestrogens in our environment has been the source of much concern in recent years. Recently, Harnagea-Theophilus and Miller (1998) hypothesized that the presence of a substituted phenol in the structure of a compound may be indicative of its estrogenic potential. Based on this hypothesis, two phenol group containing compounds were selected and assessed for estrogenic potential. Acetaminophen (APAP) is a frequently used analgesic and previous studies have demonstrated evidence of estrogenic activity in estrogen-responsive human breast cancer cell lines. Pentachlorophenol (PCP) was once extensively used as a timber-treatment product and there is only limited data investigating its potential for xenoestrogen activity. The present study used uterine wet weight, estrogen receptor binding and estrogen and progesterone receptor quantification to evaluate the estrogenic capabilities of these compounds. Uterine wet weight was measured in 23 day old female C57Bl/6 mice dosed with vehicle, 17ß-estradiol (10 µg/kg/day), APAP (100, 200, 250 mg/kg/day) or PCP (20, 30 mg/kg/day) for 3 days and sacrificed 24 hours after the last dose. The ability to compete with 17ß-estradiol for binding to estrogen receptors was assessed in hepatic and uterine cytosolic and nuclear extracts. Extracts were prepared from 21 day old female C57Bl/6 mice, and saturation and competitive binding experiments were carried out using the hydroxyapatite method. Cytosolic and nuclear extracts from the treated animals were used to quantify progesterone and estrogen receptor status. The results indicated that there was no evidence of estrogenic activity for APAP (0.03-2.5 mM) in either nuclear or cytosolic hepatic or uterine extracts, the uterotrophic assay or as indicated by progesterone or estrogen receptor quantification. However, PCP (75-115 µM) consistently exhibited evidence of weak competition with 17ß-estradiol, in both uterine and hepatic extracts, yielding IC50 values of 95.14 and 71.20 µM, for hepatic cytosolic and nuclear extract, respectively and 91.07 and 98.53 µM for uterine cytosolic and uterine nuclear extracts. Furthermore, PCP (20 mg/kg/day) induced a significant increase in uterine wet weight (0.56 ±.02) relative to control (0.31±.06, $p<0.05$). Therefore acetaminophen displayed no estrogenic potential in these experiments, while PCP was weakly estrogenic.

1229 EVALUATION OF THE UTEROTROPHIC ASSAY IN THE IMMATURE FEMALE SPRAGUE-DAWLEY RAT USING OESTRADIOL BENZOATE (OB), BISPHENOL A (BPA) AND OCTYL PHENOL (OP).

D. R. Chubb and A. G. Richardson. *Quintiles England Limited, Ledbury, United Kingdom.* Sponsor: T. Palmer.

OB, BPA and OP were used in a series of experiments to evaluate the uterotrophic assay in the immature female Sprague Dawley rat. Animals were 21 days old on the first day of dosing and were dosed once a day for up to three days. On the fourth day, surviving animals were euthanased and the uterus was excised, blotted dry and weighed. Mean uterus weight was analysed using Students or Williams 2-sided t-Tests. Oral administration of 0.4mg/kg/day OB produced highly significant increases ($p<0.001$) in uterine weight compared with controls. BPA failed to produce an increase in uterine weight when administered orally (400mg/kg/day) but did produce a significant increase in uterine weight ($p<0.05$) when administered subcutaneously (150mg/kg/day). When this experiment was repeated using an increased group size no significant increase was detected. Oral administration of OP produced very significant increases ($p<0.01$) in uterine weight at a toxic dose level (800mg/kg/day) but no increase at a lower non-toxic dose (400mg/kg/day). Subcutaneous administration of OP produced a significant increase ($p<0.05$) in uterine weight at a toxic dose level (600mg/kg/day) and a very significant increase ($p<0.01$) at a non-toxic dose (300mg/kg/day). The assay clearly demonstrated the uterotrophic effect of a known oestrogen (OB) in the Sprague Dawley strain. The results obtained with BPA and OP indicate that the route of administration and group size are important factors in the detection of weak responses. Uterotrophic activity has been reported for BPA in Alpk:AP rats (Ashby and Tindell 1998) but the doses used could not be achieved in our facility in Sprague Dawleys due to toxicity, therefore the strain of rat may also be important. Further work on OP is ongoing to assess the enhanced uterotrophic response observed at non-toxic doses.

1230 ASSESSMENT OF THE OESTROGENIC ACTIVITY OF BENZOPHENONE SUNSCREEN AGENTS.

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Over the last few years, lists of chemicals described as "known or suspected endocrine disrupters" have been produced from numerous sources. Benzophenone, a chemical used as a fixative in perfumes and cosmetics for it's UV absorbing properties, has been listed among "chemicals suspected of having endocrine disrupting effects" by the Japanese Environment Agency. However, there is currently only one report from an *in vitro* study (Jobling, et al 1995) suggesting weak oestrogenic activity. Therefore, using a combination of *in vivo* and *in vitro* assays, the possible oestrogenic effects of benzophenone and benzophenone-3 (2-hydroxy-4-methoxybenzophenone), used as a sunscreen agent, have been investigated. Competitive binding *in vitro* with the immature rat uterine oestrogen receptor (ER) was used to measure the ability of benzophenones to bind to the ER and in addition an oestrogen-inducible yeast screen, expressing the human ER, was used to examine the transcriptional activation of oestrogen responsive genes. Benzophenone and benzophenone-3 did not display any oestrogenic activity in these *in vitro* assays tested up to a concentration of 10^{-3}M. Uterotrophic assays have been conducted to investigate the potential for benzophenones to elicit an oestrogenic response when administered orally to immature female rats at doses of 50, 150 or 500 mg/kg/day for three consecutive days. Benzophenone and benzophenone-3 did not exhibit oestrogenic activity in the immature female rat using uterine wet weight as the endpoint, whereas 17beta-oestradiol (0.4mg/kg/day) consistently produced a significant increase in uterine weights over vehicle control. In conclusion, we have shown that the suggestion of endocrine disruption for benzophnones is not supported when tested in a combination of *in vivo* and *in vitro* assays for oestrogenic activity.

1231 ASSESSMENT OF ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF D4 IN IMMATURE RATS USING THE UTEROTROPHIC ASSAY.

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The estrogenic and antiestrogenic effects of D4 were evaluated by measuring changes in uterine weight and epithelial cell morphology in immature Sprague Dawley (SD) rats. These data were compared to data obtained for ethinyl estradiol (EE), diethylstilbesterol (DES) and coumestrol (CE). Beginning on postnatal day 18, the pups (12/group) received a single oral dose of either D4 (0, 10, 50, 100, 250, 500,and 1000 mg/kg/day), EE (1, 3, 10, and 30 mg/kg/day), DES (0.5, 1.5, 5, and 15 mg/kg/day), or CE (10, 35, 75, and 150 mg/kg/day) in sesame oil once daily for 4 consecutive days. Antiestrogenic effects were determined by coadministering D4 (500 mg/kg/day) with varying amounts of EE. ICI 182,780, a potent antiestrogen, was coadministered (3 mg/kg/day) with EE as a positive control. On the morning following the last dose, animals were euthanized and their uteri removed, weighed, and processed for histological examination. Uterine weight versus log dose was analyzed by linear regression analysis. The coefficients obtained were used to calculate relative potency. Absolute and relative uterine weights and epithelial cell height were statistically increased at all doses of D4 above 100 mg/kg/day. When D4 was coadministered with EE there was a statistically significant reduction in uterine weight compared to EE alone. The results indicate the following ranking based on estrogenic potency: EE > DES >>>>CE >> D4. Relative to the weak phytoestrogen CE, D4 was 83 times less estrogenic. Strain sensitivity was determined by testing Fischer rats under a similar experimental design. In conclusion, D4 showed weak estrogenic and antiestrogenic activity in both rat strains. Based on potency, SD rats were more sensitive to EE, DES, and D4 than Fischer rats. Thus, strain may play a role in the risk characterization of estrogenic or antiestrogenic compounds. (Supported in part by the Silicones Environmental Health and Safety Council.)

1232 BISPHENOL A- INDUCED INCREASES IN UTERINE WEIGHT AND STRESS PROTEIN LEVELS IN OVARIECTOMIZED B6C3F1 MICE ARE MEDIATED THROUGH THE ESTROGEN RECEPTOR.

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We have previously shown that bisphenol A (BPA), a ubiquitous and abundant contaminant of the environment and food products, increased uterine weights and altered uterine morphology and stress protein levels in ovariectomized mice. From these *in vivo* studies we concluded that BPA was weakly estrogenic. In the present study we examined the effects of the estrogen receptor antagonist ICI 182,780 (ICI), on BPA-induced increases in uterine weight and stress protein levels. Ovariectomized B6C3F1 mice were injected subcutaneously daily for 4 days with corn oil, 40 ng beta-estradiol, 2 mg BPA, or a low (20 μg) or high (200 μg) dose of ICI alone or in combination with 2 mg of BPA. Uterine wet weights were taken, and levels of the stress proteins, hsp90alpha, hsp72, hsc73, and grp94 in uterine homogenates were evaluated by Western blot analysis. BPA increased uterine weights and levels of hsp90alpha and grp94, and these effects were partially reversed by the high dose of ICI. The results of this study suggest that the BPA-induced increases in both uterine weight and stress protein levels are at least partially mediated through the estrogen receptor.

1233 IMPOSEX INDUCTION BY NEUROHORMONES.

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The peptide hormone APGWamide induces imposex more effectively than either tributyltin or testosterone in *Ilyanassa obsoleta*. Imposex, the imposition of male characteristcs on female snails, is caused by exposure to tributyltin (TBT) at concentrations as low as 2 ng/L. The mechanism of imposex induction has been proposed to be the inhibition of aromatase. However, the role of vertebrate steroid hormones in sexual development of molluscs has been questioned since only peptide hormones play a significant role in sexual development in most invertebrate phyla. Mud snails, *Ilyanassa obsoleta*, were injected (i. p.) every other day with either TBT (2 or 20 ng), testosterone (5 or 50 ng), or one of four peptide hormones: APGWamide, conopressin, LSSFVRIamide, or FMRFamide (1 or 100 picomoles). Control snails were injected with either PBS (for peptides) or ethanol (TBT and testosterone). Twenty snails were sacrificed after 7 and 14 days for each exposure group, sexed, and percent imposex and penis length measured. Imposex in control snails ranged from 7-11%, with an average penis length of 0.16 mm. Both TBT and testosterone significantly induced imposex at the 20 ng level and 50 and 500 ng levels, respectively. Maximal induction was by 500 ng testosterone on day 14 at 39%/0.25 mm. APGWamide significantly induced imposex at both doses. The 1 pmol dose imposex levels were 69%/0.32 mm after 7 days, and 80%/0.49 mm after 14 days. The 100 pmol dose imposex levels were 65%/0.24 mm after 7 days and 81%/0.28 mm after 14 days. The other neurohormones did not significantly induce imposex. From this data, it is clear that APGWamide induces imposex much more rapidly and results in longer penis length than either TBT or testosterone.

1234 MOLECULAR CLONING OF ESTROGEN RECEPTOR ISOTYPES IN LARGEMOUTH BASS, *MICROPTERUS SALMOIDES*.

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Environmental endocrine disruptors have caused a variety of adverse biological effects in wildlife species. Many of these compounds have been shown to mimic estrogens and mediate their effects via the estrogen receptor (ER). The discovery of estrogen receptor isotypes raises questions regarding the mechanisms of action of estrogen and related environmental contaminants. We have recently cloned portions of both ERα and ERβ cDNA from largemouth bass liver tissue. A third sequence with mixed homology to ERα and ERβ has yet to be characterized. Using alpha-specific primers, 553bp and 900bp sequences were amplified that contain as high as 93% homology to the ERα ligand and DNA binding domains of other fish species. Overall, a 1.4kb sequence can be pieced together representing domains C,D, and E of ERα. A portion of ERβ cDNA spanning the DNA binding domain and following loop region was amplified using primers that were specific at the 3' end. With these primers, two sequences were produced with approximate sizes of 500 bp each. Cloning and sequencing showed that one fragment shared 90% homology with ERβ of other aquatic species including gilthead and tilapia, while the other insert showed high but mixed homology to ERα and ERβ. These fragments can be used as probes in the further characterization of the receptors while concurrent work ensues to obtain the entire coding sequences. The availability of receptor isotypes will allow for determination of how xenobiotic contaminants interact with the ER's to illicit their effects.

1235 COMPARATIVE ENDOCRINOLOGY STUDY OF DEHYDROEPIANDROSTERONE (DHEA) AND A FLUORINATED ANALOGUE IN RATS.

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This study compared the endocrinologic effects of DHEA and fluasterone (fl-DHEA) in male and female rats following four weeks of daily oral (gavage) administration. DHEA is an adrenal steroid that is under development as a potential cancer chemopreventive agent. However, due to DHEA's endocrine effects, a fluorinated analogue (fl-DHEA) was synthesized as an alternate agent. Initial dose levels studied were 0, 30 and 300 mg/kg/day for each compound (N = 12/sex/group). However, due to weight loss at the high dose level for each drug, dose levels were lowered to 150 mg/kg/day for DHEA and fl-DHEA. DHEA at 30 or 300/150 mg/kg/day produced an estrogenic effect in female rats expressed as decreased plasma FSH and LH, inhibition of ovulation, prolonged estrus and increased uterine estrogen receptors. DHEA also increased plasma levels of androstenedione in both sexes. fl-DHEA decreased plasma FSH, LH and tissue estradiol, and increased plasma dihydrotestosterone levels in both sexes. fl-DHEA at 30 or 300/150 mg/kg/day increased the diestrus II phase, while 300/150 mg/kg/day fl-DHEA decreased the weights of the uterus, prostate, seminal vesicles and testes, and decreased the occurrence of estrus. Qualitatively different endocrinologic effects were produced by the compounds; DHEA produced an estrogenic response in female rats, while fl-DHEA produced complex endocrinologic effects in both sexes. (Supported by NCI Contract No. NO1-CN-75127-MAO.)

1236 CALPAIN ACTIVITY, AND CALPAIN INHIBITORS IN DELAYED NEUROPATHY INDUCED BY ORGANOPHOSPHATES (OP): MONITORING USING AUTOANTIBODIES.

H. El-Fawal and A. DeFeo. *Mercy College, Dobbs Ferry, NY.* Sponsor: J. P. O'Callaghan.

Previous studies demonstrated the attenuating effects of calcium channel blockers or calpain inhibitors on the development of OP-induced delayed neuropathy (OPIDN) in hens. This modifying effect by calcium channel blockers extended to the attenuated activity of the calcium-dependent protease calpain. Therefore, selective calpain inhibitors I (CaI) and II (CaII) were used to modify the development of OPIDN following a single dose of phenyl saligenin phosphate (PSP, 3 mg/kg, im). CaI and CaII (5 mg/kg, im) were administered immediately before PSP and 24h after PSP. Quantification of clinical signs was by measurement of stride width and length. This indicated that functional impairment was greatest in hens treated with PSP only. CaI and CaII did not attenuate the inhibition of neuropathy target esterase (NTE) produced by PSP at 24h, nor did they themselves inhibit NTE. Calpain activity in brain, spinal cord, and sciatic nerve were 140%, 130%, and 175%, respectively, compared to controls at 14 days post-PSP exposure. The amelioration of the degree of OPIDN development was confirmed by histological examination of the biventer cervicis nerve, and by assay for serum autoantibodies to neurofilaments (Nfs), and myelin basic protein (MBP). Autoantibodies to Nfs were detected as early as 6 days post-PSP exposure and predominated in hens not receiving calpain inhibitors. Autoantibody levels significantly correlated with the decline in gait length at 14 and 21 days. This data further implicates calpain in development of OPIDN, and suggests that calpain inhibitors can modify OPIDN. Furthermore, serum autoantibodies to nervous system proteins may be used to monitor neuropathy development and intervention. (Supported by NIH HD35965.)

1237 NEUROPATHY IN ORGANOPHOSPHOSPHORUS PESTICIDE (OP)-EXPOSED WORKERS: MONITORING USING AUTOANTIBODY (AAB) TO NERVOUS SYSTEM AND MUSCLE PROTEINS.

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Sponsor: J. P. O'Callaghan.

Human exposed to pesticides or heavy metals have been shown to have serum AAb to nervous system (NS) proteins that may be indicative of subclinical neurotoxicity. Some OP pesticides are suspected of causing neuropathy and/or myopathy. Agricultural workers exposed to OP for a period exceeding 5 years (n=65) and a reference population from a food processing plant (n=50, no direct exposure) were assessed for possible neuropathy and/or myopathy using serum AAb detection against neurofilaments (NF), myelin basic protein (MBP), desmin (D), choline acetyl transferase (ChAT), and acetylcholinesterase (AChE). OP pesticides included metamidophos, and chlorpyrifos, both suspected of causing neuropathy. IgG to NF, neuronal intermediate filaments, and MBP predominated in pesticide workers. IgG to ChAT, a presynaptic enzyme of motor neurons, was found only in pesticide-exposed workers. IgG to D, a muscle intermediate filament, and to AChE were detected in 45% of the pesticide-exposed group. AChE activity was 40-60% of normal (24 IU/g Hb). In contrast, few of the reference population had detectable IgG levels, only IgM to NF. None had either IgG or IgM to MBP, D, ChAT, or AChE. IgG levels to NF correlated with exposure duration. In addition, IgG levels to NF and D correlated with peripheral sensorimotor deficits (muscle tremor and reflexes). The detection of AAb to NF, and MBP suggests neuronal involvement, while those to ChAT suggest α-motorneurons. Neuromuscular damage is further supported by detection of AAb to D, possibly due to AChE inhibition. OPs are known to produce neuropathy resulting in AAb. This study suggests that AAb detection may be useful in monitoring pesticide neurotoxicity in the field. (Supported by NIH HD35965.)

1238 THE EFFECT OF REPEATED ORAL EXPOSURES TO CHLORPYRIFOS OR METHYL PARATHION ON BRAIN CHOLINERGIC AND DOPAMINERGIC SYSTEMS IN DEVELOPING RATS.

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The wide use of chlorpyrifos (CPS) and methyl parathion (MPS) is of great public health concern, particularly because juveniles are more sensitive to these compounds. The effect of CPS or MPS on cholinesterase (ChE) activity, muscarinic acetylcholine receptor (mAChR) subtypes (M1 and M2) densities, choline acetyltransferase (ChAT) activity, and dopamine (DA) receptors (D1 and D2) densities in cortex and/or striatum was studied in early postnatal rats gavaged daily with CPS or MPS in corn oil from postnatal day (PND)1 through PND 21. Rats in the low dosage groups received CPS and MPS at 1.5 and 0.3 mg/kg, respectively, for all treatments. Rats in the high dosage groups received incremental dosages from 1.5 to 6 mg/kg for CPS or from 0.3 to 0.9 mg/kg for MPS. This dosing protocol resulted in about 32% and 62% ChE inhibition in cortex and striatum, respectively, in the low dosage groups of both compounds, and about 70% and 80% ChE inhibition in cortex and striatum, respectively, in the high dosage groups of both compounds on PND 22. M1 and M2 mAChR densities in cortex were about 73% and 79% of control levels in the high CPS and MPS dosage groups, respectively, and in striatum about 59% and 76% of control levels in the high CPS and MPS dosage groups, respectively, on PND 22. ChAT activity in the high MPS dosage group was 82% of control levels on PND 22. D1 DA receptor density in striatum decreased (about 61% of control levels in both compounds) on PND 6 and increased (about 120% and 133% of control levels in the CPS and MPS groups, respectively) on PND 14. D2 DA receptor density in striatum was about 72% of control levels in all treated groups on PND 22. The results indicate that changes in cholinergic and dopaminergic systems were induced by repeated oral exposure to CPS or MPS in developing rats. (Supported by NIH RO1 ES04394.)

1239 ANALYSIS OF THE ADDITIVITY OF *IN VITRO* INHIBITION OF AChE BY MIXTURES OF CHLORPYRIFOS-OXON AND AZINPHOS-METHYL-OXON.

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Organophosphorus (OP) insecticides act through a common mechanism of toxicity, the inhibition of acetylcholinesterase (AChE) by their active metabolites. The effects of *in vitro* exposure of AChE to chlorpyrifos-oxon (C=O) and azinphos-methyl-oxon (AZM=O), was investigated to determine if concurrent exposure to these two OP compounds results in purely additive effects. Inhibition values (concentrations of inhibitor required to elicit 10-90% inhibition of AChE) were determined individually for C=O and AZM=O in increments of 10% for AChE in a rat brain membrane preparation. Then, percentage inhibition for various mixtures of the two compounds was determined by the addition of a defined concentration of C=O (110, 120, or 130) along with various defined concentrations (110-170) of AZM=O, and repeated with AZM=O held constant and C=O varied at the same concentrations. The theoretically calculated percent inhibition, which takes into account the fraction of AChE molecules assumed to be available for inhibition by the second compound following inhibition by the first compound, was calculated for the various mixtures and compared to the experimentally observed percentage inhibition. Theoretical calculations determined that the inhibition of AChE by mixtures of AZM=O and C=O would result in less than simple mathematical summation of percent inhibitions, and was confirmed by experimental results. For example, an 120 of C=O and an 140 of AZM=O yielded a theoretical inhibition of 49.35% and experimental results yielded a value of 49.4% inhibition, not the 60% which is calculated by simple summation of the individual percent inhibitions. These data suggest that simple mathematical summation of percent inhibitions is not the appropriate method for describing the combined effects of C=O and AZM=O on AChE *in vitro*.

1240 BRAIN ACETYLCHOLINESTERASE SENSITIVITY TO INHIBITION BY ORGANOPHOSPHATES IN JUVENILE AND ADULT RATS.

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Juvenile rats are more sensitive to the acute toxicity of organophosphate (OP) insecticides than are adults. Brain acetylcholinesterase (AChE) sensitivity, as measured by IC50 analysis, has been shown to be the same in both juvenile and adult rats for paraoxon (Atterberry et al., TAP 147:2, 1997) and chlorpyrifos-oxon (Padilla et al., TAP 148:1, 1998). Based on these data alone, it could be assumed that no age-related differences in AChE sensitivity occurs. AChE was assayed in whole brain homogenates spectrophotometrically using acetylthiocholine as the substrate and 5,5'-dithio-bis(2-nitrobenzoic acid) as the chromogen. IC50 values for diazoxon, azinphosmethyl-oxon, O-methoate, coroxon, paraoxon, chlorpyrifos-oxon, methyl paraoxon, fenitrooxon, malaoxon and phoxim-oxon were determined in 12 and 70 day old male rats. IC50 values for these 10 compounds ranged from 2 nM to 15,867 nM. Similar IC50's for coroxon, paraoxon, chlorpyrifos-oxon, methyl paraoxon, fenitrooxon and malaoxon were observed for both ages. However, diazoxon, azinphosmethyl-oxon, O-methoate, and phoxim-oxon exhibited 1.3-, 1.3-, 1.2-, and 1.2- fold lower IC50 values, respectively, for juveniles than adults ($p<0.05$). These data suggest that the sensitivity of brain AChE to inhibition by OP compounds may not always be the same for juveniles and adults, and that these differences are compound specific. The greater sensitivity of the AChE to inhibition by some OP compounds could contribute to the greater vulnerability of juveniles to OP insecticide toxicity.

1241 EFFECTS OF PCB EXPOSURE ON THE TOXIC IMPACT OF ORGANOPHOSPHORUS INSECTICIDES.

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Exposure to polychlorinated biphenyls (PCBs) can alter the metabolism of organophosphorus (OP) insecticides. PCB exposure not only increases cytochrome P450 levels but also increases levels of A-esterases and carboxylesterases which can detoxify the insecticides' active metabolites (oxons). Following administration of 4 mg/kg-day Aroclor 1254 in safflower

oil p.o. to female rats for 50 days, rats were injected i.p. with high sublethal dosages of the parent insecticides, parathion (P=S), methyl parathion (MP=S), or chlorpyrifos (C=S), or their respective oxons, paraoxon (P=O), methyl paraoxon (MP=O), or chlorpyrifos-oxon (C=O). Acetylcholinesterase (AChE) activity was determined in the medulla-pons, hippocampus, corpus striatum, and cerebral cortex at 2 and 24 hrs post exposure. There was induction of the protective esterases in the PCB rats. At 2 hrs, few differences in the amount of AChE inhibition were observed between control and PCB rats exposed to C=S, P=S, and MP=S. In control rats, the inhibition was higher at 24 hrs than at 2 hrs with C=S and P=S but not with MP=S. The P=S and C=S treated PCB rats had lower levels of AChE inhibition than did the controls suggesting that preexposure to PCBs has a protective effect against the acute toxicity of these two compounds but not MP=S. At 2 hrs with the oxons, no differences in the amount of AChE inhibition were observed between control and PCB rats suggesting that PCB-induction of esterases does not have a protective effect. Substantial recovery of AChE activity had occurred by 24 hrs with all three oxons. However, unlike the other oxons, AChE activity in the P=O treated PCB rats had recovered to a greater extent than had the P=O treated controls suggesting a possible PCB-mediated mechanism which allows faster recovery from P=O exposure. Thus, PCB exposure can attenuate the toxic effects of C=S and P=S but not MP=S or the respective oxons of the insecticides.

1242 ELECTROENCEPHALOGRAM (EEG), CORE TEMPERATURE (T_C) AND MOTOR ACTIVITY (MA) IN THE RAT EXPOSED TO CHLORPYRIFOS (CHP).

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There are reports of acute and chronic changes in the EEG of humans exposed to organophosphate (OP) pesticides. However, little has been done to correlate EEG changes in humans to that of experimental animals exposed to OPs such as CHP. Our study used radio telemetry to monitor cortical EEG, T_c and MA in undisturbed, unrestrained rats exposed to CHP. Transmitters (Data Sciences, St. Paul, MN; model TA10ETA-F40) were implanted intraperitoneally. EEG leads were threaded under the skin and fixed to the skull. EEG power spectra were computed in all vigilance states and averaged hourly at least a day before and after the administration of CHP (25 mg/kg; p.o.). CHP led to a significant decrease in EEG power in the 7-8, 9-13 and 14-27 Hz ranges and increases in the 1-2 and 4-6 Hz ranges. Power shifts were greatest ~4 hr after CHP and were associated with reductions in T_c and MA. The muscarinic agonist oxotremorine (OX) (0.2 mg/kg; s.c.) led to similar marked reductions of EEG power in the 7-27 Hz range. However, OX had no effect on EEG power at the low frequencies. OX also led to a significant reduction in T_c and MA. We propose that the changes in the EEG following CHP are partly attributed to stimulation of muscarinic receptors in the CNS. However, the increase in slow wave activity following CHP is apparently unrelated to muscarinic stimulation. (This abstract does not necessarily reflect US EPA policy.)

1243 COMPARISON OF NEUROLOGICAL AND COGNITIVE EFFECTS FOLLOWING SUBCUTANEOUSLY-ADMINISTERED CHLORPYRIFOS.

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Chlorpyrifos, a widely used organophosphorus pesticide, produces in rats alterations in several cognitive tasks, including delayed match-to-position and sustained attention. A profile of lowered activity, ataxia, and decreased sensory responsiveness has also been characterized using a functional observational battery (FOB) and motor activity. This study was conducted to compare the relative sensitivity of neurological and cognitive measures in detecting effects of chlorpyrifos. Adult Long-Evans rats (n=8/dose) were trained to perform an appetitive signal detection task (SDT) designed to assess sustained attention. When performance was stable, rats received a single subcutaneous dose of either 0, 60, 125, or 250 mg/kg chlorpyrifos. SDT testing continued daily after dosing. These rats were also evaluated using the FOB and motor activity before dosing, and 3, 7, and 17 days after the dose. For both types of tests, significant effects were observed only in the high dose group (250 mg/kg). In the SDT, chlorpyrifos produced an increase in the proportion of false alarms for 4 weeks after dosing, and reduced the proportion of hits at high signal intensities during week 1 only. A few behavioral effects were observed with the FOB in the same dose group. These changes included decreased rearing in the open field at all test times, and decreased response to the tail-pinch stimulus at the 3-day test only. These data reflect impaired sustained attention after administration of subcutaneous chlorpyrifos, at a dose that also produced neurobehavioral alterations. (This abstract does not necessarily reflect US EPA policy.)

1244 NEUROLOGIC ASSESSMENT OF RATS FOLLOWING LOW DOSES OF SARIN, PYRIDOSTIGMINE, CHLORPYRIFOS, AND DEET.

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Potential neurobehavioral and neuropathologic effects in rats due to exposure to low levels of Sarin (GB), alone or in combination with other compounds to which soldiers may have been exposed during the Persian Gulf War, were assessed. Doses of pyridostigmine bromide (PB) im, Sarin (GB) sc, and chlorpyrifos (CPF) topically which produced approximately 20-30, 5-10, and 5-10 percent whole blood cholinesterase inhibition (ChE-I), respectively, were used along with 15 μL N,N-diethyl-m-toluamide (DEET;≥ 97%) topically, either separately or combined, in adult Sprague-Dawley rats. Compounds were given either once a day (PB,GB) or twice a day (CPF, DEET) for four days. Blood samples were taken prior to initial dosing, the day following the last dose, and 30 days after the start of dosing to measure ChE-I. Neurobehavioral assessments were accomplished prior to dosing, the day after the last dose, and the 15th and 30th day after the start of dosing using a Functional Observational Battery (FOB) and a Figure 8 Activity Monitor. Rats were then sacrificed and selected tissues from the central and peripheral nervous systems were collected and examined microscopically. No neurobehavioral or neuropathologic effects could be attributed to dosing with GB, alone or in any combination, or to the other compounds. (This research was supported by the Department of the Army under Contract No. DAMD17-97-C-7067.)

1245 ACUTE SARIN EXPOSURE LEADS TO CHOLINERGIC DYSREGULATION IN CENTRAL NERVOUS SYSTEM OF RATS.

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The neurotoxic effects of the nerve agent sarin (isopropyl methyl phosphonoflouridate are mediated, primarily by inhibition of acetylcholinesterase (AChE). Other biological targets of sarin that are linked to acetylcholine metabolism, however, have not been evaluated in detail. In the present study, we evaluated the enzymes involved in the biosynthesis and degradation of acetylcholine after acute exposure to sarin. Male Sprague-Dawley rats were exposed to a single LD50 dose (100μg/kg, i.m.) of sarin for various time periods and their brain AChE, choline acetyl transferase (ChAT) and plasma butyrylcholinesterase (BChE) activities were studied. Brain AChE activity was significantly inhibited in cortex, mid brain, brain stem and cerebellum and plasma BChE by 1 hr and remained inhibited for extended period following single exposure. In contrast, ChAT activity registered an increase in cortex and brain stem at 1 and 3 hr post exposure, but remained unchanged in mid brain. Also, we detected only minimal activity in the cerebellum that remained unchanged after sarin exposure. These data suggest that acute sarin exposure may modulate the biosynthesis and degradation of acetylcholine differentially and thereby cause dysregulation in excitatory neurotransmission. (Supported in part by a DOD Grant DAMD 17-98-C-8027.)

1246 TOXICITIES OF ORGANOPHOSPHORUS COMPOUNDS IN A HUMAN NEUROBLASTOMA (SH-SY5Y) CELL LINE.

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Organophosphorus compounds (OPs) are widely used for the purpose of pest control. A major portion of military chemical warfare agents is also comprised of OP compounds. OP toxicity is primarily associated with the inhibition of cholinesterase activity, resulting in over-stimulation of acetylcholine that in turn could lead to death of exposed organism by suffocation. Unlike OP compounds such as paraoxon that inhibit cholinesterase activity acutely, some OP compounds, such as mipafox and DFP, can induce organophosphate induced delayed neuropathy (OPIDN) which is characterized by a 10-14 day delayed onset of symptoms such as ataxia and muscle paralysis. This study reports on

the evaluation of acetylcholinesterase inhibition of paraoxon, dementon-S, mipafox, and methyl parathion with the human neuroblastoma cell line, SH-SY5Y. Mipafox and paraoxon exhibited the highest and the lowest apparent IC_{50}s at 8.91 and 0.0084 μ M, respectively. Methyl parathion and dementon-S exhibited IC_{50}s in μ M range, 4.35 and 1.45 μ M, respectively. These findings indicate that oxidized compounds such as paraoxon have more potent anticholinesterase activity. In an amino acid incorporation assay, mipafox induced a significant reduction of [^{3}H]-leucine incorporation into protein at doses at or above 0.5 and 5 μ M for 4 and 7 day incubation, respectively. The SY5Y cell line responds to nerve growth factor (NGF) to express adrenergic activity and neurite growth. Among many proteins comprising the neurite, neurofilament (NF) is one of the major structural proteins. It was reported that NF-H is expressed much later in development than NF-L and NF-M. This study reports that NGF induced the increase of NF-H expression approximately 2 times over the control based on western analysis. It is expected that this protein can be one of the prospective tools to detect neuronal deformation related to OPIDN.

1247 DELAYED INTERACTIVE EFFECTS OF SARIN, PYRIDOSTIGMINE AND EXERCISE ON THE BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN MICE.

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Pyridostigmine (PYR), a reversible cholinesterase inhibitor, was used as a pretreatment drug for possible nerve agent exposure during the Gulf War. Military personnel were under physical stress and might have been exposed to low dose sarin. Hence, this study investigated the interactive effects of low-dose sarin, PYR and exercise training in mice. Male NIH Swiss mice were divided into five groups and treated as follows: (1) Control; (2) Sarin [0.01 mg /kg, s.c.]; 3) Sarin + exercise; (4) Sarin + PYR [1.2 mg/kg, p.o.]; (5) Sarin + PYR + 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. Tissues were dissected and processed for biochemical analysis and light and electron microscopy. Mild patchy lesions, small angulated fibers, central nucleation and foci of myonecrosis were observed only in the muscle of exercise + sarin or PYR + sarin groups. Sarin significantly decreased plasma BuChE activity, platelet, triceps muscle, and striatal AChE activity (81%, 71%, 68% and 63% of control, respectively); NTE activity in platelets, spinal cord, cortex and striatum (55%, 82%, 63% and 75% of control, respectively). Sarin significantly increased MDA levels in sciatic nerve and spinal cord (122% and 126% of control, respectively). Exercise + sarin group showed a significant reduction in plasma BuChE activity, platelets, triceps muscle, sciatic nerve and striatal AChE activity (79%, 58%, 56%, 76% and 71% of control, respectively). NTE activity in platelets, spinal cord, cortex and striatum (42%, 69%, 58% and 72% of control, respectively). Exercise + sarin group also showed a significant increase in plasma CPK activity and MDA in spinal cord (112% and 132% of control, respectively). PYR + sarin + exercise group significantly altered muscle AChE activity, MDA level, platelets, sciatic nerve spinal cord and cortical NTE activity. The data suggest that exercise potentiated the biochemical and histopathological changes in tissues of mice treated with sarin and sarin + PYR. (Supported by US Army contract # DMAD17-97-C-7066.)

1248 EFFECT OF LOW LEVEL SARIN EXPOSURE ON PHYSIOLOGICAL PARAMETERS IN RATS.

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Since the conclusion of the Persian Gulf War in 1991, some veterans have complained of diverse symptoms. This has increased interest in the potential long-term effects of single or repeated exposure to subclinical levels of nerve gas. As a part of this research effort, male F344 rats are being exposed singly (1 hour) or repeatedly (1 hour each day for 5 days or for 10 days) under normal and heat-stressed (core body temperature elevated by 1°C) conditions to 0, 0.2 or 0.4 mg/m^3 of sarin. The exposure levels represent literature values for the EC_{50} and one-half the EC_{50} for rats. Preliminary studies indicated that the exposures caused no overt symptoms of neurotoxicity (tremors) under non-heat stress conditions. Studies on temperature regulation indicated that 32°C was the ambient temperature required to raise the core body temperature of the rats (normally held at 25°C) by approximately one degree. When the rats were confined in nose-only exposure tubes, ambient temperatures of 17 and 22°C were required to maintain normal and heat-stressed core body temperatures, respectively. Analysis of changes in brain cytokine levels indicated that the cytokines (IL-1, IL-6), peaked after 3 days of heat stress; based on this information, animals were placed under heat-stress conditions for 3 days prior to exposure. During the study, the core body temperatures, as well as the motor activity of the rats, were monitored from 3 days before the beginning of the exposures until one month after the end of the exposures. Respiratory parameters were measured during the exposure. At 1 day and at 1 month after the end of the exposures, groups of rats were sacrificed and brains analyzed for total cytokines by RT-PCR and by *in situ* immunohistochemistry techniques. Brains were also analyzed for histopathology, including apoptosis and for concentration of cholinergic receptor sites. This is a report of the initial findings of the physiological response of the rats to inhaled sarin. Changes in respiration rate, core body temperature and motor activity were noted. (Research was funded by the US Army under Contract DAMD17-97-C-7054.)

1249 PERSISTENT NEUROBEHAVIORAL EFFECTS IN SPRAGUE-DAWLEY RATS FOLLOWING PRENATAL EXPOSURE TO THE ENVIRONMENTAL ESTROGEN, CHLORDECONE.

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The chlorinated pesticide, chlordecone, was banned in the USA because of its relatively strong estrogenic and neurotoxic activity. It is now useful as a model for estrogenic endocrine disruptors. Perinatal exposure of female rats to high doses of chlordecone results in altered vaginal opening, estrous cycling, and behavior. Because the developing brain is extremely sensitive to gonadal steroids, we examined the effect of prenatal chlordecone exposure on sexually dimorphic adult behaviors. We examined spontaneous activity and sex behavior in adult rats of both sexes to test if prenatal chlordecone (CD) exposure altered central nervous system function in adults. Chlordecone (5 mg/kg) was administered to pregnant rats on gestation day 16. At 50 days of age, body weight did not differ, but testis weight was significantly increased in males prenatally exposed to CD compared to controls ($p<0.001$). Behavioral tests were performed at 60-120 days of age following gonadectomy. Females prenatally exposed to CD displayed an increased ratio of inside/total crossings in an open field ($p<0.02$) with no change in total ambulation compared to controls. Males exposed to CD were not different from controls in the open field test. However, both males and females prenatally exposed to CD displayed increased levels of lordosis (female sex behavior) compared to controls when primed with estradiol benzoate and progesterone ($p<0.05$). After implanting with testosterone, a greater proportion of naïve males prenatally exposed to CD mounted stimulus females than naïve control males ($p<0.05$). These results suggest that prenatal exposure to the environmental estrogen chlordecone persistently altered sexually dimorphic behaviors in adult male and female rats. (Supported by a VA Merit Award to MMM and EKS and an American Fellowship from the American Association of University Women to SAL.)

1250 LINDANE *PER OS* INHIBITS ITS OWN ABSORPTION; PK 1195 EXACERBATES AND DEVAZEPIDE ANTAGONIZES THIS.

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Lindane is a neurotoxicant with antiGABAergic effects which include seizures, hypothermia and anorexia when given p.o. in the rat. PK 11195 (a 2-phenyl isoquinoline carboxamide) exacerbated lindane-induced hypothermia when both were given i.p., perhaps because of synergistic interaction between the 2 compounds at GABA-A receptors in temperature-regulating centers. When lindane was given p.o. and PK 11195 i.p., the effects of lindane were reduced. *In vitro* studies using intestinal strips revealed that both PK 11195 and lindane reduced the amplitude of intestinal contractions. A synergistic interaction in reducing G.I. motility, and therefore absorption of lindane, was hypothesized to account for the reduction of the effects of lindane by PK 11195. The direct effects of lindane in decreasing intestinal motility probably result from antagonizing the GABA-A receptor in the myenteric plexus. Devazepide, which is a cholecystokinin (CCK) -A receptor antagonist, given i.p. worsened the convulsant effects of lindane given p.o., at least in part by preventing constriction of the pyloric sphincter caused by lindane. This allowed more lindane to leave the stomach and be absorbed in the intestine. It is hypothesized that, when given p.o., lindane reduces its own absorption by antagonizing the GABA-A receptor and thus slowing intestinal motility. In the same way, gastric motility and the probability that the pyloric sphincter is open are also reduced. This would slow emptying of the stomach and also reduce toxicity.

1251 CHRONIC GAVAGE TOXICITY/CARCINOGENICITY STUDY OF METHYLEUGENOL IN F-344/N RATS.

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Methyleugenol (MEG) is used as a flavoring agent and to impart fragrance in cosmetic products. MEG was selected for testing because of its widespread use and structural resemblance to known carcinogens, eg. safrole. Male and female F-344/N rats (50/sex/group) were administered MEG (99% purity) in 0.5% methylcellulose by gavage at doses of 37, 75 or 150mg/Kg, 5 days/week, for 105 weeks; a control group (60 rats/sex) received only the vehicle and a stop-exposure group (60 rats/sex) received 300mg/Kg for 53 weeks followed by the vehicle only for the remaining 52 weeks. MEG decreased survival (≥150mg/Kg) and body weight (≥37mg/Kg). MEG induced liver, glandular stomach and kidney neoplasms. The overall incidence of hepatocellular adenomas or carcinomas was 7/50, 14/50, 28/50, 43/50 and 45/50 (males) and 1/50, 8/50, 14/49, 34/49 and 43/50 (females) for the control, 37, 75, 150 and 300 mg/Kg groups, respectively. The incidence of hepatocholangioma and hepatocholangiocarcinoma was increased at 300mg/Kg. The overall incidence of benign or malignant stomach neuroendocrine tumors was increased at doses of ≥150mg/Kg (males) or ≥75mg/Kg (females) and of renal tubule adenomas at doses of ≥75mg/Kg (males only). Also, a positive trend in the incidence of malignant mesotheliomas, mammary gland fibroadenomas, subcutaneous fibromas and fibrosarcomas was observed for higher dose groups of male rats. MEG caused a dose-dependent increase in the incidence of non-neoplastic lesions in the liver (eosinophilic/mixed cell foci, hypertrophy, oval cell/bile duct hyperplasia and cystic degeneration), glandular stomach (mucosal atrophy/neuroendocrine cell hyperplasia) and kidney (renal tubule hyperplasia). Under the conditions of this study, MEG produced clear evidence of carcinogenic activity in both sexes of F-344/N rats. (Supported by N01-ES-25337.)

1252 CHRONIC GAVAGE TOXICITY/CARCINOGENICITY STUDY OF METHYLEUGENOL IN B6C3F$_1$ MICE.

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Methyleugenol (MEG) is used as a flavoring agent in foods and beverages, as well as to impart a fragrance in cosmetic products. MEG was selected for testing because of its widespread use and structural resemblance to known carcinogens, eg. safrole. Male and female B6C3F$_1$ mice (50/sex/group) were administered MEG (99% purity) in 0.5% methylcellulose by gavage at doses of 0 (control), 37, 75 or 150mg/Kg, 5 days/week, for 104 weeks. Survival was similar to control for males but decreased to 37, 33 and 4%, respectively, for females. At termination, group mean body weights decreased 11 to 17% (males) and 40 to 47% (females) relative to controls. MEG induced liver and glandular stomach neoplasms. The overall incidence of hepatocellular adenoma, carcinoma or hepatoblastoma was 31/49, 47/50, 46/50 and 41/50 (males) and 25/50, 50/50, 49/49 and 49/50 (females) for the control, 37, 75 and 150mg/Kg groups, respectively. In addition, the 150mg/Kg group exhibited malignant neuroendocrine tumors of the glandular stomach (2/50 males) and hepatochol-angiocarcinomas (2/50 females). MEG caused a dose-dependent increase in the incidence of non-neoplastic liver lesions (eosinophilic foci, hypertrophy, necrosis, oval cell and bile duct hyperplasia, hemosiderin pigmentation, chronic active inflammation and hematopoietic cell proliferation) and glandular stomach lesions (glandular ectasia, mucosal atrophy, chronic active inflammation and epithelial and neuroendocrine cell hyperplasia). Under the conditions of this study, MEG produced clear evidence of carcinogenic activity in both sexes of B6C3F$_1$ mice. (Supported by N01-ES-25337.)

1253 SUBCHRONIC TOXICITY OF METHYLEUGENOL ADMINISTERED BY GAVAGE TO F-344 RATS AND B6C3F1 MICE.

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Methyleugenol (MEG) is used as a flavoring agent in foods and as a fragrance in perfumes. It is similar in chemical structure to the carcinogen safrole and was found to induce neuroendocrine (ECL cell) tumors of the glandular stomach and liver tumors in a recent NTP bioassay. MEG was administered to F344 female rats at 0, 37, 75, 150, 300 and 1000 mg/kg/day and to B6C3F1 male mice at 0, 9, 18.5, 37, 75, 150 and 300 mg/kg/day for 30 and 90 days. Stomach pH and serum gastrin levels were measured. Cell proliferation and histopathology were analyzed in the squamous epithelium of the forestomach, gastric pits and fundic glands of the glandular portion of the stomach, pyloric region and the liver. Male mice demonstrated significantly increased serum gastrin levels at 150 and 300 mg/kg/day at 30 days as did female rats at 300 and 1000 mg/kg/day and 150, 300 and 1000 mg/kg/day at 90 days. A significant increase in stomach pH was also observed in female rats at 300 and 1000 mg/kg/day at 30 and 90 days. Treatment related increases in cell proliferation were observed in the fundic glands of mice and rats and liver of rats. These data support the hypothesis that the stomach tumors are secondary to parietal cell necrosis causing a rise in stomach pH resulting in chronic gastrin release which is trophic for ECL cell proliferation. Increased cell proliferation may contribute to liver tumors.

1254 CHEMOPREVENTION OF B[A]P-INDUCED MORPHOLOGICAL TRANSFORMATION IN SYRIAN HAMSTER EMBRYO CELLS BY STRAWBERRY EXTRACTS.

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Recent studies have demonstrated potential cancer chemopreventive activity of berry derivatives including strawberries. To date the exact chemical agent(s) responsible for the chemopreventive activity in the strawberries has not been defined. In the present study, we utilized the Syrian Hamster Embryo (SHE) cell Transformation model to examine the potential activity of several strawberry extracts. For strawberry extract preparation, freeze-dried strawberries were combined with methanol and either extracted with water (FA003), dichloromethane and methanol (FA-DM) or methanol only (FA-ME). These extracts were analyzed for anti-transformation activity in the SHE cell assay. Benzo(a)pyrene (B(a)P) was used as the positive carcinogen. In initial studies, the relative toxicity and transformation ability of the extracts was examined. The maximum subtoxic concentration for all extracts was found to be 100 μg/ml. None of the extracts by themselves produced an increase in morphological transformation. For chemopreventive activity assessment, SHE cells were treated with each extract and B(a)P for 7 days. FA-ME (5-100 μg/ml) produced a dose dependent decrease (60-84%) in transformation compared with B(a)P treatment only, while FA-003 and FA-DM did not induce a significant decrease in transformation. In a separate study, the FA-ME was examined for chemopreventive activity after a 24 hr co-treatment with B(a)P and for 6 days after a 24 hr treatment with B(a)P. Under this protocol, FA-ME reduced the transformation rate in a dose dependent manner when coincubated with B(a)P for 24 hrs but failed to prevent transformation when incubated for 6 days following the B(a)P treatment. These results suggest that several fractions of the strawberry extract are potentially chemopreventive with the FA-ME extract having the most potency. The FA-ME fraction was also shown to function during metabolism, activation and/or DNA binding of B(a)P.

1255 REVERSIBILITY OF PRENEOPLASTIC NODULES INDUCED IN RAT PANCREAS BY SOY FLOUR.

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Rodents fed soy flour (SF) diet develop numerous pancreatic nodules and represent a model for the study of pancreatic tumorigenesis. This work presents data on the putative reversibility of rodent pancreatic nodules. Groups of rats were fed SF diet for 45 weeks. Progression of pancreatic nodules was assessed thereafter by serial laparotomy at 3-month intervals. Post-laparotomy at Week 45, one group (A) was maintained on SF and a second group (B) was on regular rodent chow. At Week 45, SF-fed rats averaged 6 nodules ranging from 2 to 10 mm per pancreas. By Week 58, the number and size of nodules increased in 80% of the rats in Group A. In contrast, Group B rats averaged 3 nodules ranging from 2 to 5 mm. BrdU-containing osmotic pumps were implanted SC 4 days after discontinuing SF diet and acinar and duct cell proliferation were assessed by morphometry. Apoptotic cell death was quantitated in sections stained by terminal nucleotidyl transferase. Acinar cell proliferation decreased in nodules from rats in Group B relative to nodules from rats in Group A. Duct cell proliferation also decreased in nodules from rats from Group B. Increased apoptotic acinar cells were observed concurrently with decreased BrdU labeling in nodules from Group B rats, suggesting that cell proliferation and nodule development, may be reversed or arrested.

1256 EFFECT OF GREEN AND BLACK TEA POLYPHENOLS ON CYCLOOXYGENASE (COX)- AND LIPOXYGENASE (LOX)-DEPENDENT METABOLISM OF ARACHIDONIC ACID IN HUMAN COLON TISSUES.

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Ingestion of green tea has been shown to rapidly decrease prostaglandin E_2 (PGE_2) levels in human colorectum, suggesting that tea polyphenols might modulate arachidonic acid metabolism. In this study, we investigated the effects of green and black tea polyphenols on COX- and LOX- dependent arachidonic acid metabolism in normal human colon mucosa and colon cancers. At 30 μg/ml, (-)-epigallocatechin-3-gallate(EGCG), (-)-epigallocatechin (EGC), and (-)-epicatechin-3-gallate (ECG) from green tea and theaflavins (TFs) from black tea inhibited LOX-dependent activity by 35 - 75%. The green tea polyphenols are stronger inhibitors of LOX compared to TFs. The formation of different LOX metabolites was affected evenly by tea polyphenols. Tea polyphenols inhibited COX-dependent arachidonic acid metabolism in normal colon by 37 - 62%, at 30 μg/ml. ECG showed the strongest inhibition. It also inhibited ovine COX-1 in a non-competitive manner with a Ki of 16.9±1.3 μM. Compared to other metabolites, thromboxane (TBX) and 12-hydroxyheptadecatrienoic acid (HHT) formation was more severely inhibited by EGCG, ECG, and TFs. The inhibitory effects of EGCG and TFs on the metabolism were, however, less pronounced in tumors. TFs increased the production of PGE_2 and decreased the formation of TBX and HHT. Among TF mixtures, purified TF showed the most potent increase of PGE_2. The effect was related to COX-2 activity and its expression level in microsomes. The results suggest that inhibition of arachidonic acid metabolism may be one of the possible anticarcinogenic mechanisms of green tea in the prevention of human colon cancer. (Supported by NIH grant CA56673.)

1257 EVALUATION OF THE TUMORIGENIC ACTIVITY OF A CRUCIFEROUS VEGETABLE JUICE IN A SHORT-TERM BIOASSAY USING TRANSGENIC MICE.

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Cruciferous vegetables such as broccoli are generally believed to be a beneficial component of the human diet by virtue of their protective activity in carcinogenesis. However, recent studies indicated that juices and extracts of these vegetables have genotoxic activity in some *in vitro* test systems (Gupta et al., Reg. Toxicol., 23, 14, 1996; Kassie et al., Chemico-Biol. Interact., 102, 1, 1996). We have previously demonstrated that broccoli juice/extracts, while not mutagenic in the Ames test, exhibited considerable cytotoxicity and genotoxicity in the mouse lymphoma forward mutation assay and the CHO chromosomal aberration test. Since a causal relationship between genotoxicity and carcinogenicity is generally assumed, we have investigated the tumorigenic potential of broccoli juice in a short-term bioassay using the transgenic Tg.AC mouse model. Homo- and hemizygous female Tg.AC mice (15 mice/group/zygosity) were dosed dermally (200 ul/mouse, 5 days/week, 26 weeks) either with acetone; or crude broccoli juice (1:1 ratio in acetone). Mice treated with the phorbal ester TPA (2.5 μg/mouse, 3 days/week for up to 26 weeks) were used as positive controls. No papillomas were observed at the dermal test site of mice treated with broccoli juice or acetone for 26-weeks. However, mice treated with TPA began to develop tumors at approximately 5 weeks after the start of treatment with 100% of genotypically responder mice developing papillomas by the end of the 26-week study. These data suggest that the genotoxic agent(s) present in broccoli juice were efficiently detoxified *in vivo*, a result consistent with the abrogation of broccoli-induced genotoxicity *in vitro* in the presence of S9. Current efforts are underway to examine the antitumorigenic effects of broccoli in Tg.AC mice treated with benzo(a)pyrene or TPA.

1258 RESVERATROL, AN ANTIOXIDANT FOUND IN RED WINE, PREVENTED THE INHIBITION OF GAP JUNCTION INTERCELLULAR COMMUNICATION BY THE TUMOR PROMOTING AGENTS DICUMYL AND BENZOYL PEROXIDES.

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Dicumyl and benzoyl peroxides act as tumor promoters in SENCAR mice, while di-tert-butyl-peroxide does not have tumor promoting activity. Tumor promotion requires the removal of growth suppression by decreasing gap junction intercellular communication (GJIC) and the induction of mitogenic intracellular pathways. We showed that dicumyl and benzoyl peroxides both reversibly inhibited GJIC at noncytotoxic doses in WB-F344 rat liver epithelial cells, while the non-tumor promoting di-tert-butyl peroxide did not inhibit GJIC. Both dicumyl peroxide and benzoyl peroxide also transiently activated mitogen-activated protein kinase, specifically extracellular receptor kinase. The consumption of red wine is believed to be responsible for the low incidence of cancer and heart disease among the French population and the active component of red wine is thought to be the antioxidant resveratrol. We found that pretreating WB cells with resveratrol prevented the GJIC-inhibitory effects by dicumyl and benzoyl peroxides. (This research was supported by the NIEHS Superfund grant #P42 ES04911-07and USAFOSR grant no. F49620-97-1-0022.)

1259 CHEMOPREVENTIVE EFFECTS OF ORALLY ADMINISTERED IMPERATORIN AND ISOPIMPINELLIN.

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Several naturally occurring coumarins, to which humans are routinely exposed in the diet, were previously found to inhibit P450-mediated metabolism of benzo[a]pyrene (B[a]P) and 7,12-dimethylbenz[a]anthracene (DMBA) *in vitro*, block DNA adduct formation, and inhibit skin tumor initiation by B[a]P and/or DMBA when applied topically to SENCAR mice. The present study was designed to investigate the effects of orally administered naturally occurring coumarins (70 mg/kg, p.o., 4 successive daily doses) on phase I and II enzyme activities, and DNA adduct formation by B[a]P and DMBA in various SENCAR mouse tissues. Orally administered imperatorin and isopimpinellin inhibited both P450 1A1-mediated 7-ethoxyresorufin O-dealkylase and P450 2B1/2-mediated pentoxyresorufin O-dealkylase activities (except in liver) at 1 hr after the last of 4 consecutive doses in forestomach, liver, lung and skin epidermis. Hepatic glutathione S-transferase (using 1-chloro-2,4-dinitrobenzene as substrate) was elevated at both 1 and 24 hr after the last oral dose of both imperatorin and isopimpinellin. Orally administered imperatorin inhibited DMBA DNA adduct formation in epidermis and forestomach. Orally administered isopimpinellin inhibited B[a]P DNA adduct formation in forestomach, liver, and lung. Certain naturally occurring coumarins may be effective anticarcinogens in several tissues when administered orally. (CA79442)

1260 EFFECTS OF INTERMITTENT EXPOSURE TO AFLATOXIN B1 (AFB1) ON DNA AND RNA ADDUCT FORMATION IN RAT LIVER.

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Little is known about the health risks posed by intermittent exposure to chemical carcinogens. AFB_1, a known animal carcinogen, was chosen as the chemical model to assess the effects of intermittent exposure. Fisher-344 male rats were intermittently fed 0.01, 0.04, 0.4, and 1.6 ppm of AFB_1 for 8, 12, 16, 20, and 40 weeks with alternate 4-week dosing periods and 4-weeks no-dosing periods. Other groups of rats were continuously fed 1.6 ppm of AFB_1 for 4, 8, 12, 16 and 40 weeks. DNA and RNA adducts were measured at 4-week intervals for up to 20 weeks of intermittent exposure and for up to 16 weeks of continuous exposure. DNA and RNA were extracted from the liver, purified, and acid hydrolyzed. The hydrolyzed DNA and RNA were extracted by solid-phase extraction before being injected into a Waters HPLC system equipped with fluorescence and photo-diode array detection. Under the HPLC conditions used, AFB_1-N^7-Gua did not separate well from AFB_1-FAPyr1 but AFB_1-FAPyr2 did. Therefore, the terms AFB_1-DNA or -RNA adducts are used here to include both AFB_1-N^7-Gua and AFB_1-FAPyr1 adducts. The extent of AFB_1-

DNA adducts was dose-dependent from 0.01 to 1.6 ppm regardless of the exposure regime. AFB_1-DNA adducts increased 1.5- to 2-fold from 4 to 20 weeks of intermittent exposure only when the rats were dosed the last 4 weeks before sacrifice. When the rats were off-treatment for the last 4 weeks, the levels of AFB_1-DNA adducts decreased but remained significantly above control levels. In comparison, the levels of DNA adducts increased markedly from 4 to 16 weeks of continuous feeding with 1.6 ppm AFB_1 and did not appear to reach a steady-state at 16 weeks of treatment. The extent of AFB_1-RNA adducts was also dose-dependent from 0.01 to 1.6 ppm regardless of the exposure regime. While these results appear significant, further research is needed to determine the overall implications for health risk assessment.

1260A COMPARATIVE ANALYSIS OF CHEMICAL:DNA ADDUCT FORMATION IN MOUSE LUNG FOLLOWING DERMAL APPLICATION OF COAL TAR SHAMPOO AND MGP TAR.

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Recent studies have demonstrated that manufactured gas plant (MGP) tar is a potent lung tumorigen when ingested by animals. In addition, lung tissue has been determined to be particularly susceptible to chemical:DNA adduct formation following the ingestion or dermal application of MGP tar. The similarity in the chemical composition of MGP tar and pharmaceutical grade coal tar suggests that coal tar containing shampoos would exhibit similar properties. Thus, the present study evaluated the potential of over-the-counter coal tar containing shampoos to induce chemical:DNA adduct formation in mouse lung following dermal application. Six coal tar shampoos along with shampoo mixtures prepared with pharmaceutical grade coal tar or MGP tar were evaluated. Shampoos were applied to the shaved backs of CD-1 mice fitted with Elizabethan collars to prevent shampoo ingestion. Mice were housed individually for 24 hrs in metabolism cages and urine was collected for 1-hydroxypyrene analysis while chemical:DNA adduct formation was evaluated in lung and skin tissue. In the case of DNA adduct formation, all of the shampoos evaluated produced easily detectable levels of adducts in skin and lung tissue. Adduct levels varied according to the tar content of each shampoo. In some instances, the over-the-counter coal tar shampoos produced lung DNA adduct levels comparable to those observed with mice treated with a similar dose of a shampoo prepared with MGP tar. These results demonstrate that coal tar containing shampoos produce significant levels of chemical:DNA adducts in lung tissue following topical application. In addition, the similarity in the ability to produce lung DNA adducts between MGP tar and coal tar containing shampoos also suggests that these shampoos may exhibit similar biological properties. (EPRI Grant WO2963-06.)

1261 INDOLE-3-CARBINOL DEMONSTRATES ESTROGENIC PROPERTIES IN THE RAINBOW TROUT: CONTRIBUTION OF 3,3'-DIINDOLYLMETHANE.

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Indole-3-carbinol (I3C), a compound found in *Brassica* vegetables has been widely studied for its chemopreventive properties. In previous studies, I3C blocked tumor initiation and promotion; however, it can also act as a tumor promoter. I3C and some of its acid condensation products have been shown to be antiestrogenic, particularly 3,3'diindolylmethane (I33'), in several cell line assays. We report that I33' acts as an estrogen in the rainbow trout liver *in vitro* and *in vivo* by inducing vitellogenin (Vg), a well-characterized biomarker for estrogens. Precision-cut liver slices from male rainbow trout, *Oncorhynchus mykiss*, 8 mm x 250 μm, were incubated in Hank's media of 25% fetal bovine serum supplemented with HEPES, $NaHCO_3$ and 1% bovine serum albumin at 14°C for 96 hr. I3C, I33' and a mixture of I3C acid condensation products (RXN) (0-250 μM) were added to the medium with DMSO as the vehicle. I33' and RXN increased Vg levels in rainbow trout liver slices by over 100 and 10 fold, respectively, vs vehicle. The efficacy of I33' induction of Vg was comparable to 17β-estradiol with 500 fold less potency. *In vivo*, juvenile male rainbow trout were fed I3C, RXN (0- 2000 mg/kg) or I33' (0-250 mg/kg) for two weeks. At 2000 mg/kg, I3C induced Vg by over 100,000 fold compared to controls, which was comparable to 5 mg/kg 17β-estradiol (the dose resulting in maximum induction). Compared to I3C, RXN was one-tenth as potent, but I33' was three times as potent. I33' may account for Vg increases observed in trout fed I3C as it is present in liver after oral dosing, at concentrations (70 μM) expected to maximally induce Vg. In trout, results *in vitro* and *in vivo* document that I33' is estrogenic, consistent with our hypothesis that I3C promotes liver cancer in trout by estrogenic pathways. I33' apparently functions as a pro- or antiestrogen in a tissue- and/or species-specific manner. (Supported by ES03850, ES04766 and ES07060.)

1262 EFFECTS OF COMBINED TREATMENT WITH ANTIOXIDANTS AND SODIUM NITRITE ON THE RAT FORESTOMACH.

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It has been shown that the combined administration of ascorbic acid or phenolic compounds with sodium nitrite ($NaNO_2$) induces cell injury and hyperplasia in the rat forestomach epithelium, which subsequently leads to squamous cell tumor development. We have investigated the effects of $NaNO_2$ in combination with nine environmental antioxidants, *i.e.* α-tocopherol, sodium erythorbate (SE), β-carotene, quercetin, green tea catechins (GTC), hydroquinone (HQ), *o*-phenylphenol (OPP), curcumin and bisphenol A on the rat forestomach. Five-week old male F344 rats were fed diet containing the antioxidants at 1% or basal diet with or without 0.2% $NaNO_2$ in the drinking water for 4 weeks. Using ELISA, urinary 8-hydroxydeoxyguanosine (8-OHdG) levels were measured. We also investigated the expression of 8-OHdG, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and nitrotyrosine (N-Tyr) by immunohistochemistry. Microscopically, no changes were seen in any of the antioxidant or $NaNO_2$ alone group. However, combined treatment with α-tocopherol, SE, GTC, HQ or OPP with $NaNO_2$ induced squamous cell hyperplasia with inflammation and erosion in the forestomach. Most remarkable changes were observed in the groups receiving SE or HQ with $NaNO_2$. PCNA-labeling indices were significantly increased by the combination of SE, GTC, HQ or OPP with $NaNO_2$ as compared with the $NaNO_2$ alone value. However, no variation in 8-OHdG levels was detected among the groups. Immunohistochemical analysis showed positive expression of iNOS, COX-2 and N-Tyr in the inflammatory cells but not in the forestomach epithelium. These results indicate that co-administration of a-tocopherol, SE, GTC, HQ or OPP with $NaNO_2$ induces forestomach hyperplasia, and suggest that production of NO and COX-2 in the inflammatory cells may be secondarily involved in this process.

1263 INDUCTION OF TUMORS IN THE COLONS AND LIVERS OF FEMALE SCID MICE BY 2-AMINO-3-METHYLIMIDAZO[4,5-*f*]QUINOLINE (IQ), AND THEIR MODULATION BY FATTY ACIDS.

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2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), one of the most potent mutagenic heterocyclic aromatic amines, was found to be carcinogenic to several organs including the liver in various mammals, however, the colon has not been shown to be a target for IQ carcinogenicity in any mouse strain tested. We recently reported the sensitivity of mice with severe combined immunodeficiency (SCID) to a low-dose site-specific induction of aberrant crypt foci (ACF), preneoplastic lesions, in the colon by IQ. In the present experiment we tested the carcinogenic effect of dietary administration of 300 ppm IQ alone and the modifying effects of fatty acids in combination with IQ for 32 weeks on SCID mice. A total of 75 female SCID mice were divided into 5 groups. Group 1 received IQ mixed with the basal diet. Groups 2-4 received 20% corn oil, perilla oil or olive oil respectively mixed with the basal diet and IQ, while group 5 received the basal diet only as control. Treatment with IQ only (group 1) caused mainly 50% incidence of colonic adenomas and 58% incidence of liver adenomas and adenocarcinomas in the mice in addition to 100% incidence of preneoplastic lesions in both organs. Treatment with corn oil and perilla oil inhibited significantly the incidence of colon tumours into 9% and 0% and liver tumours into 0% and 8% respectively along with a significant reduction in the tumoricidal activities in the two organs such as cell proliferation, mitotic indices and colonic crypts heights. Olive oil treatment didn't show similar inhibitory effects. Colonic tumours were mainly induced in the distal colons. These results support the carcinogenic potential of IQ in the colon of SCID mice and that corn oil and perilla oil, but not olive oil, perform strong suppressing effects on the tumoricidal activity of IQ in the colon and liver of SCID mouse.

1264 A REVIEW OF MORTALITY IN TUMORIGENICITY STUDIES, COMPLETED OVER THE PERIOD 1987 TO 1999, USING THE CHARLES RIVER ORIGINAL OR INTERNATIONAL GENETIC STANDARD SPRAGUE-DAWLEY RAT.

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At Huntingdon Life Sciences, the mortality data have been analysed from the control groups of 81 tumorigenicity studies using the original or International Genetic Standard (IGS) strain designations of Sprague-Dawley rat, Crl:CD®BR (VAF). The data obtained from the IGS rat have been closely monitored since the introduction of this new strain designation in 1996 and 11 studies completed in 1998 or 1999 have been compared against up to 70 studies using the original strain designation (completed over 1985 to 1997). Low protein maintenance diet was used in these dietary and oral gavage studies. The analyses have indicated that the IGS rat is showing a similarly high mortality pattern to that seen in the original strain studies completed over the period 1996-97, but an increased pattern when compared with studies completed 1993-95. In the original rat studies, review of the terminal mortality against time has demonstrated an increasing trend towards higher values over the period of 1987 to 1997, particularly in females. The 11 completed IGS rat studies show a similar trend to the original rat studies. From the results currently available, it can be concluded that the IGS rat is not remarkably different from the original strain of rat, showing the same high mortality pattern, particularly in female rats.

1265 A REVIEW OF THE IN-LIFE PARAMETERS AND TUMOUR DATA IN TEN GANG-HOUSED DIETARY TUMORIGENICITY STUDIES USING THE CHARLES RIVER INTERNATIONAL GENETIC STANDARD OR ORIGINAL STRAIN DESIGNATIONS OF SPRAGUE-DAWLEY RAT.

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At Huntingdon Life Sciences, data obtained from the Charles River International Genetic Standard (IGS) strain of Sprague-Dawley rat have been closely monitored since the introduction of this new strain designation in 1996. The in-life and tumour data from the control groups of ten gang-housed dietary tumorigenicity studies (using low protein maintenance diet) have been assessed, with five IGS rat studies (completed 1998-99) compared against five studies (completed 1994-97) using the original strain of Sprague-Dawley rat. These comparisons have shown that the IGS rat is showing a similarly high mortality pattern to that seen in the original strain studies. The bodyweight growth pattern, bodyweight gain and food consumption data analysed over the first year, have only shown minor differences between the IGS rat and the original strain of rat. Assessment of the tumour profile (tumour incidence, number of tumour bearing rats and factors contributory to death) has not shown any major differences between the two groups. From the results available, it can be concluded that the IGS rat is not remarkably different from the original strain of rat.

1266 MECHANISM OF NEPHROCARCINOGENICITY OF A SHORT CHAIN CHLORINATED PARAFFIN.

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Short-chain chlorinated paraffins cause kidney tumours in male rats but not in female rats or mice of either sex. To investigate whether the tumours were caused via the accumulation of a male rat specific protein, alpha 2u globulin, groups of male and female Fischer 344 rats were dosed by gavage for 28 consecutive days with Chlorowax 500C (C12:60Cl%,C500) or 1,4-dichlorobenzene (DCB), dissolved in corn oil, at doses of 625 and 300 mg/kg of body weight respectively. Control animals received corn oil vehicle alone. DCB is known to cause male rat specific kidney tumours via the accumulation of alpha 2u globulin. All animals were implanted with osmotic pumps containing BrdU to label proliferating cells. On termination, kidneys were removed and sections were immunohistochemically stained for alpha 2u globulin and BrdU. The remainder was homogenised and analysed by polyacrylamide gel electrophoresis (PAGE) and two dimensional PAGE (2D-PAGE). Western blots were performed using anti-alpha 2u globulin antibody on both PAGE and 2D-PAGE gels. The immunohistochemical staining showed an increase in alpha 2u globulin and cell proliferation in both C500- and DCB-treated male rats. The increases were greater in the DCB-treated male rats. No changes in these parameters were seen in the treated female rats. Analysis of data from individual rats indicated that the increase in cell proliferation was directly correlated with the increase in alpha 2u globulin. Western blotting showed five different isoelectric isoforms of alpha 2u globulin in control male rat kidney. Increases in all five isoforms were observed in the C500-treated male group. The increase in total alpha 2u globulin was greater in the DCB males and a specific increase in the more basic isoform was observed. Thus, alpha 2u globulin appears to be involved in the nephrocarcinogenicity of C500, however, there appears to be both a quantitative and qualitative difference between the C500- and the DCB-induced renal responses.

1267 EKER RAT MODEL CHARACTERIZATION, PHASE II-THE RESPONSE TO A GENOTOXIC CARCINOGEN AND TWO NON-CARCINOGENIC NEPHROTOXINS FOLLOWING FOUR OR SIX MONTHS OF DOSING.

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The Eker rat carries a germline mutation in the Tsc-2 tumor suppressor gene, which predisposes it to the development of renal carcinoma (RC). In phase I, 4 months treatment with 2,4-Diaminotoluene or 2-Nitropropane (genotoxic non-renal carcinogens) increased renal hyperplastic tubules (HT) and RC or increased total tumor count (TT), respectively (Youssef et al., Toxicol. Sci. 43:233, 1999). Phase II characterized the responses of the Eker rat after 4 or 6 months exposure to another genotoxic non-renal carcinogen (furan) and two non-carcinogenic nephrotoxins (aluminum nitrilotriacetate (Al-NTA) and cyclosporine A (CsA)). Three groups were treated with either furan, 8 mg/kg/day orally (p.o.) 5x/week, CsA, 30 mg/kg/day p.o. 7x/week or Al-NTA, 2 mg/kg/day intraperitoneally (i.p.) 3x/week, for 4 or 6 months. Three control groups received either no treatment or vehicle (sterile water, i.p. for Al-NTA or olive oil p.o. for furan and CsA) daily for 4 or 6 months. Histopathology included: counting of all renal proliferative lesions (i.e., HT, RA and RC), and TT in all animals. Statistical analysis (two-tailed) showed increased HT, RA and TT after Al-NTA or CsA compared to their controls, and increased RA and TT after 6 months vs. 4 months. There was no change in incidence of RC. Furan did not result in renal proliferative lesions or in biliary neoplasms. The model did not show promise as an alternative for screening of compounds for carcinogenic potential (Sponsored by TAP Holdings, Inc.).

1268 EFFECT OF CHLOROFORM WITH DICHLOROACETIC ACID OR TRICHLOROACETIC ACID ON N-METHYL-N-NITROSO-UREA-INITIATED LIVER AND KIDNEY TUMORS IN FEMALE AND MALE B6C3F1 MICE.

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Chloroform, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are common byproducts found in chlorinated drinking water. All three chemicals have been shown to induce liver tumors in B6C3F1 mice. Therefore, we evaluated mixtures containing chloroform with either DCA or TCA in the drinking water. Male and female B6C3F1 mice were administered 30 mg/kg *N*-methyl-*N*-nitrosourea on day 15 of age. At four weeks of age, the mice started to receive in their drinking water 3.2 gm/l DCA or 4.0 gm/l TCA with 0, 800 or 1,600 mg/l chloroform. The mice were sacrificed at 44 weeks of age and their livers and kidneys evaluated for tumors. Both DCA and TCA promoted liver tumors in female and male mice. The presence of chloroform greatly reduced the level of yield of liver tumors promoted by DCA but not TCA. In contrast, chloroform greatly enhanced the yield of kidney tumors in DCA-treated male mice. The kidney tumors were mainly solid and papillary cystic adenomas. Chloroform did not effect the yield of kidney tumors in TCA-treated mice. Kidney tumors were not found in female mice. Hence, in DCA-treated mice chloroform prevented liver tumors and promoted kidney tumors male, while in TCA-treated mice chloroform did not affect the tumor yield in either organ. Funded in part by the US Environmental Protection Agency Grant R 825384 and not subjected to the Agency's review nor necessarily reflects the views of the Agency.

1269 A MULTISTAGE BIOLOGICALLY BASED MODEL FOR MOUSE LIVER TUMORS RESULTING FROM EXPOSURE TO DICHLOROACETIC ACID.

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Dichloroacetic Acid (DCA) is a major byproduct of the chlorine disinfection of humic acid containing drinking water sources. It is a hepatocarcinogen in mice and rats at exposure concentrations in drinking water that are at least 4 orders of magnitude above the concentrations in drinking water to which humans are exposed. Under the dosing regime that results in the formation of tumors in B6C3F1 male mice, DCA causes a spectrum of benign, premalignant and malignant lesions of the liver that changes during the lifetime of the subjects. To aid in the extrapolation from these exposure conditions to drinking water exposures a biological based model for DCA carcinogenesis has been developed. Histopathologic analysis of the livers of exposed mice suggests that following chronic exposure to DCA, carcinomas can arise directly from single initiated cells in the liver as well as within hyperplastic nodules and adenomas. Based on these studies, a biologically based model for DCA carcinogenesis that contains at least 3 precancerous stages and 6 transitions was developed. With the large number of parameters in this model, it is clear that any method for numerical parameter estimation would not provide meaningful results. The PCNA and TUNEL assays were used to obtain birth and death rates for cells in each precancerous stage. A general transition rate was obtained from the mutation frequency induced in the livers of lacI B6C3F1 transgenic mice. Many of these parameters were a function of DCA exposure. Using the experimentally determined parameters and the Kolmogorov backwards equations, there is agreement with the time-to-tumor data for mice exposed through their drinking water for two years. It is more difficult to obtain agreement with the experimental determination of the number of tumors/animal as a function of time in that study. Dose related extrapolation of the model may be obtained from extrapolation of the parameters. (This abstract does not necessarily reflect US EPA policy).

1270 SODIUM CHLORATE TREATMENT RESULTS IN A DOSE-DEPENDENT INCREASE IN RAT THYROID FOLLICULAR CELL HYPERPLASIA FOLLOWING SUBCHRONIC EXPOSURE IN DRINKING WATER.

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Chlorine dioxide treatment is an effective water disinfection method but sodium chlorate has been identified as a potentially harmful disinfection by-product. Subchronic toxicity tests have identified the rat thyroid as the primary target organ. Studies were performed at EPA or sponsored by the National Toxicology Program (NTP) to describe the development of thyroid lesions in animals exposed to various doses of sodium chlorate in the drinking water. Under the NTP dosing regimen, male and female F344 rats and B6C3F1 mice were exposed to 0, 0.125, 1.0, or 2.0 g/L sodium chlorate for 21 or 90 days. Under the EPA dosing regimen, male F344 rats were exposed to 0, 0.001, 0.01, 0.1, 1.0, or 2.0 g/L sodium chlorate for 90 days. Female F344 rats were exposed to 0, 0.5, 1.0, 2.0, 4.0, or 6.0 g/L of sodium chlorate for 105 days. Thyroid follicular cell hyperplasia and significant colloid depletion were present in all male and female F344 rats at doses of 1.0 g/L or greater of sodium chlorate following 21 days and 90 days of treatment in the NTP study. While serum T_3 and T_4 levels were not altered in male or female rats after 90 days, TSH levels increased slightly in a dose-dependent manner. Thyroid follicular cell hyperplasia was present in female F344 rats at doses of 1.0 g/L or greater and in male F344 rats at all treatment doses in the EPA study. Significant thyroid colloid depletion was present in female F344 rats at doses of 2.0 g/L or greater. Increased cell proliferation was not evident in male rats but female rats had a 5-19 fold increase in positively stained S-phase cells compared to concurrent controls. Follicular cell hyperplasia was not present in male or female B6C3F1 mice. Sodium chlorate treatment induced a dose-dependent increase in the incidence and severity of thyroid follicular hyperplasia associated with colloid depletion and increased serum TSH levels. Male rats appear to be more sensitive to the effects of sodium chlorate treatment than females. (This abstract does not reflect EPA policy.)

1271 EFFECT OF 2,2',4,4',5,5'-HEXACHLOROBIPHENYL (PCB-153) AND 3,3',4,4'-TETRACHLOROBIPHENYL (PCB-77) ON NF-KB AND AP-1 ACTIVATION, ALTERED HEPATIC FOCI FORMATION, CELL PROLIFERATION AND APOPTOSIS IN RATS.

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PCBs are environmental pollutants that have been withdrawn from use but still persist in nature. They have been shown to be complete carcinogens and tumor promoters in rodent liver. In this study we investigated the mechanisms of tumor promoting activity of two different PCB congeners. After initiation with diethylnitrosamine (DEN), rats were injected biweekly with either PCB-153 or PCB-77 (100 or 300 μmol) or with both PCBs (100 μmol each). All animals were sacrificed ten days after the fourth injection; three days before sacrifice all animals were implanted with osmotic pumps containing 5-bromo-2'-deoxyuridine (BrdU). NF-kB and AP-1 were found to be strongly activated by both PCBs and by their combination. While the number of placental glutathione S-transferase-positve foci was increased in all PCB treated animals, the mean focal volume in all groups remained unchanged. The cell proliferation index showed a significant increase only in high-dose PCB-77 treated animals. The apoptotic index (measured using the TUNEL assay) was increased by PCB-77 but decreased by PCB-153. These results show that NF-kB and AP-1 activation is increased following the application of PCBs as tumor promoters. (Supported by ES 07380 and ES 07266.)

1272 COMPARATIVE 30-WEEK DERMAL TUMOR PROMOTION EVALUATION OF CIGARETTE SMOKE CONDENSATE FROM A REFERENCE CIGARETTE AND AN ECLIPSE PROTOTYPE (9-014) TEST CIGARETTE IN FEMALE SENCAR MICE.

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A 30-week dermal tumor promotion assay was conducted to compare the potential dermal tumor promotion activity of mainstream cigarette smoke condensate (CSC) collected from an ECLIPSE prototype cigarette (9-014), which primarily heats tobacco, and a 1R4F (University of Kentucky) reference cigarette. Mainstream CSC's were collected by cold trap from smoke generators using the Federal Trade Commission puffing regimen. Female SENCAR mice were "initiated" with a single 75 μg application of DMBA to the shaved dorsal skin. The CSC's were then applied to the skin three times per week for 29 weeks. Each CSC was administered at 9, 18, or 36 mg tar/application to groups of 40 animals. End-points included body weights, clinical observations, organ weights, dermal tumor development data and histopathology. The numbers of dermal tumors and the numbers of dermal tumor-bearing animals for the 1R4F reference CSC were statistically different from the "DMBA-initiated" control group and increased with increasing exposure. When corresponding doses of 1R4F reference and 9-014 CSC were compared, the 9-014 CSC groups had significantly fewer dermal tumors and tumor-bearing animals. In this assay, the dermal tumor-promotion potential of the 9-014 CSC was, therefore, substantially reduced when compared to the 1R4F reference CSC.

1273 REVERSIBILITY STUDY OF THE HEPATIC AND PULMONARY EFFECTS OF PERMETHRIN IN MICE.

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The study was designed to investigate the autonomous growth of permethrin-induced hepatic eosinophilic adenomas and pulmonary adenomas in mice. Groups of 50-100 female mice were fed control diet or 5000 ppm permethrin for 9, 12, 15, or 18 months and sacrificed. Additional groups receiving treatment for these intervals received untreated diet following the treatment interval and then were sacrificed at 18 or 24 months to monitor for recovery/reversal of effects. All groups of mice were examined for the incidence of hepatic and pulmonary lesions. At each of the interim kills following treatment with permethrin, the mice had increased liver weights, centrilobular hypertrophy and karyomegaly. Eosinophilic adenomas were seen at 12 months, with the highest incidence at 18 months. Satellite groups at 12 months showed increases of hepatic P450, CYP1A, CYP2E1, CYP3A, and CYP4A. Following withdrawal of treatment the centrilobular hypertrophy and karyomegaly reversed. No progression or increase in hepatic eosinophilic adenomas was seen. The

number of basophilic adenomas increased with age. PCNA immunocytochemistry showed that the labeling of eosinophilic nodules was less than basophilic nodules with a different distribution within the nodule. These data show eosinophilic adenomas in the liver are not associated with hepatocarcinomas, suggest adenomas are end-stage lesions, and do not satisfy the requirement for autonomous growth. The lungs showed consistent Clara Cell hypertrophy and hyperplasia at all times on treatment which reversed in the recovery groups. Pulmonary adenomas were seen at 9 months but only increased at the 18-month interim kill. Following withdrawal of treatment there was no progression of pulmonary adenoma to carcinoma at the final kills at 18 and 24 months.

1274 PROMOTING ACTIVITY OF XYLAZINE ON RAT THYROID CARCINOGENESIS AND ITS MECHANISM OF ACTION.

K. Yasuhara, K. Mitsumori, K. Takegawa, T. Koujitani, H. Onodera, H. Takagi, M. Hirose and M. Hirose. *National Institutes of Health Science, Tokyo, Japan.* Sponsor: T. Shirai.

We have reported that thyroid hypertrophy was induced in rats given xylazine hydrochloride (XZ), an alpha-2-adrenoceptor agonist which is used for food-producing animals as a sedative agent, for 28 days. In order to examine the thyroid tumor promoting potential of XZ, male F344 rats received diet containing 0 or 1000 ppm XZ for 52 weeks after initiation with 2400 mg/kg N-bis(2-hydroxypropyl)nitrosamine (DHPN). Focal follicular cell hyperplasias, adenomas and carcinomas were observed in 100, 60 and 25% of the animals of the DHPN+XZ group, respectively, and these incidences were significantly increased as compared to the DHPN alone group. As a second experiment to clarify the mechanism of the promotion effects, male F344 rats received diet containing 0 or 1000 ppm XZ for 1-4 weeks to examine serum levels of T3, T4 and TSH as well as thyroid iodine uptake and organification. In the XZ group, a significant increase in thyroid weights and decreases in serum T3/T4 were observed from week 1, and inhibition of thyroid iodine uptake and organification from week 2. These results indicate that XZ exerts a tumor-promoting effect on the thyroid follicular cells, and suggest that alterations of thyroid-related hormone levels in the XZ group are due to inhibition of thyroid iodine uptake and organification, the thyroid tumor-promoting effects being probably secondary to prolonged serum TSH stimulation resulting from negative feedback through the pituitary-thyroid axis.

1275 4-CHLOROBIPHENYL HAS CANCER-INITIATING ACTIVITY.

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A modified Solt-Farber protocol was selected to investigate the initiating activity of several lower chlorinated PCBs including PCBs 3, 12, 38, and 77 in male Fischer 344 rats. Rats received a standard 2/3 partial hepatectomy followed by a single bolus dose of the suspected initiating agent, or vehicle. Two weeks later all selection groups received 3 daily doses of 2-acetylaminofluorene then a single dose of carbon tetrachloride via gavage, followed by 3 additional daily treatments of 2-AAF. Only PCB 3 induced grossly visible nodules as well as induced gamma-glutamyltranspeptidase (GGT) positive foci per cm3 and per liver in treated rats. Histological staining of liver sections further revealed the presence of foci and nodules in hematoxylin and eosin-stained sections in all DEN treated animals and in 80% of 4-CBP treated animals. The number of GGT-positive foci (expressed per liver and per cm3) and focal volume of these foci (in % of liver volume) in DEN-treated animals were 5500 foci/liver, 425 foci/cm3, and 34%, respectively, while 4-CBP treated animals had 1500 GGT-positive foci/liver, 200 foci/cm3, and 3% of the total liver. No GGT-positive foci were detected in vehicle or non-selected groups, and all rats that were administered PCB 77 failed to survive the selection portion of the experimental treatments. We must conclude that under the conditions and time course of this experiment, PCB 3 has initiating activity in male Fischer 344 rats.

1276 CONSIDERATION OF THE POTENCY CLASSIFICATION OF ACRYLAMIDE (ACR) BASED ON THE INCIDENCE OF TUNICA VAGINALIS MESOTHELIOMAS (TVMS) IN MALE FISCHER 344 RATS.

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The T25 approach is used by the European Community to classify cancer potency as low, medium or high. Using TVM incidence data in male F344 rats, the T25 for ACR was "borderline" between the high and medium categories. For borderline chemicals, modifying factors (dose-response, species/strain activity, genotoxicity, mechanistic relevance to humans and toxicokinetics) are considered qualitatively to determine if the chemical should be in the lower or higher potency category. To recommend placement of ACR into the appropriate category, a weight-of-evidence evaluation of all of the relevant data for ACR was conducted. The T25 calculated using the Benchmark approach, an alternative to the T25 approach, indicated that ACR should be classified as medium. An evaluation of the mode of action strongly indicated that the presence of Leydig cell tumors (LCTs) was an obligatory, precursor step in the production of TVMs. The formation of LCTs was related to changes in normal hormone levels and alterations in Leydig cell feedback loops, conditions unique to the aging male F344 rat. ACR likely exacerbates this process via dopamine-mediated decreases in prolactin levels, resulting in a down-regulation of LH receptors on the testes, a mechanism not operative in man. The down-regulation of LH receptors is followed by a compensatory increase in LH Leydig cell stimulation, and eventually more metabolically active LCTs. LCTs further alter hormonal balances triggering alterations in growth factor receptors leading to autocrine stimulation of mesothelial cells and TVMs. Based on these data, ACR should remain in the medium potency category.

1277 THE U.S. HIGH PRODUCTION VOLUME (HPV) CHEMICAL VOLUNTARY CHALLENGE PROGRAM.

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On Earth Day, 1998, Vice President Al Gore challenged the U.S. chemical industry to provide a minimum set of hazard data on the approximately 2800 U.S. HPV chemicals (manufactured or imported at > 1 million pounds per year) and make the information publicly available. The "Challenge Program" is unique because: 1) it is based on a cooperative effort among government, industry, and environmental groups; 2) chemical manufacturers are volunteering to make hazard data publicly available; and 3) the data will be placed on the internet for right-to-know purposes. The program is based upon the Screening Information Data Set (SIDS) which has been a mainstay of the Organization for Economic Cooperation and Development's (OECD) existing chemicals testing program. The SIDS includes tests for physicochemical properties (melting point, boiling point, vapor pressure, partition coefficient, and water solubility); environmental fate (photodegradation, biodegradation, stability in water, and transport/distribution estimates); ecotoxicity (acute toxicity to fish, daphnids and aquatic plants); and human health effects (acute and repeat dose toxicity, genotoxicity, and reproductive/developmental effects). Discussions about determining the adequacy of existing data, the use of structure-activity relationships primarily through their use in chemical categories, and developing appropriate summaries of test reports which will be placed on the worldwide web will be presented. If available, the first round of data will be presented and discussed. (The views expressed are those of the authors and do not represent EPA policy. Visit the Challenge homepage at http://www.epa.gov/chemrtk/volchall.htm.)

1278 THE FDA PROCESS IN ADDRESSING ACCEPTANCE AND IMPLEMENTATION OF ICCVAM-RECOMMENDED TOXICOLOGICAL TESTS.

L. M. Schechtman and B. A. Schwetz. *USFDA, Rockville, MD.*

ICCVAM (the Interagency Coordinating Committee on the Validation of Alternative Methods), established in 1997, is charged with establishing criteria and processes for the validation and regulatory acceptance of sponsored toxicological test methods. In response to that mandate, 14 federal regulatory and research agencies and programs that would consider utilizing such methods or the data derived from them participate in trans-agency efforts associated with the validation, acceptance and harmonization of toxicological test methods. The FDA initiative in responding to ICCVAM issues involves input from ORA (Office of Regulatory Affairs) and all FDA research and product-based centers, i.e., NCTR (National Center for Toxicological Research), CDER (Center for Drug Evaluation and Research), CBER (Center for Biologics Evaluation and Research), CDRH (Center for Devices and Radiological Health), CFSAN (Center for Food Safety and Applied Nutrition, and CVM (Center for Veterinary Medicine), coordinated through CDER's Office of Testing and Research (OTR). FDA product Centers function as distinct units regulating different products and responding to different regulatory directives. ICCVAM-validated methods are reviewed by each Center for applicability regarding research potential, intended use, and relevance to products regulated within their purview. The organization and nature of the

FDA is such that the diversity of regulated products necessitates that each product Center (and not the Agency as a whole), in its mission to ensure product safety, determines which new ICCVAM-recommended test(s) are appropriate for implementation to achieve their regulatory obligations. Centers promote internal and external awareness of ICCVAM information, pending ICCVAM-recommended procedures, and their anticipated application to the Center's safety assessment processes. Upon adoption of a test by a given Center, relevant staff scientists are informed and educated regarding the availability and utility of the test and data interpretation. Sponsored by the SOT Regulatory and Safety Subspecialty Committee.

1279 THE ROLE OF THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS IN THE REGULATORY ACCEPTANCE OF NEW TOXICOLOGICAL TESTING METHODS.

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New toxicological test methods offer benefits of improved safety assessments and potential cost and time savings, and the refinement, reduction, and replacement of animal use. Before new test methods are used to generate information to support regulatory decisions, validation studies must be conducted to determine their performance characteristics, limitations, and usefulness for specific proposed uses. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) were established by the U.S. government to work with test developers and Federal agencies to facilitate the evaluation and adoption of new test methods. Test developers are encouraged to interact with ICCVAM/NICEATM throughout test method development, pre-validation, and validation to maximize the likelihood of generating information needed by agencies to assess the usefulness and limitations of new test methods. Test method submission guidelines have been established and are periodically updated to assist developers in designing test method protocols and validation studies that will adequately address established validation and acceptance criteria. Following validation, ICCVAM and NICEATM coordinate the independent peer review of test methods by an independent international panel of expert scientists. The review process provides for stakeholder participation through public meetings and the opportunity for public comments. The peer review report and ICCVAM recommendations are then provided to Federal agencies for regulatory acceptance consideration. These initiatives seek to facilitate the development, validation, and acceptance of new methods that will provide enhanced protection of public health and the environment and benefit animal welfare whenever feasible. Sponsored by the SOT Regulatory and Safety Subspecialty Committee.

1280 GOOD RESEARCH SCIENCE: CRITICAL TO EFFECTIVE REGULATORY DECISION-MAKING.

S. C. Fitzpatrick and B. Schwetz. *USFDA, Rockville, MD.*

One of FDA's highest priorities is to ensure that the strongest, most up-to-date science underpins all of its decision-making. From the reviewer to the inspector, FDA can only be effective if it has a strong, scientifically skilled workforce. FDA faces many challenges that demand the development and application of sound scientific regulatory principles. For example, FDA must continually identify and respond to an ever-changing array of public health hazards. FDA is also called upon to review products that result from cutting-edge science. FDA must constantly evaluate the public health impact of its prevention and control measures. If FDA has to respond on the basis of incomplete information, it may be forced to compensate for uncertainty by requiring more regulation. More regulation is not only costly to the agency in terms of time and personnel, but it imposes additional financial burdens on industry, and ultimately increases the price to the consumer. To meet this challenge, FDA has developed many innovative regulatory research programs. FDA believes that good laboratory science and research builds a strong foundation for its scientists to anticipate and comprehend new technologies, detect and address risks, and find science-based strategies for their regulatory decisions. FDA has achieved many of its research goals through the strong partnerships it has forged with its sister regulatory agencies. These partnerships have resulted in collaborative research projects on the cutting edge of toxicological science. (Supported by the SOT Regulatory and Safety Evaluation Specialty Section.)

1281 INCORPORATING CHILDREN'S HEALTH CONCERNS INTO STATE ENVIRONMENTAL REGULATIONS: THE MICHIGAN PERSPECTIVE.

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President Clinton issued an Executive Order in 1997 mandating that federal agencies ensure that environmental standards are protective of children's health although federal guidance has been slow in coming. Michigan has taken the lack of guidance as an opportunity to develop innovative approaches to achieving the Order's goals. In December,1998, the Governor convened a Panel to evaluate the protectiveness of current environmental standards, administered by the Michigan Department of Environmental Quality, for children's health and to identify and prioritize standards that may need to be reevaluated. The Panel found that the standards of one state agency could not be evaluated in the absence of consideration of standards of other state agencies as well as consideration of unregulated exposures. It was also clear that there are far too many regulations for the Panel to realistically evaluate and the Panel concluded that it could best fulfill its charge by developing an approach for identifying the most significant exposures and thus for selecting for re-evaluation those regulations having the greatest impacts on children's health. This approach is based on the sensitivity analysis concept. Exposures to the same chemical (or class of chemical) by different exposure routes are semi-quantitatively assessed to determine if one or a small number of routes are responsible for the majority of the risk. Once the largest risk contributors for each chemical are identified, risk comparisons between chemicals or chemical groups can be made and used in assigning priorities for re-assessments.

1282 OCCUPATIONAL EXPOSURE LIMITS (OELS) FOR 30 ORGANOPHOSPHATE PESTICIDES (OPS) AND SUPPORTING RATIONALE.

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Dose- and time-dependent red blood cell (RBC) acetylcholinesterase (AChE) inhibition was evaluated for 30 OPs to clarify the dose-time-response relationship in general; and, to recommend inhalation OELs (mg/m3). Data from 1400+ studies indicated departures from equivalent toxicity across species (humans, rats, dogs), exposure routes (oral, inhalation) and exposure duration (suchronic, chronic) for specific OPs were common, but were readily explained by consideration of OP-specific rates of toxicokinetic (TK; e.g., toxification or detoxification rates) and toxicodynamic (TD; e.g., rates of binding, aging, etc.) processes in relation to exposure duration and observation period. OELs based on peak RBC AChE inhibition were derived using a weight-of-evidence NOEL approach which considered data from all pertinent oral and inhalation studies as well as pertinent TK and TD processes. Overall, OELs were poorly correlated with inhalation NOELs derived from oral NOELs in dogs, rats or humans using species-specific physiologic parameters, consistent with the observation that oral studies often failed to consider OP-specific TK and TD processes in their design. OELs were better correlated with inhalation NOELs from rat inhalation studies which tended to minimize the confounding influence of TK and TD processes by appproaching constant dose. (Not enough dog or human inhalation studies were available for analysis.) Thus, although more readily available, oral studies are a poor basis for OELs unless relevant TK and TD processes are incorporated into their design. (JES current address: NY State Dept. of Health).

1283 VARIATION IN CANCER CLASSIFICATION BETWEEN EUROPEAN COUNTRIES AND ORGANIZATIONS.

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This study a) compared cancer classification systems and their use in different European countries and agencies and b) identified reasons for differences in classifications for the same chemical. Countries and agencies considered were: the International Agency for Research on Cancer (IARC), the European Union (EU), the German MAK (maximum workplace concentration) Commission (German MAK), the Netherlands, Norway, and Sweden. We

evaluated 14 chemicals. IARC, the EU, and the German MAK classify carcinogens according to potential for carcinogenicity in humans, which may affect labeling, use restrictions, and occupational exposure levels (OELs). Norway classifies carcinogens according to potency, for labeling and establishing use restrictions, without distinguishing between animal and human carcinogens. The Netherlands classifies carcinogens according to genotoxicity, for specifying risk assessment approaches and deriving OELs. Different countries and agencies place different weight on data from epidemiological and animal studies, and mechanistic studies. There is general agreement on classification for chemicals with good evidence of carcinogenicity in humans (e.g. asbestos, benzene, and vinyl chloride) and less agreement for chemicals with positive animal data and inadequate or limited human data (e.g. 1,2-dichloroethylene, tetrachloroethylene, and trichloroethylene). Differences appear to be due to consideration of mechanisms, consideration of negative epidemiology data, and date of the classification (i.e., data availability). For example IARC recently downgraded its classification of acrylonitrile from probably to possibly carcinogenic to humans (2A to 2B) based on epidemiology data, but the classification of acrylonitrile has not been adjusted by either the EU or the German MAK.

1284 EUROPEAN DIFFERENCES IN DERIVATION OF OCCUPATIONAL EXPOSURE LEVELS.

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This study a) compared derivation of occupational exposure levels (OELs) in European countries and agencies, and b) identified reasons why OELs may differ for the same chemical. Countries and agencies considered were: the European Union (EU), the United Kingdom (UK), the German MAK (maximum workplace concentration) Commission (German MAK), the Netherlands, Sweden, Denmark, Norway, and Ireland. Most countries and the EU use separate committees for evaluating scientific criteria, and technical and socioeconomic factors. Composition of the committee evaluating scientific data consists of: independent scientific experts (EU, German MAK); scientists representing employers, labor, and trade organizations (UK, Sweden); or scientists appointed by government, or a governmental organization (Netherlands, Denmark, Norway). OELs for genotoxic carcinogens consider: technological feasibility (UK, German MAK); cost-benefit analysis (UK); and acceptable risk level with consideration of socioeconomic factors (Netherlands, EU). OELs for non-carcinogens and non-genotoxic carcinogens are generally health-based, using a No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed-Adverse-Effect-Level (LOAEL) and uncertainty factor (UF) approach. The EU uses unique species-specific UFs, and UFs to account for the nature of the dose-response relationship. The UK uses UFs that consider severity of the critical effect. OELs for respiratory sensitizers may be based on feasibility (UK) or acceptable risk (EU). The selection of critical endpoint varies among countries and agencies. For example, Sweden is more likely to select irritation as a critical effect, which may be the basis for the lower OEL for trichloroethylene in Sweden (10 ppm) vs. other countries (30 - 100 ppm). Other differences in OELs reflect differences in both risk assessment and risk management approaches. For example, the difference in the OEL for ethylene oxide between the UK (5 ppm) and the German MAK (1 ppm) appears to reflect differences in risk management rather than risk assessment.

1285 JOURNAL ARTICLES: IMPROVING THE USEFULNESS FOR RISK AND REGULATORY ASSESSMENTS.

S. M. Taulbee. *SkeleTech, Inc., Bothell, WA.* Sponsor: R. W. Tyl.

Risk and health affects assessment professionals rely on published literature for primary and supporting documentation in evaluating compounds. In performing assessments, the usefulness of a journal article is first effected by the quality of the data and completeness of information and second by the value of the research results. A review of 97 reproductive and developmental toxicology articles was performed to evaluate the completeness of data presentation. The evaluation used regulatory and Institute for Evaluating Health Risk (IEHS) criteria to review presentation of materials and methods, results and discussion. The review showed that ~99% of the articles lacked at least one of the evaluation criteria. Results showed that ~4 to 8% of the articles did not adequately describe the test, control or reference substance; and ~40% did not describe characterization analyses. Approximately 30% of the articles did not fully describe the number of animals on study. While these results are dramatic, they must be tempered with an understanding that many articles are written to describe research results of in a certain context unrelated to risk evaluations. However, data are potentially used in other than the intended context, e.g., risk assessment, and journal instructions and editors encourage condensation and summarization. The primary purpose of this poster is to demonstrate the need for, and to present a list of, key criteria to be used by authors and journal reviewers as a checklist, thereby improving the availability of useful data.

1286 PROPOSED GUIDELINES FOR THE GLOBAL PRECLINICAL DEVELOPMENT OF VARIOUS NUTRACEUTICALS.

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Nutraceuticals have been defined by Stephen DeFelice as food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease. While there is still some debate about what a nutraceutical is, medical foods, functional foods and dietary supplements are all considered to be specific types of nutraceuticals. Due to a variety of socio-economic pressures, there is a rapidly growing popularity and prevalence of nutraceuticals, especially dietary supplements. Companies have perceived a significant market opportunity with these products because of attractive economic returns and an extremely lax global regulatory environment. As the lines of demarcation begin to pale between these new types of products and foods, food additives and traditional drugs, the need for regulatory guidance is becoming increasing more profound. Proposed here the details of preclinical development packages for a variety of different types of nutraceuticals. Before proceeding with the development of any potential nutraceutical, it is important to ascertain whether the potential product is a food, a drug or a dietary supplement. It is the intended use of a proposed nutraceutical rather than the type of ingredient in the nutraceutical that determines the applicable review process. Drugs must be proven to be safe and effective for a particular indication before marketing according to well-known guidelines. In parallel fashion, food additives incur a FDA premarket review of ingredient safety. Specific programs are outlined for medical and functional foods. Dietary supplements have no FDA premarket review of ingredients or finished products. They also incur no regulations concerning good manufacturing practice, identification, characterization or standardization of ingredients, efficacy or safety. From a regulatory viewpoint, the treatment of a dietary supplement is dependent on how it is labeled and what claims are stated on the label or in the course of marketing the material. In order to market dietary supplements, testing included in a developmental package can range from a basic pharmacology profile, acute toxicology data, antigenicity and hypesensitivity testing results and other specific tests as indicated to complex programs similar to those for pure pharmaceuticals. The proposed programs attempt to achieve balance between regulation, safety, cost and liability.

1287 RISK ASSESSMENT-BASED APPROACH FOR THE BIOLOGICAL EVALUATION OF MEDICAL DEVICE MATERIALS.

M. E. Stratmeyer and R. P. Brown. *Health Sciences Branch, CDRH/USFDA, Rockville, MD.* Sponsor: P. L. Goering.

Systemic toxicity tests of medical device materials are usually conducted using an extract obtained from the material. The use of relatively dilute extracts limits the ability of these tests to detect all but the most overt signs of toxicity. Further, these tests are often conducted with little or no knowledge of the chemical composition of the extract obtained from the device. As a result, it is not possible to anticipate the toxic effects that may be produced following exposure to the medical device material. To address these needs, a risk assessment-based approach has been developed to assess the potential for a medical device material to produce adverse systemic effects. The hallmarks of this approach are: 1) chemical characterization of the device, 2) estimation of the dose of each chemical constituent received by a patient, 3) derivation of a Tolerable Intake (TI) value for each constituent, and 4) comparison of the dose of each compound received by a patient to it's TI. While this proposed approach has not yet been formally accepted in CDRH as an alternative to systemic toxicity testing, it shows promise as a means to provide relevant data for regulatory decision making and should reduce the number of animals required for premarket testing. Further, this proposed approach is consistent with a number of regulatory initiatives currently underway in the FDA, including the use of knowledge bases and consensus standards for the biological evaluation of medical devices.

1288 DOSE-DEPENDENT HYPOTHYROXINEMIA IN FEMALE SD RATS FOLLOWING A SINGLE ORAL DOSE OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN.

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In order to assess the effects of 2,3,7,8-tetrachlorodibenso-p-dioxin (TCDD) on thyroid function, low doses of TCDD, just sufficient to affect hormonal status were employed in this study. Female Sprague-Dawley (SD) rats (6-week old) were given a single oral dose of TCDD ranging from 1.0 to 4.0 μg/kg bw, and serum and tissue samples were collected 7 days after dosing. Serum levels of thyroxine (T4) were decreased significantly at doses of 2.0 and 4.0 μg TCDD/kg, but triiodothyronine (T3) levels were not changed. Serum thyroid stimulating hormone (TSH) levels and thyroid weights were significantly increased at dose of 4.0 μg TCDD/kg bw. A marked increase of hepatic TCDD-responsive genes, UDP-glucuronosyltransferase-1 (UGT1) and cytochrome P4501A1 (CYP1A1), were found at as low as 1.0 μg TCDD/kg bw in a dose-related fashion. TCDD concentrations in the serum and adipose tissues were detected dose-dependently by gas chromatography-mass spectrometry. Interestingly, we found the intensive immunostaining of TSH by TCDD in the βcells of the pituitary anterior lobe. These results show that the induction of TCDD-response gene UGT1 (1.0 μg/kg bw) via aryl hydrocarbon (Ah) receptor may cause an enhancement of biliary excretion of T4. The subsequent depression in serum T4 levels results in an elevation of TSH production. The present studies demonstrate for the first time that a single low dose of TCDD induce hypothyroxinemia as a result of liver-pituitary-thyroid axis in a dose-dependent manner.

1289 MODIFICATION OF 2,3,7,8-TETRACHLORO-DIBENZO-*P*-DIOXIN INDUCED CYTOCHROME P-4501A1 BY ESTROGEN IN LIVER OF FEMALE LONG-EVANS RATS.

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induces liver tumors in female rat liver and is classified as a potential human carcinogen. In the present study, we examined the effects of estrogen (E2) on TCDD-induced CYP1A1 gene expression in the liver of female Long Evans rat. Induction of CYP1A1 by TCDD was observed following oral administration of single doses of 100, 300 and 1000ng/kg body weight. The induction of CYP1A1, determined as EROD activity, was significantly increased by E2 (17b-estradiol, 5μg/kg body weight) treatment to both intact and ovariectomized rats. Immunoblot analysis of liver cytosol showed no apparent change in arylhydrocarbon receptor (AhR) levels by treatment of E2 or TCDD alone or both compounds. While there was an increase in nuclear AhR by E2 or TCDD alone or both, no difference between TCDD alone and E2 plus TCDD treatment was observed. Additionally, immunoblot analysis also showed that treatments by E2 alone and E2 plus TCDD, but not TCDD alone, significantly increased E2 receptor (ER) levels in the nucleus of ovariectomized rat liver. These observations suggest that the liganded ER in the nucleus may be involved in the activation of CYP1A1 gene expression after the formation of Ah receptor TCDD complex. In conclusion, estrogen *in vivo* can enhance the TCCD induced CYP1A1 expression.

1290 DIFFERENTIAL EFFECTS OF ESTRADIOL CONGENERS ON THE EXPRESSION OF CYP1A1 INDUCED BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN A MOUSE OVARIAN EPITHELIAL CANCER CELL LINE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a well known environmental pollutant, promoter of carcinogenesis and reproductive toxicant. TCDD induced ethoxyresorufin-o-deethylase (EROD) activity dose-dependently in a mouse ovarian epithelial cancer cell line (ID8). Induction of EROD by TCDD was inhibited by a-naphthoflavone, an aryl hydrocarbon receptor (AhR) antagonist. Interestingly, estradiol (E2) congeners increased dose-dependently the induction of EROD by TCDD in order of estrone (E1) > 17b-estradiol (E2) > 4-hydroxyestradiol (4-OHE2) @ 2-hydroxyestradiol (2-OHE2), whereas estriol (E3) decreased EROD activity. E2 congeners did not affect the number of ID8 cells, as shown by similar DNA and protein concentrations during 48hr in culture. Based on Western and Northern blots, E2 increased CYP1A1 protein and mRNA dose-dependently whereas E3 decreased these parameters. Transfection with a dioxin response element (DRE)-containing a luciferase reporter revealed that E2 did not alter the luciferase activity induced by TCDD whereas E3 decreased luciferase activity dose-dependently, indicating differential mechanisms between E2 and E3. RT-PCR revealed that CYP1A1 mRNA was induced by TCDD in ID8 cells whereas CYP1B1 mRNA was constitutively expressed and was not affected by TCDD. Progesterone and gonadotropins had no effect on the induction of EROD by TCDD. Based on RT-PCR, estrogen receptor (ER) a is constitutively expressed in ID8 cells, but ERb and progesterone receptors were not detectable. These results indicate that most of the E2 congeners tested in this study enhanced induction of EROD by TCDD wherease estriol reduced its ability to do so. This differential effect may provide insight into the mechanism(s) of how estrogens interact with TCDD.

1291 INCREASED OXIDATIVE DNA DAMAGE IN FEMALE SPRAGUE-DAWLEY RATS CHRONICALLY TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN AND 17 β-ESTRADIOL.

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Hepatocarcinogenesis induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is influenced by ovarian hormones. The induction of cell proliferation and the formation of oxidative DNA damage by TCDD are ovarian hormone-dependent. Estrogen is hypothesized to contribute to the mechanism of TCDD-induced hepatocarcinogenesis through multiple mechanisms, including the increased production of reactive oxygen species from the redox cycling of catechol estrogen metabolites. The aim of this current study was to test the hypothesis that estrogen enhances TCDD-induced formation of oxidative DNA damage. Diethylnitrosamine-initiated intact and ovariectomized female Sprague-Dawley rats were co-treated with 17β-estradiol and TCDD for 30 weeks and 8-hydroxydeoxyguanosine (8-OHdG) adduct formation in hepatic nuclear DNA was measured by HPLC with electrochemical detection. Preliminary analyses demonstrated an increase in 8-OHdG adduct formation in TCDD-treated intact, but not ovariectomized rats. The formation of 8-OHdG was increased in TCDD-treated ovariectomized rats supplemented with continuous 17β-estradiol and equivalent to levels observed in TCDD-treated intact rats. These data support previous observations of the dependency of TCDD-induced 8-OHdG adduct formation on ovarian hormones and suggest that 17β-estradiol is a key factor in TCDD-induced oxidative DNA damage.

1292 INHIBITION OF ESTROGEN-INDUCED RETINOIC ACID RECEPTOR α1 GENE EXPRESSION BY TCDD MECHANISMS OF ACTION.

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17β-Estradiol (E2) induces retinoic acid receptor α1 (RARα1) mRNA levels (2-fold) in MCF-7 human breast cancer cell lines, and after cotreatment with 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the E2-induced response was completely inhibited in these cells. pRARA12 is an estrogen-responsive construct containing an insert from the -509 to +105 region of RARα1 gene promoter linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene. In MCF-7 cells transiently transfected with pRARA12 and an estrogen receptor α (ERα) expression plasmid, treatment with 1 nM E2 resulted in a 4-fold induction of CAT activity. Cotreatment with 1 nM E2 + 10 nM TCDD significantly decreased (> 50%) the hormone-induced response. Promoter deletion and mutation analysis was further used for identifying functional inhibitory dioxin-responsive elements (iDREs) (GCGTG) that interact with the Ah receptor complex and mediate inhibitory effects of TCDD for other E2-induced genes including cathepsin D, c-*fos* and *pS2*. Results showed that a potential iDRE at -110 was not functional; however, inhibition of E2-induced transactivation using several constructs was dependent on the Ah receptor. The mechanisms associated with inhibition of ERα/Sp1-dependent activation of RARα1 gene promoter-derived constructs by TCDD were investigated and shown not to be related to modulation of kinase-dependent

activation of Sp1 or alteration of cellular Sp1/Sp3 ratios. The results suggest that Ah receptor-mediated downregulation of ERα levels may be a possible mechanism, and this was supported by DNA footprinting studies showing that the Ah receptor complex partially blocked ERα/Sp1 interactions with E2-responsive GC-rich sites. (ES04176 and ES09106)

1293 3',4'-DIMETHOXYFLAVONE AS AN ARYL HYDROCARBON RECEPTOR ANTAGONIST IN BREAST CANCER CELLS.

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Previous studies have shown that several substituted flavonoids, including some 3'-substituted flavones, exhibit aryl hydrocarbon receptor (AhR) antagonist activities. The AhR antagonist activity of substituted flavones can be both structure- and cell context-dependent, and interpretation of results can be difficult due to other activities of these compounds. We have been investigating the AhR agonist/antagonist activities of 3',4'-dimethoxyflavone (DMF) in estrogen receptor (ER)-positive MCF-7 and T47D breast cancer cell lines. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (1 nM) induced a 20- to 40-fold increase in CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity in MCF-7 and T47D cells, whereas 0.1 to 10 μM 3',4'-DMF alone did not induce EROD activity. In cells cotreated with 1 nM TCDD plus 0.1 to 10 μM 3',4'-DMF, there was a concentration-dependent decrease in the induced response, with total inhibition at the 10 μM concentration. In parallel experiments; 3',4'-DMF (10 μM) also inhibited chloramphenicol acetyltransferase (CAT) activity in breast cancer cells treated with 1 nM TCDD and cotransfected with pRNH11c, an Ah-responsive construct containing a human CYP1A1 gene promoter insert (-1142 to +2434) linked to a CAT reporter gene. 3',4'-DMF alone did not induce CAT activity in this transfection assay. 3',4'-DMF did not significantly inhibit estrogen-induced growth of breast cancer cells, and ongoing studies are further investigating the AhR antagonist activities of 3',4'-DMF and the effects of this compound in blocking AhR-mediated antiestrogenic responses in breast cancer cells. (ES04176 and ES09106)

1294 TESTICULAR TOXICITY OF TCDD IN A TCDD- SENSITIVE AND A TCDD-RESISTANT RAT STRAIN: A STEREOLOGICAL ANALYSIS.

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Testicular toxicity of TCDD was studied in the sensitive Long-Evans (Turku/AB; L-E) and the resistant Han/Wistar (Kuopio; H/W) rats, differing >1000-fold in their sensitivity to the acute lethality of TCDD. Adult male rats were given a single oral dose of TCDD at 0, 0.1, 2 or 20 μg/kg (L-E), and 0, 0.1, 20 or 1000 μg/kg (H/W). The study was terminated and samples collected 4 or 17 days after dosing. Testes were fixed in Bouin's solution and examined using a stereological optical disector/fractionator method. Sertoli cells, Leydig cells and spermatogenic cells at stages VII and VIII of the seminiferous epithelial cycle were quantified. Serum testosterone, estradiol, LH and FSH levels were analyzed using RIA/FIA. Neither strain showed any changes in seminiferous epithelial cell types 4 days after dosing. On day 17, the number of Sertoli cells and all spermatogenic cell types (spermatogonias, preleptotene and pachytene spermatocytes, round spermatides) at stages VII and VIII were significantly decreased at 20 μg/kg in L-E rats. The number of Leydig cells was not significantly affected. In H/W rats no changes were observed at 1000 μg/kg on day 17. Decreases were observed in serum testosterone, estradiol and LH concentrations in L-E rats at 20 μg/kg on day 17 (estradiol and LH levels showed some decrease already on day 4), but not at all in H/W rats. It is concluded that H/W rats that are resistant to acute lethality of TCDD are also resistant to the toxic effects of TCDD to the seminiferous epithelium. (Supported by the Academy of Finland, the Finnish Research Programme on Environmental Health.)

1295 TUMOR PROMOTION IN THE LIVERS OF MALE RATS CHRONICALLY EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD).

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Chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces the formation of liver tumors in female but not male rats. The aim of this study was to investigate the mechanism of the gender-specific hepatocarcinogenicity of TCDD. The development of putatively preneoplastic altered hepatocellular foci (AHF) was analyzed in the livers of diethylnitrosamine-initiated male Sprague-Dawley rats that were treated weekly with 700 ng TCDD/kg, or corn oil vehicle, for 30 weeks. Mean hepatic TCDD levels (ppt wet weight), measured by GC/MS, were 35 ± 26 ppt and 12595 ± 4225 ppt in control and TCDD-treated male rats, respectively. AHF expressing the placental form of glutathione-S-transferase (PGST) were measured immunohistochemically. The mean number of PGST AHF/cm^3 in TCDD-treated male rats (4921±1074) was significantly higher than that observed in control animals (2559±1904) ($P<0.01$ Fisher PLSD test). These levels were significantly higher than those observed in similarly treated female rats. In contrast there was no significant difference in mean PGST AHF liver volume fraction between corn oil-treated (0.84±0.81) and TCDD-treated (1.63±1.08) animals. Furthermore these volume fractions were similar to that observed in similarly treated female rats. These data suggest that in males, TCDD is able to promote the formation of these putatively preneoplastic AHF but is unable to promote the growth of them. The increase in number of AHF by TCDD may be due to an increase in mutation of normal cells to an initiated phenotype and/or due to a suppression of apoptosis of initiated cells.

1296 LIVER TUMOR PROMOTING ACTIVITY OF TCDD IN A TCDD-SENSITIVE AND A TCDD-RESISTANT RAT STRAIN.

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Liver tumor promoting activity of TCDD was studied in the sensitive Long-Evans (Turku/AB; L-E) and the resistant Han/Wistar (Kuopio; H/W) rats differing >1000-fold in their sensitivity to the acute lethality of TCDD. Female rats were partially hepatectomized, initiated with nitrosodiethylamine and, after 5 weeks, treated with weekly s.c. doses of TCDD (total doses 0, 0.17, 1.7, 17 (both strains), 170 and 1700 (H/W only) μg/kg) for 20 weeks. Liver TCDD concentrations at the end of the study reflected accurately the doses administered. Dose-responses for liver EROD activity were largely similar in both strains. Liver tumor promoting activity, measured as glutathione S-transferase P (GST-P) positive foci, was significantly and dose-dependently increased in L-E rats at 1.7 and 17 μg/kg, but in H/W rats only at 170 μg/kg. Histopathological findings and elevation of plasma transaminase activities followed the same pattern, suggesting an association between liver tumor promotion and hepatotoxicity in these rat strains. Frequency of micronucleated erythrocytes in bone marrow and peripheral blood were increased in L-E rats at 17 μg/kg and in H/W rats at 170 and 1700 μg/kg. The results demonstrate that although H/W rats are extremely resistant to the acute lethality of TCDD, they are relatively sensitive to liver tumor promotion by the same compound, and the sensitivity difference between L-E and H/W rats in liver tumor promotion (about 100-fold) is clearly smaller than that in lethality. (Supported by the European Commission, Contract No. ENV-CT96-0336.)

1297 ANEMIA AND LUNG CANCER IN 1,2,3,4,6,7,8-HEPTACHLORODIBENZO-*P*-DIOXIN (HPCDD)-TREATED FEMALE SPRAGUE-DAWLEY RATS AFTER VARIOUS SINGLE AND MULTIPLE ORAL DOSES.

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Groups of 30 to 60 adult female Sprague Dawley rats were administered single doses of HpCDD ranging from 2.8 to 3.4 mg/kg in corn oil (4 ml/kg) by gavage. Another group of 30 females received a loading dose of 2.8 mg/kg HpCDD followed by biweekly maintenance doses of 0.086 mg/kg also by gavage. Body weight and feed intake were monitored at least 5 times a week. Animals were monitored for signs of wasting/hemorrhage (25% or more body weight loss/bleeding around nose or in intestine), anemia (exceptional paleness) and lung cancer (visible only as an abscess upon death) by daily observations. The different effects were allowed to run their time course unless rats were in severe distress (e.g., large or bleeding [mainly mammary] tumors) in which case they were euthanized. Upon death heart, lungs, kidneys, upper GI tract, sternum and femur, liver and any macroscopically observable tumor were harvested, embedded, sectioned and stained with H & E. Microscopic examination confirmed in every instance macroscopic observations regarding anemia in that bone marrow cellularity was always less than 20% in exceptionally pale rats. Additional, macroscopically not identifiable squamous cell carcinomas of the lungs were also detected by light microscopy. Times to first wasting/ hemorrhage (70.3±8.5 mg/kg x day) and to first anemia (435.8±15.4 mg/kg x day) and to first lung cancer (544.9±66 mg/kg x day) were in reasonably good agreement with c x t=k. Steady state vs. single dose exposure resulted in a steepening of the dose response for wasting/hemorrhage and for anemia with an increase in incidence approximately proportionate to the AUC. More time will be needed to obtain corresponding information for lung cancer.

1298 2,3,7,8- TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ACCELERATES DIFFERENTIATION, BUT NOT APOPTOSIS, OF HUMAN KERATINOCYTES IN ORGANOTYPIC CULTURE.

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Human exposure to the environmental toxin TCDD produces a severe skin pathology known as chloracne. Historically the primary tool employed to explore the molecular basis of TCDD's effect upon skin has been monolayer culture of keratinocytes. Because monolayer culture cannot recapitulate the three dimensional architecture or epithelial-mesenchymal interactions found in skin, organotypic culture, which accounts for these factors, would be an ideal system in which to investigate the effect of TCDD on intact skin. In new experiments we have shown that TCDD causes accelerated differentiation in a three dimensional, organotypic skin model. Analysis at both the light and electron microscope levels reveals a fully differentiated cornified layer in TCDD-treated organotypic cultures at earlier time points than observed in vehicle (DMSO)-treated controls. In situ 3' end labeling reveals no increase in nucleosomal fragmentation, a characteristic of apoptosis, in TCDD-treated organotypic cultures. Furthermore, basal cells in TCDD- and DMSO-treated organotypic cultures show no differences in proliferation as measured by quantification of BrdU positive nuclei. We hypothesize that TCDD exerts its effects upon intact skin through transcriptional modification of a mesenchymally-produced growth factor, which in turn could produce changes in the differentiation program of the epithelial layer. The described *in vitro* organotypic skin model will be used to test both the validity of this hypothesis and the cell type-specific role that the aryl hydrocarbon receptor (AhR) plays in TCDD toxicity in skin.

1299 TCDD INDUCES EXPRESSION OF MATRIX REMODELING PROTEASES.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in a number of pathological conditions including chloracne, a hyperkeratinization of the skin, as well as an increased incidence of liver and skin cancer. It is generally accepted that the pathological effects of TCDD are mediated through its binding to the aryl hydrocarbon receptor (AhR). TCDD binding to AhR results in translocation into the nucleus, where it dimerizes with ARNT and becomes an active transcription factor binding to specific sequences known as xenobiotic response elements (XRE). How this pathway results in the pathological effects of TCDD in target tissues is unclear. Using human skin as a model, we have focused on the effect of TCDD on a family of matrix proteases, the matrix metalloproteinases (MMP). These enzymes are responsible for the majority of extracellular matrix degradation, and their expression is required for processes involving tissue remodeling such as wound healing and development. We identified two potential XRE elements in the promoter of the human MMP-1 (collagenase) gene, suggesting a role for the AhR/ARNT pathway in regulation of this gene. Northern analysis demonstrates that expression of MMP-1, as well as other MMPs, is increased in TCDD-treated keratinocytes, but not in TCDD-treated dermal fibroblasts. Mobility shift analysis shows binding to both the putative XRE elements in the MMP-1 promoter. Our findings have identified a new class of target genes that may be involved in mediating the pathological response of human skin to TCDD exposure.

1300 DERMAL AND ORAL EXPOSURE OF TCDD IN TG.AC MICE: DOSE- AND TIME-RESPONSE STUDIES.

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Dose-response and exposure route studies were performed with the ubiquitous, bioaccumulative, and carcinogenic agent-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-using the Tg.AC transgenic mouse model in order to evaluate the development of papillomas over time. Female hemizygous Tg.AC mice (20/group) received 0, 5, 17, 36, 76, 121, 166, 355, or 760ng TCDD/kg in acetone three times a week, for 26 weeks, by dermal application or 0, 105, 450, or 1250ng TCDD/kg in corn oil five times a week, for 26 weeks, by gavage administration. With either route of exposure, TCDD caused neoplastic lesions that were confined to the skin and included squamous cell papillomas and carcinomas. The average number of papillomas per animal and the time to papilloma occurrence were dose-dependent. An increase in the number of papillomas was observed at doses ≥17ng TCDD/kg (dermal) or 1250ng TCDD/kg (oral) by study termination. A dose-dependent increase in hepatic and pulmonary cytochrome P4501A activities was observed. TCDD significantly increased hepatic CYP1A1 and CYP1A2 activities in all dose groups (dermal and oral). Pulmonary CYP1A1 activities were significantly increased at doses ≥36ng TCDD/kg in the dermal study and at all dose levels in the oral study. A remarkable finding in the gavage study was the absence of neoplastic lesions in organs other than the skin.

1301 IDENTIFICATION AND CHARACTERIZATION OF TIG1, A NOVEL TCDD-INDUCIBLE GENE.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a prototype of a class of halogenated aromatic hydrocarbons, which are mostly byproducts of industrial processes and combustion of fuels. Many of the chemicals are persistent environmental contaminants. TCDD elicits a wide range of toxic responses in animals and impose a threat to human health. The aryl hydrocarbon receptor is a ligand-activated receptor/transcription factor, which, together with other factors, mediates the biological effects of TCDD and related chemicals. The mechanism of action of AhR involves transcriptional regulation of gene expression of target genes. However, current knowledge on the target genes and their roles in TCDD toxicity is limited and does not adequately explain the wide spectrum of TCDD toxicity. To gain new insights into the mechanism of TCDD action, we have utilized the mRNA differential display technique to identify new genes that are regulated by TCDD. We report here the identification of a new TCDD-inducible gene (TIG1) in mouse cell lines. Analyses of the partial sequence of TIG1 suggest that it represents a novel gene product. The induction of TIG1 by TCDD is both time- and dose-dependent. Cycloheximide, an inhibitor of protein synthesis, superinduces TIG1 in the presence of TCDD. Cell genetic analyses of the induction reveal that it requires AhR and Arnt. Furthermore, these analyses demonstrate that the transcription activation functions of both AhR and Arnt are required for the induction of the gene. Finally, treatment of C57BL/6 mice with low doses of TCDD induces TIG1 mRNA expression in liver. Thus, TIG1 is a novel target gene of

TCDD in both cultured cell and intact animal; the induction of the gene by TCDD is mediated through the AhR/Arnt signaling process. These findings provide new insights into the mechanism of gene regulation by AhR. Current research is aimed at analyses of the sequence and cellular function(s) of TIG1.

1302 INDUCTION OF METALLOTHIONEIN IN LIVER OF FEMALE RATS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN.

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Metallothioneins (MTs) are cysteine-rich metal-binding proteins that exert cytoprotective effects against metal toxicity and oxidative stress. Since 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known to cause an exaggerated oxidative stress response in the rats and mice, we have thus studied a possible involvement of MT in oxidative responses induced by TCDD. Female Sprague-Dawley (SD) rats (6-week old) were administered a single oral dose of TCDD varying from 1.0 to 4.0 μg/kg and the serum and tissues were collected 7 days after dosing. In the liver MT protein as well as mRNAs of MT-I and MT-II, the major forms of MT, were dose-dependently induced at as low as 1.0 μg TCDD/kg bw. Significant increases in serum 8-OHdG levels, a biomarker for DNA oxidation, were observed in the rats dosed at 2.0 and 4.0 μg TCDD/kg bw. TCDD administration resulted in a significant increase in metal concentrations (Cu, Fe, Zn and Ca) in the liver. Serum cytokines (TNF-α, IL-1β, IL-6 and IL-10), GOT and GPT were not affected at any doses of TCDD. These results indicate that a single low dose of TCDD caused an oxidative stress response as shown by an increase in serum 8-OHdG levels, and suggest that induced MT in the liver may be responsible for the reduction of the toxicity for an oxidative stress caused by TCDD. (This work was supported in part by STA Special Fellowship to N. Nishimura.)

1303 MECHANISTIC MODELING OF TCDD-INDUCED CYTOCHROME P450 GENE EXPRESSION IN RAT LIVER.

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The dose-response relationships of cytochromes (CYP) P450 1A1, 1A2, and 1B1 in the livers of female Sprague-Dawley rats chronically exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have been characterized. The aim of this study was to evaluate CYP P450 gene expression across several chronic tumor initiation/promotion studies in a mechanistic model and to use the model to identify possible mechanisms for the previously observed differences in the dose-dependent expression of CYP1A1, CYP1A2, and CYP1B1. In the two continuous studies, rats were treated with either low or high doses of TCDD via biweekly gavage for 30 continuous weeks. In the "discontinuous" study, rats were treated with the same approximate daily dose of TCDD via biweekly gavage but for varying exposure durations. TCDD-induced gene expression was modeled using biochemically realistic parameters such as multiple binding sites for the TCDD/aryl hydrocarbon receptor (AhR) ligand/AhR nuclear translocating protein (ARNT) complex at dioxin responsive elements (DREs) on DNA, stabilization of mRNA by a poly(A) tail, a lag time for mRNA translation, and saturation kinetics of protein synthesis. The best fit of the model to the observed data predicted: (1) the TCDD/AhR/ARNT complex had a higher affinity to DREs of CYP1A1 and CYP1A2 compared to CYP1B1, (2) CYP1B1 mRNA was less stable due to its estimated shorter poly(A) tail, and (3) saturation kinetics was due to the limited number of ribosomes available for binding to RNA. The ability of this model to reproduce the observed TCDD-induced responses demonstrates its utility for providing mechanistic insights into receptor-mediated gene induction.

1304 MARKERS OF EXPOSURE AND EFFECT IN REPRODUCTIVE AND NON-REPRODUCTIVE TISSUES OF FEMALE CHICKENS FOLLOWING *IN VIVO* TCDD TREATMENT.

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While the overt toxicity of TCDD to avian species has been clearly demonstrated, the associated biochemical and molecular changes have been much less studied. To address this issue, twenty-one week old Rhode Island Red - White Feather F1 hybrid hens were treated with 10 (low dose) or 100 (high dose) μg/kg body weight TCDD or an equal volume of the corn oil vehicle and sacrificed after 24 hours to determine the effect of exposure on cellular function and signal transduction pathways. Liver, adipose, and ovarian tissues were collected and analyzed for glutathione-S-transferase, lipoprotein lipase, glucose transporter, and cellular signaling protein kinases that are well-characterized as targets for TCDD in mammals. The kinases we specifically focused on are two important components of signaling protein pathways: -tyrosine kinase, an upstream signaling protein that mediates cytosolic signals in response to changes in epidermal growth factor receptor (EGFR)-ERK2, one of the MAPK family members that can transduce from cytosolic to nuclear signal, activates the transcriptional activity of several transcription factors including early response genes and their protein complex AP-1, and phosphorylates and activates the estrogen receptor. Glutathione-S-transferase (GST) activity was increased in adipose tissue, not effected in the liver, and decreased in the ovarian tissue of birds exposed to the high dose of TCDD, but was not changed in either tissue with 10 μg/kg TCDD. Lipoprotein lipase activity in adipose tissue was decreased in birds treated with 100 μg/kg TCDD but not with 10 μg/kg exposure. Glucose transporters in liver (GLUT2) of birds in the high dose group bound more cytochalasin B than the respective controls, while glucose transporter binding in adipose tissue (GLUT4) decreased in TCDD-treated birds. Finally, tyrosine phosphorylation of a currently unidentified protein with M_r between 116 and 97 consistently increased in liver, ovarian, and adipose tissue of 100 μg/kg TCDD-treated birds. Future experiments will also measure ethoxyresorufin-O-demethylase (EROD) activity as classical marker of exposure, and lipid peroxidation and epidermal growth factor receptor binding as additional indicators of biochemical effects.

1305 AGONIST ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR COMPARED TO AVIAN CARDIOTOXICITY MEDIATED BY TCDD.

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Polyhalogenated aromatic hydrocarbons, such as 2,3,7,8 tetrachlorodibenzo-p-dioxin, (TCDD) are ubiquitous environmental contaminants whose toxicity has been correlated with their ability to bind and activate the aryl hydrocarbon receptor (AHR). The AHR activates specific genes such as cytochrome p450 1A1 and 1A2 (CYP1A1 and CYP1A2) by binding to specific DNA response elements. Avian species are especially sensitive to TCDD exposure which results in a myriad of toxic effects including edema disease and cardiotoxicity, as well as increased levels of CYP1A4 and CYP1A5, the avian homologues to mammalian CYP1A1 and CYP1A2. However, the direct activation of the avian AHR has yet to be characterized or correlated with observed physiological effects of TCDD exposure in avian systems such as cardiotoxicity. Therefore we have designed a model to determine the relative affinity of five well-defined AHR agonists, including TCDD, for the chicken AHR by comparing the ability of each compound to induce DNA binding of the AHR complex in either LMH (chicken liver carcinoma) cells or HepG2 (human liver carcinoma) cells by electrophoretic mobility shift assay. These results were quantitated by phosphorimaging analysis to determine the toxic equivalency factors from the apparent EC50 of each of these compounds between the chicken and human AHR. Our initial results indicated no dramatic difference between the binding affinities for the chicken and human AHR. To correlate activation of the DNA binding form of the AHR to cardiotoxicity, the chicken AHR cell culture data were compared to the ability of the same compounds to increase heart wet weight in chick embryos. Our initial results indicate that the rank/order for these compounds may differ between the *in vitro* and *in vivo* models for AHR mediated toxicity in the developing chicken. These data suggest that factors, in addition to AHR activation, may contribute to the cardiotoxicity induced by AHR agonists *in vivo*.

1306 TCDD INDUCED NF-KB BINDING TO THE KB CONSENSUS MOTIF IN MURINE B-CELL LINES.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a prominent environmental toxicant with effects including lymphoid involution, chloracne, teratogenesis, cancer, and immunosuppression. The mechanism which are responsible for these effects is thought to be mediated by the aryl hydrocarbon receptor (AhR). However, previous studies in our laboratory demonstrated an AhR-independent effect of TCDD on NF-kB binding in the 3'a-hs4 enhancer

region of the 3'a immunoglobulin heavy chain enhancer. NF-kB is a transcription factor that is involved in the regulation of genes participating in immune and inflammatory responses, many of which have been shown to be affected by TCDD. The aim of the present study was to further characterize TCDD-mediated induction of kB binding using a kB consensus probe. kB binding was evaluated, by an electrophoretic mobility shift assay, in two B-cell lines, the AhR-expressing CH12.LX cell line and the AhR-deficient BCL-1 cell line. TCDD induced kB binding in a concentration-dependent manner in the CH12.LX cells. TCDD- inducible binding to the kB consensus probe was competed away with both the unlabeled kB consensus probe, as well as, the unlabeled 3'a-hs4 probe, indicating that the binding observed at the 3'a hs4 enhancer was specific for the kB motif identified within the enhancer. Since LPS is a polyclonal stimulator for B-cells and is known to induce kB binding, the effect of LPS stimulation on kB binding alone or in costimulation with TCDD were also evaluated. In the CH12.LX cells, LPS induced kB binding; however, a 30nM TCDD treatment resulted in greater kB binding as compared to that induced by LPS alone. Interestingly, costimulation with both TCDD and LPS resulted in reduced binding to the kB motif as compared to TCDD alone. TCDD-induced kB binding was also observed in the BCL-1 cells. These results demonstrate a concentration dependent induction of kB binding by TCDD that is AhR-independent. (This work was supported by NIEHS Grant ES02520.)

1307 ANTAGONISTIC EFFECTS OF DI-*ORTHO* POLY CHLORINATED BIPHENYLS ON ARYL HYDROCARBON RECEPTOR-DEPENDENT CYP1A1 AND IGM GENE EXPRESSIONS IN CH12.LX B CELLS.

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Halogenated aromatic hydrocarbons (HAHs) are ubiquitous contaminants in our environment. Many of the toxic effects produced by HAHs are believed to be elicited by binding to aryl hydrocarbon receptor (AhR). However, several recent investigations have demonstrated that certain HAH congeners, primarily di-orthosubstituted polychlorinated biphenyls (PCB), inhibited the AhR-mediated responses induced by other toxic HAHs. The mechanism responsible for this inhibition by certain PCBs is presently unknown. Here we present the antagonistic effects of di-orthosubstituted PCBs on AhR-mediated biological responses in the murine B cell line, CH12.LX. CYP1A1 mRNA expression induced by TCDD or coplanar PCBs [PCB77 (3,3',4,4'-tetrachlorobiphenyl) and PCB126 (3,3',4,4',5-pentachlorobiphenyl)]was inhibited by PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) and another di-orthosubstituted PCB congeners [PCB47 (2,2',4,4'-tetrachlorobiphenyl), PCB52 (2,2',5,5'-tetrachlorobiphenyl), and PCB128 (2,2',3,3',4,4'-hexachlorobiphenyl)] also antagonized CYP1A1 mRNA induction in co-treatment with TCDD. Di-orthosubstituted PCB52 antagonized TCDD- or PCB126-mediated inhibition of IgM secretion in LPS-activated cells. This inhibition of IgM secretion by PCB52 was found to correlate well with a change in mRNA expression level of IgM heavy chain. Furthermore, in TCDD-treated CH12.LX cells, PCB52 inhibited nuclear translocation of AhR from cytosol and subsequent DNA binding of AhR to the dioxin responsive element (DRE) in the nucleus. Collectively, these results suggest that certain di-orthosubstituted PCBs inhibit AhR ligand-modulated CYP1A1 and IgM heavy chain gene expression in B cells through the inhibition of AhR activation. (This work was supported in part by NIH grant PO1 P42ES04911-08.)

1308 THE AHR SIGNALING PATHWAY AND TRANSCRIPTIONAL REGULATION OF IGM EXPRESSION BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN.

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The immune system has been identified as a sensitive target for the toxic effects produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Furthermore, the B-cell has been identified as a sensitive cellular target of TCDD by previous cell-type fractionation studies from this laboratory. The actual molecular mechanism responsible for the immunotoxic effects produced by TCDD is unclear; however, many of the biological effects produced by TCDD are thought to be mediated by the aryl hydrocarbon receptor (AhR). Our previous results describing the CH12.LX and BCL-1 B-cell lines, which differ markedly in their expression of the AhR and in their sensitivity to TCDD, have implicated a role for the AhR in TCDD-induced inhibition of B-cell function. The purpose of the present studies was to further investigate this role of the AhR through a characterization of the structure activity relationship (SAR) of several polychlorinated dibenzo-p-dioxin (PCDD) congeners on three endpoints, inhibition of both IgM secretion and μ heavy chain expression and AhR-mediated CYP1A1 induction, in the CH12.LX cells. The effects of the PCDD congeners followed a SAR for each endpoint with the following rank order potency, TCDD > 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HxCDD) > 2,3,7-trichlorodibenzo-p-dioxin (TriCDD) > > 1-monochlorodibenzo-p-dioxin (MCDD). Among the three endpoints, there was also a general concordance between the EC_{50} and IC_{50}'s for a particular congener. In addition, TCDD, HxCDD, TriCDD and MCDD had no effect on IgM secretion from the AhR-deficient BCL-1 cells. These results further support an AhR-dependent mechanism for the inhibition of IgM secretion and of μ expression. Furthermore, two dioxin responsive enhancer (DRE)-like sites were identified within the Ig heavy chain 3'α enhancer which might mediate the PCDD-induced inhibition of μ heavy chain expression and thus of IgM secretion. EMSA-Western analysis with the CH12.LX cells demonstrated TCDD-induced binding of the AhR nuclear complex to both DRE-like sites as well as TCDD-induced binding of several NF-κB/Rel proteins to a κB site which overlaps one of the DRE-like sites. Interestingly, κB binding in the BCL-1 cells was also induced by TCDD demonstrating an AhR-independent effect of TCDD on κB binding. These results support an AhR/DRE-mediated mechanism for TCDD-induced inhibition of μ heavy chain expression. Although the effect on κB binding may not contribute to this effect, induction of κB binding by TCDD may have profound effects on the vast number of genes that are regulated by NF-κB/Rel proteins. (These studies were supported by the NIH grant ES02520.)

1309 MODULATION OF MOUSE IgH 3'α-HS4 ENHANCER ACTIVITY BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN AND LPS.

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Immunoglobulin heavy chain (IgH) gene expression is controlled by a variable region promoter, intronic enhancer and additional regulatory region recently identified in the 3' end of the IgH locus. 3' regulatory region of IgH locus includes several enhancer elements and these enhancer elements function as a locus control region which shows tissue-specific and position-independent activity. All of these promoter and enhancer elements are B lymphoid-specific and regulation of IgH gene expression by these elements is extremely complex. 3'α-hs4 is one of the important enhancer elements of IgH 3' regulatory region and shows enhancer activity throughout B cell development. Here, we demonstrate the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and LPS on 3'α-hs4 enhancer activity in CH12.LX B cells using transient transfection and luciferase reporter gene assay. We amplified IgH variable region promoter and 3'α-hs4 enhancer element from B6C3F1 mouse genomic DNA and subcloned to upstream and downstream multicloning site of luciferase structural gene, respectively. Transient transfection of 3'α-hs4 to CH12.LX cells showed relatively high basal level activity and this 3'α-hs4 activity was enhanced by TCDD and LPS treatment. Furthermore, the enhancement of 3'α-hs4 activity by TCDD was partially blocked by alpha-naphthoflavone which is known as partial blocker of AhR activation. These results show that TCDD and LPS modulate 3'α-hs4 enhancer activity and suggest that IgH gene expression may be regulated by TCDD in an AhR-dependent manner. (This work was supported in part by NIH grant ES02520.)

1310 EXOGENOUS GONADOTROPIN RELEASING HORMONE (GNRH) INDUCES LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH) SURGES AND PARTIALLY RESTORES OVULATION IN AN ECG-PRIMED IMMATURE RAT MODEL TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Immature (23 day old) female Sprague-Dawley rats were dosed with TCDD (32 mg/kg) in corn oil or vehicle alone. Equine chorionic gonadotropin (eCG) was injected (5 IU, sc) 24-h later to induce follicular development. 54-h after

eCG injection, half of TCDD- or corn oil-treated rats were injected with GnRH (2 mg/rat, sc) to induce LH and FSH surges. TCDD (32 mg/kg) inhibited ovulation by ~75% (2 to 3 ova/rat) when compared with vehicle-treated control rats (~10 ova/rat). GnRH (2 mg/rat) increased the number of ova shed to ~7 ova/rat in TCDD-treated rats, although this was lower than vehicle-treated control rats receiving GnRH (~15 ova/rat). The LH and FSH surges triggered by exogenous eCG in controls was abolished by TCDD (32 mg/kg) at 54 to 60-h after eCG injection and the gonadotropin surges were restored when GnRH was given. GnRH alone increased serum concentrations of estradiol (E2) and progesterone (P4) at 56 to 58-h after eCG injection, but in rats treated with both TCDD and GnRH, serum concentrations of E2 and P4 were reduced. Because TCDD blocks ovulation by preventing the LH surge and through direct inhibitory action on follicular rupture, GnRH could only partially restore the blockade of ovulation induced by TCDD. In addition, it appears that a lack of hypothalamic GnRH secretion may account for the absence of an LH surge in TCDD-treated rats through mechanisms not yet identified.

1311 BLOCKAGE OF OVULATION BY POLYCHLORINATED DIBENZOFURANS (PCDFS), BIPHENYLS (PCBS) AND THEIR MIXTURE WITH DIBENZO-*P*-DIOXINS (PCDDS)SUPPORTS THE TOXIC EQUIVALENCY (TEQ) CONCEPT.

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Immature female rats were administered various doses of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PeCB) or 2,2',5,5' tetrachlorobiphenyl (TCB) and their mixture with polychlorinated dibenzo-p-dioxins (PCDDs) including 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzo-p-dioxin (PeCDD) and 2,3,4,6,7,8-hexachlorodibenzo-p-dioxin (HxCDD). Equine chorionic gonadotropin (eCG; 5IU) was injected 24-h later to induce follicular development. Blood and ovaries were harvested, ovarian weights and the number of ova shed were determined at various times after eCG. Serum concentrations of 17ß-estradiol (E2), progesterone (P4), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were determined by radioimmunoassay. PeCDF, PeCB, TCB and their mixture with PCDDs blocked ovulation dose dependently. The slopes of the dose responses for inhibition of ovulation generated by the individual PeCDF, PeCB and/or their mixture with PCDDs were similar. PeCDF, PeCB and the mixture increased serum concentrations of E2 at 72-h after eCG injection, the day of expected ovulation; in contrast, serum P4 and FSH were decreased at that same time point. Only the high doses of TCDD, PeCDF, PeCB and the mixture blocked LH and FSH surges at 58-h after eCG. The ovarian histology revealed that the effect of PeCDF, PeCB and the mixture was very similar to that of PCDDs, consisting of ova trapped in preovulatory follicles and a lack of or reduced number of corpora lutea. These results provide further support of the TEQ concept and indicate a similar mechanism of action among PCDDs, PCDFs, and PCBs in this ovulation model.

1312 INTERACTION OF ESTRADIOL AND 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN AN OVULATION MODEL: EVIDENCE FOR SYSTEMIC AND LOCAL EFFECTS.

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Immature (day 25) hypophysectomized or intact rats were treated subcutaneously with a long acting estrogen (estradiol cypionate, ECP, 0-2 mg/kg, s.c.) followed 24 hours later by TCDD (0 or 10 mg/kg, p.o.). On the next day, follicular development was induced with equine chorionic gonadotropin (eCG, 5 or 10 IU, s.c.) followed by an ovulatory dose of human chorionic gonadotropin (hCG, 10 IU, s.c.). Systemic exposure to TCDD alone tended to retard weight gain in hypophysectomized rats. The inhibition of ovulation by systemic TCDD was potentiated by systemic pretreatment with ECP (2 mg/kg) in hypophysectomized but not in intact rats. Furthermore, only hypophysectomized rats exposed systemically to TCDD and ECP exhibited actual weight loss. Pair feeding mimicked the combined effects of TCDD and ECP on ovarian function in hypophysectomized rats. In another experiment, rats received ECP subcutaneously (0 or 2 mg/kg) and TCDD into the ovarian bursa (0 or 250 ng). Still another group of rats received TCDD orally (10 mg/kg) and ECP into the ovarian bursa (0 or 1.5 mg). In contrast to systemic TCDD exposure, for which ECP pretreatment potentiated the effect on ovulation, blockade of ovulation by intrabursal treatment with TCDD was alleviated by local or systemic ECP pretreatment. Overall, the results indicate that estrogens increase the systemic toxicity of TCDD in hypophysectomized rats while antagonizing direct effects of TCDD in the ovary.

1313 A MAJOR HUMAN ARSENIC METABOLITE, DIMETHYLARSINIC ACID (DMA), REQUIRES REDUCED GLUTATHIONE (GSH) TO INDUCE APOPTOTIC CELL DEATH.

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Inorganic arsenicals are potent toxicans and carcinogens in humans. In mammals, including humans, inorganic arsenicals often undergo methylation, forming compounds such as dimethyarsinic acid (DMA). Some evidence indicates DMA is carcinogenic in rodents, possibly through formation of radical species. Thus, in this study we examined the role of reduced glutathione (GSH) in the *in vitro* cytotoxicity of DMA compared to that of the inorganic arsenical, arsenite, using murine TRL 1215 liver cells. Arsenite was very cytotoxic in these cells (IC_{50} ~ 35 :M after 48 hours of exposure). With arsenite about 20% of dead cells were apoptotic while 80% were necrotic. Arsenite cytotoxicity increased when GSH was depleted with the glutathione synthase inhibitor, buthionine-sulfoximine (BSO). In contrast, DMA was much less cytotoxic (IC_{50} ~ 1.5 mM) than arsenite and most cells died through apoptosis. BSO actually decreased the DMA-induced cell death. The glutathione reductase inhibitor, carmustine, and a glutathione peroxidase activator, sodium selenite, also prevented DMA-induced cytolethality. Conversely, the addition of GSH or a GSH precursor, N-acetyl-cysteine, enhanced DMA-induced cell death. These data imply that DMA requires GSH to induce apoptosis *in vitro*. Furthermore, DMA markedly increased cellular GSH levels, but, despite this, DMA also induced lipid peroxidation. Ethacrynic acid, an inhibitor of glutathione S-transferase that catalyses GSH-substrate conjugation, and acivicin, an inhibitor of (-glutamyltranspeptidase which catalyses the initial breakdown of GSH-substrate conjugates, also suppressed DMA-induced cell death. These findings indicate that DMA induces apoptosis *via* conjugation with GSH and DMA enhances the cellular accumulation of GSH. Increased apoptosis can be associated with development of proliferative lesion but further study is required to define any such role in DMA-induced neoplasia.

1314 ACUTE ARSENIC-INDUCED FREE RADICAL PRODUCTION AND OXIDATIVE STRESS-RELATED GENE EXPRESSION IN MICE.

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This study determined free radical production and oxidative stress-related aberrant gene expression in mice acutely treated with inorganic arsenicals. Mice were treated with a high dose of arsenite (As^{+3}, 100 : mol/kg, sc) or arsenate (As^{+5}, 500 : mol/kg, sc). To evaluate free radical production, mice were simultaneously given the spin trap agent, *N-tert*-butyl-A-phenylnitrone (PBN, 250 mg/kg, ip, in DMSO) with the arsenicals, and livers were removed 30 minutes later. The PBN-trapped free radicals were extracted with methanol:chloroform (1:2), and detected with electron spin resonance (ESR). Arsenical treatment resulted in the appearance of free radicals, with stronger signals seen in As^{+3}-treated mice than in As^{+5}-treated mice. To examine oxidative stress-related aberrant gene expression, livers were removed 3 hours after arsenic treatment, and total RNA was extracted. The Clontech Atlas Toxicology/Stress array indicated that acute arsenic treatment markedly induced heme oxygenase 1 (HO-1; 6-fold), heat-shock protein (HSP60; 3-fold), the DNA damage-induced protein gadd45 (20-fold), along with DNA excision repair proteins such as ERCC-1 (7-fold). In contrast, DNA mismatch repair protein MSH3, MHR23, MmRad51, 8-oxy-quanine DNA glycosylase and breast cancer susceptibility locus 2 product (BRCA2) were down-regulated 50-90%. Expression of many cytochrome P450 enzymes and NADPH-cytochrome P450 reductase were reduced, while CYP3A25 was slightly

increased. Arsenic-induced HO-1, HSP70, and nuclear factor 6 B were observed by Western-blot analysis. Thus, a variety of aberrant gene expressions are associated with acute arsenic-induced oxidative stress and these could play a critical role in acute toxicity.

1315 THE ROLE OF TRANSPORT PROTEINS AND GLUTATHIONE *S*-TRANSFERASE Pi IN ARSENIC TOLERANCE SEEN IN CELLS CHRONICALLY EXPOSED TO LOW LEVELS OF ARSENITE.

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Arsenic (As) is a known human carcinogen. Our recent work showed that chronic (>18 week) low level (500 nM) arsenite (As^{+3}) exposure induced malignant transformation in a rat liver epithelial cell line (TRL 1215). These cells also developed self-tolerance to As, with a significant reduction in the accumulation of cellular As. The purpose of this study was to examine the role of transport proteins and glutathione *S*-transferases (GST) in decreased As accumulation in these chronic As exposed (CAsE) cells. The Clontech Atlas cDNA microarray analysis indicated that the expression of GST-pi was markedly increased in CAsE cells. The expression of multidrug resistance gene (MDR1) that codes for the transporter P-glycoprotein (P-gp) and multidrug resistance protein (Mrp2) was also increased in CAsE cells, but to a lesser extent than the GST-pi gene. Increased protein levels of GST, Mrp2 and P-gp in CAsE cells were confirmed by Western-Blot analysis. The increased levels of GST suggest that an As-glutathione complex may be formed and effluxed by Mrp2 and/or P-gp transporter. Furthermore, the CAsE cells showed cross-resistance to several anticancer drugs, such as cisplatin, vinblastine, adriamycin, and actinomycin D. Resistance to these anticancer drugs is known to be associated with the increased GST and efflux via Mrp2 or P-gp transporter. These results indicate that chronic As exposure induces GST, which may facilitate the formation of an As-glutathione conjugate for efflux by increased Mrp2 and/or P-gp transporter, thus decreasing As accumulation in the CAsE cells.

1316 EARLY MOLECULAR AND CELLULAR EFFECTS OF ARSENIC IN SKIN CULTURES.

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The effects of topically applied As(V) and As(III) in Earle's balanced salt solution on cytotoxicity, apoptosis, morphology, permeation rate, biosynthesis of DNA, RNA and protein were studied in human pseudoepidermis (PSED) grown on Puropore® nylon membranes at the air-liquid interface and in submerged cultures of human keratinocytes (HK). Topical exposure of PSED to 100 -250μg/L of As(V) results in the focal disruption of the basal layer with occasional karyopyknotic cells 24 hours later. With 500-1000 μg/L, cells progressively deteriorate with disintegration of the basal layer pyknotic nuclei and abundant mitotic figures. Cornified layers remain intact. Marked DNA fragmentation consistent with apoptotic changes are seen in lifted cultures at 250-1000μg/L exposure starting at 24hr. HK grown in submerged culture for 24 hr and exposed to 500 and 1000μg/L As(V) for the following 4 days developed the most pronounced changes involving striking stratification and differentiation of HK. As(III) exposure produced marked, dose dependent cytotoxicity as early as 24 hours. Permeation of arsenic was used to evaluate the degree of barrier function in PSED. Most of the applied As(V) was adsorbed at concentrations of <500μg/L; at 1000μg/L As(V) ~30% was retained by the culture. LDH leakage in PSED (determined in the aliquots of the same effluent) increased during 2hr exposure to 250μg/L As(III) and 500μg/L As(V). In contrast, the submerged 24hr old cultures of human keratinocytes (HK) revealed LDH release at 10μg/L As(V) after 2hr exposure. 3H-thymidine incorporation in PSED exposed to 10-1000μg/L As(V) revealed a biphasic response with an initial decrease (ca 50%) with subsequent stimulation (ca 2.5-fold) of the incorporation at 24hr exposure. Variability of 3H-uridine and 14C-leucine incorporation in short-term exposures was not statistically significant. Long-term exposure experiments are under way. (This research is funded by AWWA.)

1317 MONOMETHYLARSONOUS ACID (MMA^{III}) IS MORE TOXIC THAN ARSENITE IN CHANG HUMAN HEPATOCYTES.

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Methylation has been considered by many to be the primary detoxication pathway of inorganic arsenic. Inorganic arsenic is methylated by many, but not all animal species to monomethylarsonic acid (MMA^{V}), monomethylarsonous acid (MMA^{III}) and dimethylarsinic acid (DMA^{V}). Yet the relative toxicity of MMA^{III} has remained unknown. *In vitro* toxicities of arsenate, arsenite, MMA^{V}, MMA^{III} and DMA^{V} were determined in Chang human hepatocytes. Leakage of lactate dehydrogenase (LDH) and intracellular potassium (K^+) and mitochondrial metabolism of the tetrazolium salt XTT were used to assess cytotoxicity due to arsenic exposure. The mean LC_{50} based on LDH leakage in phosphate media was 6.0 μM for MMA^{III} and 68.0 μM for arsenite; for K^+ leakage in phosphate media, it was 6.3 μM for MMA^{III} and 19.8 μM for arsenite. The mean LC_{50} based on XTT metabolism in phosphate media was 13.6 μM for MMA^{III} and 164 μM for arsenite. LC_{50}'s were also determined in phosphate-free media. The results of the three cytotoxicity assays (LDH, K^+ and XTT) using phosphate media reveal the following order of toxicity in Chang human hepatocytes: MMA^{III}>Arsenite>Arsenate>MMA^{V}= DMA^{V}. The results demonstrate that MMA^{III} is a highly toxic intermediate in inorganic arsenic methylation and again raises the question as to whether methylation of inorganic arsenic is a detoxication process. (Supported in part by the NIEHS Superfund Basic Research Program Grant ES-04940 and NIEHS Center Grant ES-06694.)

1318 METHYLATED ARSENIC IN URINE AS A FUNCTION OF EXPOSURE TO INORGANIC ARSENIC IN DRINKING WATER.

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A salient feature of inorganic arsenic (iAs) metabolism is its conversion to monomethyl (MAs) and dimethylated (DMA) forms. Although methylation is often considered a means of detoxification of iAs, methylated arsenicals exert distinct adverse effects, including tumor initiation and promotion. Hence, methylation might also be regarded as a means of activation of iAs to more toxic forms. Here, we examined the profiles of iAs, MAs, DMA in urines collected from 96 individuals residing in Millard County, Utah, who used drinking water supplies that contained from 2 to 600 μg of iAs/L. The percentage of As in urine that was accounted for by MAs and DMA was stable for repeated urine samples collected on a single day and also stable across a five-day experimental period. A strong correlation between the concentration of iAs in the drinking water supplies used by participants and the concentration of MAs and DMA in urine suggested that in this population ingestion of iAs-containing drinking water was the major determinant of the amount of methylated As excreted in urine. The percentage of As in urine that was present as MAs or DMA ranged from 74 to 94 percent among study participants. The ratio of the concentration s of DMA to MAs in urine ranged from about 2 to 14 and was not strongly correlated with the concentration of all forms of As (iAs+MAs+DMA) in urine. Hence, factors other than the concentration of As in the drinking water supply or intake of As from drinking water likely determines the interindividual differences in the capacity for the methylation of iAs or for the retention of these arsenicals. These differences may be genetically determined or related to differences in other environmental factors that determine metabolic capacity. (This abstract does not necessarily reflect EPA policy.)

1319 POTENTIATION OF AGONIST-INDUCED PLATELET AGGREGATION BY ARSENIC.

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Chronic ingestions of arsenic by drinking water have been reported to induce cardiovascular disease such as blackfoot disease, atherosclerosis and hypertension, but the exact mechanism has not been elucidated yet. In order to investigate the possible causes of cardiovascular disease by arsenic, we examined the effects of arsenic on platelets which play an important role in development of cardiovascular disease. Sodium arsenite (iAsIII), trivalent inorganic arsenic, did not induce either aggregation or cytotoxicity to rat platelets directly, whereas iAsIII potentiated platelet aggregations induced by various

agonists, such as thrombin, collagen, ADP and arachidonic acid in concentration- and time-dependent manners. Thrombin-induced platelet aggregation was also enhanced by sodium arsenate (iAsV) or monomethylarsonic acid (MMA) in relatively higher concentrations compared to iAsIII. Treatment of iAsIII resulted in a dose-dependent increase of thrombin-induced serotonin secretion from platelets, while the formation of thromboxane A_2 from platelets did not altered significantly. Consistent with these findings, the *in vivo* studies revealed that ingestion of drinking water containing iAsIII in mouse elevated blood serotonin levels significantly, which is indicative of platelet aggregation *in vivo*. These results suggest that arsenic exposure makes platelets more susceptible to aggregatory stimulus and readily enhance the serotonin secretion from platelets and thus these effects by arsenic may contribute to the pathogenesis of cardiovascular disease.

1320 INDUCTION OF C-MYC PROTEIN BY ARSENITE IN PRECISION-CUT MOUSE KIDNEY SLICES.

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Arsenic [As] is a ubiquitous environmental contaminant with many adverse human health effects, including cancer, cardiovascular diseases and nephrotoxicity. Although the mechanism(s) underlying the acute toxicological response of the kidney to arsenite [As(III)] and arsenate [As(V)] has been elucidated over the past three decades, our understanding of the potential molecular effects of lower, non-cytotoxic levels of As remains sparse. Previous studies have shown that nanomolar levels of both As(III) and As(V) enhance c-myc gene expression in precision-cut rabbit renal cortical slices following 6 hr of exposure. In the current study, experiments were designed to further characterize the effect of As(III) on expression of c-myc. There is a well-established relationship between overexpression of c-myc and renal diseases, including cancer and polycystic kidney disease, suggesting that c-myc may be a critical mediator of the nephrotoxicity of As(III). Precision-cut kidney slices were prepared from male CD-1 mice and following a 1 hr pre-incubation, As(III) [0-5μM] was added for 6-24 hr. Nuclear extracts were prepared from kidney slices and c-myc protein expression was examined by Western blot analysis. Following 6 hr of exposure, 5μM As(III) enhanced c-myc protein expression, however, 8 hr of As(III) challenge resulted in a concentration-dependent increase in c-myc protein, with the peak effect observed at 1μM. Although c-myc protein levels were elevated in As(III)-challenged renal slices following 10 hr of exposure, by 12 hr no effect of As(III) on c-myc protein was seen. These data suggest that induction of c-myc protein may be a critical target of As(III) in the kidney.

1321 ALTERATIONS IN THE UBIQUITIN-DEPENDENT PROTEOLYTIC PATHWAY CAUSED BY LOW-LEVEL ARSENITE EXPOSURE IN RABBIT RENAL CORTICAL SLICES.

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Our recent studies have shown low-level arsenite concentrations to affect several genes in the ubiquitin-dependent proteolytic pathway in rabbit renal cortical slices. Other studies suggest that disruption of ubiquitin-dependent protein degradation by arsenite may contribute to both its genotoxicity and carcinogenicity. While changes in the ubiquitin pathway following chemical and oxidative stresses are well documented, effects of low-level arsenite exposure on the ubiquitin-dependent proteolytic pathway remain unclear. Thus, cortical slices from the rabbit kidney were incubated in concentrations of As(III) between 100nM and 10μM. One hr prior to collecting slices for protein analysis, a peptide-aldehyde proteosome inhibitor (Z-Leu-Leu-Leu-al, 50μM) was introduced to allow the cellular accumulation of ubiquitin-tagged proteins for analysis. Slices were collected at timepoints 0, 4, 8 and 24 hr. Using an antibody recognizing ubiquitin in the free and conjugated forms, protein homogenates from control and As(III) treated kidney slices were analyzed by Western blot. Renal cortical slices exposed to As(III) in levels as low as 1μM show detectable decreases in the accumulation of ubiquitin-conjugated proteins in the cell at early timepoints. At the later time points, accumulation of ubiquitin-tagged proteins appears to be elevated in slices exposed to arsenic at all concentrations, possibly as a recovery mechanism that degrades proteins damaged by arsenite treatment. Ongoing studies seek to understand if alterations in ubiquitin-dependent proteolysis represent response to and recovery from subtoxic levels of arsenite, or a precursory response signaling the onset of toxicity. (NIH ES04946.)

1322 ARSINE TOXICITY IN THE PERFUSED RAT KIDNEY AND CORTICAL EPITHELIAL CELLS.

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Arsine (AsH_3) is the most toxic form of arsenic. It is used extensively in the semiconductor industry for epitaxial growth of gallium arsenide and as a dopant for silicon-based electronic devices. In these situations accidental exposure to AsH_3 may occur. Exposure to AsH_3 is fatal in 25% of the reported human cases, and usually results in acute oliguric renal failure. The mechanism of renal toxicity is unknown. This study investigated acute AsH_3 toxicity in the rat perfused kidney and in the renal cortical epithelial cells. The hypothesis was that early AsH_3 toxicity is caused by unchanged AsH_3, but at later time points, it requires the formation of hemolysate containing arsenite (AsIII) as a metabolite. Perfusion for 10 minutes with AsH_3, but not with the hemolysate, produced toxicity in the isolated kidney. The main AsH_3 target was the glomerular and peritubular microvasculature as determined by electron microscopy. AsH_3 toxicity in the cortical epithelial cells required 24 hours to appear. However, the AsH_3-produced hemolysate and the AsIII-spiked hemolysate produced toxicity in these cells as early as 1 hour. Toxicity in these cells was investigated by XTT bioreduction (mitochondrial marker), intracellular potassium (early toxicity) and LDH leakage (cell death). It was concluded that unchanged AsH_3 damages the renal microvessels at an early time compromising renal filtration. The extent of damage will determine the delay time for the symptoms to appear (2-24 hours). At a later time, the hemolysate products along with As(III), will further compromise renal function leading to oliguric renal failure. (Supported by NIEHS Grant ES06644, and Center Grant P30 ES06694.)

1323 INTERACTIONS OF DIMETHYLARSINIC ACID WITH RAT ERYTHROCYTES.

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Unlike other mammalian animal models, rats demonstrated significant accumulation of dimethylarsinic acid (DMA) in blood over time. However, the actual binding site of DMA in the rat erythrocyte is not clear. Our laboratory has shown that, in spectral studies of oxyhemoglobin (oxyHb), DMA did not bind oxyHb on the iron or the porphyrin ring of heme, nor to the globin chains in a manner that denatured the protein. In this study, it is hypothesized that hemoglobin reduced DMA(V) to DMA(III), which could bind hemoglobin at sulfhydryl groups on its globin chains. This investigation examined the binding of DMA with rat oxyHb in solution and the distribution and binding of DMA in the Sprague Dawley rat erythrocyte. Rat oxyHb solutions, dosed with DMA (1 to 10mM), were incubated for up to 60 minutes at 37°C. Free and bound DMA were separated by ultrafiltration, and DMA was measured by hydride generation atomic fluorescence spectroscopy. Little binding occurred (1-3% of the total DMA dose), and no significant changes in binding were observed with increasing dose or time. Uptake and cellular distribution of DMA were examined by exposing rat erythrocytes to 1 and 10mM DMA at 37°C for 30 minutes or 24 hours. DMA uptake was slow, with 30-40% of the dose taken up by 30 minutes, increasing to 40-65% by 24 hours. Most of the intracellular DMA was localized to the intracellular side of the membrane and bound to high molecular weight cytosolic proteins. The percent of the DMA dose found in these two cellular compartments significantly increased over time. Levels of glutathione (GSH) and glutathione disulfide (GSSG) were measured in erythrocytes dosed with DMA to determine if GSH was reducing DMA(V). GSH depletion was observed but was not accounted for by increases in GSSG, indicating the formation of another product of GSH oxidation. (Supported by NIH Grant ES06644, NIEHS Grant ES07091, and Center Grant P30 ES06694.)

1324 STUDY ON METABOLISM OF ARSENIC AND DNA DAMAGE IN THE PATIENTS WITH ACUTE ARSENIC POISONING.

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The presentation is results of toxicology area concerning mass acute arsenic poisoning in Japan on 1998. The patients were 67 people and 32 men and 35 women. The age was from 1 to 67 years old. Four people died around half a day after intake of the arsenic trioxide. The curry was contaminating with the arsenic trioxide. The patients ate several items of the curry with the teaspoon. Arsenic trioxide intake dose in 63 patients was presumed to be 20-140mg. The mean of the urinary arsenic (inorganic arsenic; iAs + methylated arsenic; MA

+ dimethylated arsenic; DMA) levels after intake of the arsenic trioxide at 1, 10 and 30 days, 9029, 1148 and 160 μg As/g creatine, respectively. In the arsenic ratio in the urine after intake of the arsenic trioxide during a day, iAs 86%, MA 9% and DMA 3%, respectively. The concentration of arsenic in urine recovered at the level of normality two or three months later. The methylation capacity in the acute arsenic-poisoning patients was compared. The 2-nd methylation (MA to DMA) was observed in the age difference. The low age person of the methylation capacity was higher than the senior persons. On the other hand, the sex difference was not indicated. The DNA damage by the exposure of arsenic trioxide in the acute arsenic-poisoning patients was evaluated. DNA damage in the body was caused by arsenic trioxide intake, and 8-hydroxy-2-deoxyguanosine (8-HOdG) levels in urine increased remarkably. The determination of 8HOdG levels in urine was effective to the evaluation of DNA damage by the arsenic exposure.

1325 ROLE OF METALLOTHIONEIN IN ORGAN DISTRIBUTION AND EXCRETION OF CADMIUM COMPLEXES.

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The role of metallothionein (MT) in the distribution and excretion of cadmium (Cd) was studied in MT-I/II knockout (null) mice after a single i.p. injection of one of four different Cd-complexes: Cadmium chloride, Cd- cysteine (1:2), Cd- glutathione (1:2), and Cd-MT. Each of these compounds was injected (0.9mg/kg) to wild type (control) and knockout mice, and mice were killed after 24 hours. The amount of cadmium was measured in blood, liver, kidney, pancreas, spleen, heart, lung, stomach and intestine; and MT content determined by Cd-hem assay. There was no detectable MT in any organs of MT-null mice. The tissue distribution (%) of cadmium was similar between control and MT-null mice within each treatment group, although the concentration of cadmium was low in MT-null mice. There was a greater excretion of cadmium into the intestine of MT-null mice as compared to the control, suggesting an increased bilary excretion of cadmium. In mice injected with Cd-MT, most of the Cd was detected in the kidney (53-66%) while in all other treatment groups most of the cadmium was accumulated in the liver (46-62%). Injection of Cd-MT caused renal damage in both control and MT-null mice. The results suggest that the intracellular MT plays only a minor role in the initial organ distribution of Cd. However, in the absence of two major isoforms of MT, more cadmium is excreted into the intestine, and less Cd is retained in the body. (Supported by MRC, Canada.)

1326 EXPRESSION OF IMMEDIATE EARLY GENES IN CULTURED HUMAN PROXIMAL TUBULE CELLS AND THE DERIVED CELL LINE, HK-2, EXPOSED TO CADMIUM.

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Many of the immediate early genes, such as c-fos, c-jun and c-myc, are induced in response to cellular stress. The expression of these three genes was assessed in cultures of human proximal tubule (HPT) cells and an immortalized human proximal tubule cell line, HK-2, exposed to lethal and sublethal concentrations of cadmium for durations of several hours to 16 days. HK-2 cells required lower levels of cadmium to elicit similar toxicities compared to HPT cells. It was determined that the expression of c-fos was induced at 1-2 hr and returned to near control levels by 24 hr. For c-jun the expression profile was similar; however, in the case of c-myc, there was no induction observed. The response in the HK-2 cell line was similar to that of the HPT cells except that induced c-fos and c-jun levels were attained with lower levels of cadmium. During long term cadmium exposure there was cell death in both culture systems without induction of any of these genes.

1327 CADMIUM-INDUCED ALTERATIONS IN CHRONICALLY EXPOSED HUMAN PROSTATE EPITHELIAL CELLS.

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Human prostate is a presumptive target of cadmium (Cd) carcinogenesis, although the mechanisms involved remain elusive. To define the molecular events associated with chronic Cd exposure in the prostate, the normal human prostate epithelial cell line RWPE-1 was continuously exposed to 10 μM Cd *in vitro* for 8 weeks and compared to passage-matched control cells. The Cd-treated prostate epithelial (CTPE) cells showed altered morphology, increased Cd resistance and decreased dependence on growth factors compared to controls. Specifically, the CTPE cells grew more efficiently in growth factor-free medium and, unlike control RWPE-1 cells, were unresponsive to epidermal growth factor (EGF). The latter is significant since EGF receptor levels also appear to be reduced in prostate tumors. In addition, CTPE cells showed a propensity to form foci of piled-up cells in culture, which is often seen with malignantly transformed cells. Data from cDNA array experiments indicated altered expression of a variety of genes in CTPE, including down-regulation of several pro-apoptotic genes, such as those coding for several caspases, as well as retinoic acid receptor γ and retinoid X receptor β. As predicted from the array data, CTPE cells were more resistant than RWPE-1 cells to the apoptosis inducer, etoposide (ETS). This resistance was accompanied by a decrease in caspase-3 enzymatic activity in ETS-treated CTPE cells compared to similarly treated RWPE-1 cells. CTPE cells also showed resistance to the growth inhibitory effects of all-trans retinoic acid. Retinoids are important regulators of prostate growth *in vivo* and are being investigated as chemopreventative and chemotherapeutic agents. In summary, chronic Cd exposure induces growth and gene expression alterations in human prostate epithelial cells indicative of transformation and loss of growth regulation that could be important in the development of prostate malignancies.

1328 EFFECT OF CADMIUM ON PANCREATIC PROTEASE ACTIVITIES IN MICE.

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The pancreatic effects of cadmium (Cd) in mice were studied. A single sc injection of 1 mg Cd/kg had no obvious toxic effects on the liver, kidney, and pancreas at both 1 and 5 days after Cd treatment. However, within the pancreas the activities of trypsin, chymotrypsin, and carboxypeptidase A were significantly decreased at 1 day after Cd treatment, while the activity of carboxypeptidase B was not changed. The concentrations of Cd in the pancreas were very similar at 1 and 5 days after Cd treatment, indicating a stable deposition of the metal. Metallothionein levels in the pancreas were increased but only at 5 days after Cd treatment. In order to more fully examine the inhibitory effects of Cd on these protease activities in the pancreas, the direct effects of Cd on purified proteases were also studied *in vitro*. Contrary to the results *in vivo*, Cd increased the activity of purified trypsin in a concentration-dependent manner. Consistent with the *in vivo* results, the activity of purified carboxypeptidase A was decreased by *in vitro* Cd treatment in a concentration-dependent fashion. The activities of chymotrypsin and carboxypeptidase B did not change with *in vitro* Cd exposure. The enhanced activity of trypsin by Cd was returned to the control levels by subsequent treatment with EDTA, indicating that this effect was reversible. In addition, the zinc normally contained in purified carboxypeptidase A and carboxypeptidase B was released by the Cd exposure. These results indicate that Cd inhibits protease activities within the pancreas *in vivo* at doses which do not induce overt hepatic, renal, or pancreatic toxicity. Based on additional *in vitro* study, the decreases seen in trypsin and chymotrypsin activities might be due to indirect effects of Cd while the decreases in carboxypeptidase A activity are probably due to a direct inhibition of the enzyme by the metal.

1329 CADMIUM-INDUCED HEPATOTOXICITY IN TUMOR NECROSIS FACTOR-α KNOCKOUT MICE.

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Cadmium (Cd) is an environmental pollutant that causes hepatotoxicity. Recent reports indicate that Kupffer cells, the resident macrophages of the liver, participate in the manifestation of chemical-induced hepatotoxicity. Tumor necrosis factor-alpha (TNF) is a proinflammatory cytokine that is a major product of Kupffer cells and mediates the hepatotoxic effects of bacterial endotoxin (LPS). LPS, in combination with d-galactosamine (LPS/Gln), causes severe hepatic necrosis and apoptosis. Mice deficient in TNF have been shown to be less sensitive to the hepatotoxic effects of several hepatotoxicants, including carbon tetrachloride and acetaminophen and resistant to LPS and LPS/Gln. It has been speculated that cadmium also may exert its hepatotoxicity, in part, via the production of TNF by the Kupffer cells. Therefore, this study was undertaken to determine whether mice deficient in TNF are resistant to Cd-induced hepatotoxicity. TNF knockout (TNF-KO) and wildtype (TNF-WT) mice were dosed ip with saline, LPS (0.1 mg/kg)/Gln (700 mg/kg), or CdCl2 (2.2, 2.8, 3.4, 3.9, and 4.5 mg Cd/kg). Serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH)

activities were quantified to assess liver injury. Caspase-3 activity was quantified to assess hepatocellular apoptosis. LPS/Gln-treatment increased ALT (17 fold) and SDH (21 fold) in TNF-WT mice. In contrast, LPS/Gln-treatment did not significantly increase ALT or SDH in TNF-KO mice. LPS/Gln-treatment caused a 7.8-fold increase in caspase-3 activity in TNF-WT mice, but did not increase caspase-3 in TNF-KO mice. Cd caused a dose-dependent increase in liver injury in both TNF-WT and TNF-KO mice. However, the liver injury produced by Cd in the TNF-KO mice was not different from that in TNF-WT at any dose. No significant increase in caspase-3 activity was detected in any of the Cd- treated mice. These data indicate that, in contrast to LPS/Gln-induced hepatotoxicity, TNF does not appear to mediate Cd-induced hepatotoxicity. (Supported by NIH grants ES-01142 and ES-07079.)

1330 THE MECHANISM OF GLYCINE PROTECTION AGAINST CADMIUM-INDUCED CYTOTOXICITY IN LLC-PK_1 CELLS.

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We have reported previously that glycine protects against chronic cadmium (Cd)-induced nephrotoxicity *in vivo*. The purpose of the present study was to examine the mechanism of glycine protection. Confluent monolayers of LLC-PK_1 cells were treated with 30 μM $CdCl_2$ in DME medium, with or without pretreatment with 10-100 mM glycine for 16 hr, and/or cotreatment with glycine. Treatment with Cd alone was toxic and caused 56% LDH leakage over 24 hr. Pretreatment and cotreatment with 10 mM glycine offered no protection, however, 25, 50, and 100 mM glycine decreased LDH leakage to 26, 22, and 23%, respectively. Pretreatment or cotreatment with 50 mM glycine also reduced LDH leakage to 28 and 25%, respectively. The cells accumulated 0.55 μg Cd/mg protein after incubation with 30 μM Cd for 4 hr. Pretreatment, cotreatment, or a combination at 50 mM glycine decreased Cd accumulation by 25, 51, and 63%, respectively. This suggested that glycine protected against Cd cytotoxicity by reducing Cd accumulation. The reduction in Cd accumulation could be due to either reduced uptake because of complex formation between glycine and Cd or increased efflux in the presence of glycine. The complex formation was examined by incubating 50 mM glycine with 30 μM Cd in Tris-HCl buffer (pH 8.4) at room temperature for 20 min and chromatography on a Sephadex G-10 column. Cd formed a stable complex with glycine as all of the Cd co-eluted with glycine. To examine the effect of pretreatment with 50 mM glycine on the efflux of Cd, the cells were exposed to 30 μM radiolabeled Cd for 30 min followed by incubation in Hank's buffered salt solution containing 30 μM non-radiolabeled Cd. Glycine pretreatment increased the efflux of Cd. As compared to the non-pretreated cells, 17% more Cd was released into the medium by glycine pretreatment in 5 min. Thus, glycine appears to protect against Cd-induced cytotoxicity in LLC-PK_1 cells not only by reducing Cd uptake but also by increasing its efflux. (Supported by PHS grant # ES 03187.)

1331 MITOCHONDRIAL DAMAGE UPON CADMIUM-METALLOTHIONEIN ADMINISTRATION IS CAUSED BY CD^{++}.

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Acute cadmium-metallothionein (CdMT) injection causes renal proximal tubular damage and mitochondria are considered the earliest target. The purpose of the present study was to examine whether the mitochondrial damage *in vivo* was due to Cd^{++} released upon CdMT degradation. Sprague-Dawley rats were injected ip with 0.3 μmol Cd as CdMT/kg. Urine was collected at 4°C and the animals were sacrificed after 8 and 12 hr. Mitochondria were isolated from renal cortex and respiratory function was measured. Urinary protein, a marker of renal function, increased 2.5- and 11.4-fold after 8 and 12 hr of CdMT injection, respectively. Similarly, urinary LDH, a marker of cytotoxicity, increased 2- and 29-fold. At these time points, mitochondrial respiratory control ratio decreased 16.5 and 51%. State 3 oxygen consumption rate also decreased by 23 and 52% suggesting that Cd inhibited mitochondrial electron transfer. As compared to the controls, the P/O ratio decreased 8 and 24% indicating that mitochondrial oxidative phosphorylation was uncoupled. In a parallel study, mitochondria were isolated from untreated Sprague-Dawley rats and incubated with 0.1-2 μM $CdCl_2$ for 1 min. Cd treatment caused a dose-dependent decrease in respiratory control ratio, state 3 oxygen consumption, and an increase in state 4 oxygen consumption. Treatment with 2 μM Cd decreased the respiratory control ratio by 67% and state 3 oxygen consumption by 25%, suggesting that mitochondrial respiratory function and electron transfer were inhibited. In comparison, state 4 oxygen consumption rate was 129% of controls, indicating that mitochondrial oxidative phosphorylation was uncoupled. The similarity of the mitochondrial damage caused by CdMT administration *in vivo* and $CdCl_2$ treatment *in vitro* suggests that it is the Cd^{++} liberated from CdMT degradation that is responsible for the renal toxicity. (Supported by PHS grant # ES03187.)

1332 CADMIUM (CD)-INDUCED ACUTE HEPATIC INJURY IS EXACERBATED IN HUMAN INTERLEUKIN-8 TRANSGENIC MICE (HIL-8TG).

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It is generally accepted that severe hepatocellular necrosis along with infiltration of neutrophils occurs after acute Cd exposure. In order to investigate the role of neutrophils in Cd-induced liver injury, we first prepared hIL-8Tg which overexpressed IL-8 and displayed an inability of neutrophil migration due to the lack of chemotactic gradient and the downregulation of L-selectin on the surface, and then examined Cd-induced hepatotoxic effects in these mice. A significantly lower survival rate was observed in hIL-8Tg compared with wild-type mice after subcutaneous administration of Cd. Evident liver injury characterized by abrupt increases in plasma GOT and GPT levels was found in hIL-8Tg at 18 hours after Cd administration. Histological examinations including H-E staining and esterase staining revealed the infiltration of numerous neutrophils into the damaged liver tissues in wild-type mice, and the inhibition of the neutrophil migration into the liver as well as enhanced hepatocellular necrosis in hIL-8Tg. Peripheral white blood cell and polymorphonuclear cell counts increased in wild-type mice, whereas the increase in those blood leukocyte counts was delayed in hIL-8Tg. There was no significant difference in the amounts of Cd accumulated in liver and kidneys between wild-type mice and hIL-8Tg. Metallothionein expression was not suppressed in the liver of hIL-8Tg, although the initial synthesis was a little delayed. In conclusion, an acute Cd hepatotoxic effect was exacerbated in hIL-8Tg, suggesting that neutrophils play a protective role in an acute Cd-induced liver injury.

1333 DIETARY EXPOSURES OF CADMIUM IN THE UNITED STATES.

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The Total Dietary Study (TDS), FDA's yearly market basket program, monitors levels of toxic chemical contaminants and nutrients in the U.S. food supply. It provides information on trends in concentrations in the food supply and dietary exposures for the general population. Retail food samples are collected 4 times a year, once each from 4 geographic areas of the U.S., and are analyzed either after preparation/cooking or as ready-to-eat. The latest TDS (1991-1997, 18 baskets) data show that mean cadmium concentrations range from below detection (≤0.002 ppm) to 0.125 ppm, with very low levels detected in most foods. Only 15% of the 264 foods/mixed dishes had a cadmium concentration ≥ 0.02, and 21% between 0.01 and 0.02 ppm. The highest concentrations (0.05 to 0.125 ppm) are found in leafy vegetables, liver, peanuts, and grain cereals. Based on the United States Department of Agriculture's 1987-1988 Nationwide Food Consumption Survey, the estimated daily cadmium average intakes are (μg/day):6-11 month-old, 1.3; 2 year-old, 4.8; 6 year-old, 7.5; 10 year-old, 8.6; 14-16 year-old boys, 10.6; 14-16 year-old girls, 8.3; 25-30 year-old men, 11.2; 25-30 year-old women, 8.7; 40-45 year-old men, 12.2; 40-45 year-old women, 8.9; 60-65 year-old men, 11.5; 60-65 year-old women, 8.9; 70+ year-old men, 10.8; and 70+ year-old women, 8.1. Major sources of cadmium exposure are vegetables, especially lettuce, spinach and potatoes, and grain products. The estimated average cadmium exposures over this period have remained low and are well within the World Health Organization's provisional tolerable weekly intake of 7 μg/kg body weight/week for cadmium.

1334 HIGHLY SENSITIVE MINIATURIZED ASSAY FOR CADMIUM IN BLOOD OR URINE.

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Cadmium is a toxic metal with widespread use in batteries, pigments for plastics and paints, and as coatings on metal products. Cadmium workers are

monitored for elevated cadmium levels in blood and/or urine to ensure that they are not receiving excessive doses. In some areas where mining or smelting is carried out, food, water and air may contain elevated levels of cadmium that is unknowingly ingested or inhaled by the human population. A highly sensitive assay (detection limit=0.02μg/L) for blood/urine cadmium has been published (Peterson et al., Anal. Biochem., **192**, 434-40, 1991). Its main drawback is the relatively large amount of blood needed, up to 8mL. We report here a miniaturized version that uses only 1mL. Blood is deproteinated directly with hydrochloric acid (HCl), converting cadmium to the anionic hexachloride form, which immediately binds to AG1-X8 anion-exchange resin. Use of 15mg resin in a centrifugeable column greatly simplifies eluate collection. Other blood ions are washed out with a small HCl volume. Conversion from the anionic cadmium hexachloride to the cationic cadmium nitrate is begun by a 1N HNO_3 wash. The final sample treatment step involves extraction of resin-bound cadmium into 100μL of 1N HNO_3 with 70% cadmium recovery, affording a 7-fold concentration of the sample cadmium. Cadmium in the final acid extract is quantitated by graphite furnace atomic absorption spectrophotometry. This miniaturized assay is simplified, retains the high sensitivity of the original assay, and, with its small sample volume (1mL), can be applied to pediatric studies. (Work supported by NIEHS grant ES04816.)

1335 CADMIUM-INDUCED LUNG INJURY: EVIDENCE FOR A SPECIFIC INCREASE IN THE PERMEABILITY OF E CADHERIN AND VE-CADHERIN DEPENDENT CELL-CELL JUNCTIONS.

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Although it is well-known that respiratory exposure to Cd^{2+} results in pulmonary edema, the specific mechanisms underlying this effect have yet to be elucidated. Studies from our laboratory have shown that Cd^{2+} can disrupt the tight junctions between epithelial cells in culture by interfering with the normal function of the Ca^{2+}-dependent cell adhesion molecule E-cadherin. The objective of the present study was to determine if the early pneumotoxic effects of Cd^{2+} might result from specific changes in the permeability of E- and VE-cadherin-dependent cell-cell junctions in the lung. Male CF1 mice were given either saline or $CdCl_2$ (65 nmoles) via intratracheal instillation. After 24 hours, the animals were euthanized, and the lungs were removed and either subjected to bronchoalveolar lavage or analyzed for histopathologic changes. The results showed that Cd^{2+} caused an increase in lung weight and in the protein content of the lavage fluid. The microscopic analyses showed evidence of edema and capillary congestion but little evidence of injury to the alveolar epithelial cells or the capillary endothelial cells. In addition, the samples from the Cd^{2+}-treated mice showed a decrease in the amount of E-cadherin in the epithelial cells of the alveoli and small bronchioles and of VE-cadherin in vascular endothelial cells. These findings suggest that Cd^{2+}-induced pulmonary edema may be a consequence of relatively specific increases in the permeability of the cadherin-dependent cell-cell junctions in the alveolar epithelium and the vascular endothelium. (Supported by Grant #R01 ES06478 from the NIEHS.)

1336 CADMIUM DISRUPTS VE-CADHERIN-DEPENDENT CELL-CELL JUNCTIONS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS.

P. C. Lamar, C. A. Pearson and W. C. Prozialeck. *Midwestern University, Downers Grove, IL.*

Many of the acute toxic effects of cadmium (Cd^{2+}) *in vivo* seem to involve an increase in microvascular permeability. However, the mechanisms by which Cd^{2+} increases endothelial permeability are not well understood. The recent finding that Cd^{2+} can selectively damage cadherin-dependent cell-cell junctions in many types of epithelial cells lead us to examine the effects of $Cd2^{2+}$ on the vascular endothelial (VE)-cadherin-dependent cell-cell junctions in human umbilical vein endothelial cells (HUVEC's). Primary cultures of HUVEC's were grown to confluence on glass coverslips and then exposed to Cd^{2+} (0-180 microM in serum-free medium) for up to 24 hours. The results showed that exposure to Cd^{2+} for 2-8 hours caused the cells to separate from each other without detaching from the growing surface. This effect coincided with the loss of vascular endothelial (VE) cadherin from the cell borders, the reorganization of the actin cytoskeleton, and the appearance of the gaps between the cells. Additional results showed that the cells were still viable and were not in metabolic distress at the time in which these changes were occurring. These results are similar in many respects to those we have recently observed in several other types of epithelial cells, and they suggest that VE-cadherin-dependent intercellular junctions may be specific targets for Cd^{2+} toxicity in endothelial cells. (Supported by Grant # R01 ES06478 from the NIEHS.)

1337 CADMIUM DISRUPTS CADHERIN-DEPENDENT CELL-CELL JUNCTIONS IN ROS 17/28 CELLS.

W. C. Prozialeck, P. C. Lamar and C. A. Pearson. *Midwestern University, Downers Grove, IL.*

While the osteotoxic effects of Cd^{2+} have long been recognized, the mechanisms by which Cd^{2+} affects bone are not well-understood. Recent studies from our laboratory have shown that Cd^{2+} can selectively damage the E-cadherin-dependent junctions between many types of epithelial cells in culture. At the same time, studies from other laboratories have shown that E-cadherin and related cadherins are present in osteoblast-like cells and play a key role in the differentiation of the cells into their epithelial phenotype. The objective of the present study was to determine if $Cd2^{2+}$ could disrupt cadherin-dependent cell-cell junctions in the rat osteoblast-like cell line, ROS 17/28. The cells were grown to confluence on glass coverslips in DMEM and then exposed to 0-20 microM Cd^{2+} in a physiological saline solution. The integrity of cell-cell junctions was assessed morphologically, and cadherin-like cell adhesion molecules were visualized by immunofluorescence using a pan-cadherin primary antibody. Exposure to Cd^{2+} for 2-4 hours caused the cells to separate and retract from each other without detaching from the growing surface. This effect coincided with the loss of pan-cadherin immunoreactive material from the cell borders and a reorganization of the actin cytoskeleton, but occurred well before the cells showed evidence of serious injury or the loss of membrane integrity. These results indicate that Cd^{2+} can disrupt the cadherin-dependent cell-cell junctions in ROS 17/28 cells, raising the possibility that some of the osteotoxic effects of Cd^{2+} might result from the disruption of cadherin dependent cell-cell junctions in bone. (Supported by Grant RO1 ES06478 from the NIEHS.)

1338 CADMIUM CHLORIDE ($CdCl_2$) INDUCES REGIONAL EXPRESSION OF HEAT SHOCK PROTEIN-72 (hsp72) IN RAT LIVER.

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Many proteotoxic agents enhance the expression of hsps, a response that may constitute a cellular defense mechanism. $CdCl_2$ is a well-known hepatotoxicant after acute exposure and produces a general necrotic response across the liver acinus. We have shown previously that, in response to acute $CdCl_2$ exposure, the *de novo* synthesis of hsp72 (an inducible member of the hsp70 family) is induced in rat liver prior to detection of hepatic injury using classical functional and clinical indices. The objective of this study was to determine the cell-specific localization of hsp72 within the liver acinus in response to $CdCl_2$. Male SD rats were injected with $CdCl_2$ (0.5, 1, and 2 mg Cd/kg, iv), and livers were removed at 4, 8, 16, and 24 hr after injection. Tissue sections were fixed and prepared for histopathological and immunohistochemical analyses. No treatment-related histopathological changes were observed. A monoclonal antibody specific to hsp72 was used for immunohistochemical analyses. No immunostaining signal specific for hsp72 was observed in untreated controls. In rats receiving 2 mg Cd/kg, no signal was observed after 4 hr; however, a low-intensity signal was first detected after 8 hr. At 16 after exposure, no signal was observed in rats injected with 0.5 mg Cd/kg, but a low-intensity signal was detected at the 1 mg/kg dose. The most intense hsp72 signal was observed 16 hr after exposure to 2 mg Cd/kg. Hsp72 accumulation was predominantly localized in the cytoplasm of centrolobular hepatocytes, primarily around venules. At 24 hr after exposure, the hsp72-specific signal was less intense. These results demonstrated that hsp72 expression in response to acute Cd exposure occurs in hepatocytes located in centrolobular areas and adjacent to larger intrahepatic venules prior to the onset of overt cytotoxicity.

1339 SUPPRESSION OF A HIGH-AFFINITY MANGANESE TRANSPORT PATHWAY IN CADMIUM-RESISTANT METALLOTHIONEIN NULL FIBROBLASTS.

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We have established a Cd-resistant cell line (Cd-rB5) from immortalized metallothionein null fibroblasts, and found that Cd-rB5 cells exhibited a marked decrease in cadmium (Cd) uptake. This cell line may be useful in elucidating the mechanism of Cd transport in mammalian cells. A multitracer technique developed at RIKEN, Japan, was applied to examine whether the incorporation of other metals than Cd was also altered in Cd-rB5 cells. Among 20 elements added to the medium, incorporation of Mn(II) into Cd-rB5 cells was approximately 10% of that of parental cells. However, the reduced uptake of Mn(II) in Cd-rB5 cells was observed only at low manganese concentration, suggesting that a high-affinity component of manganese transport system is suppressed in Cd-rB5 cells. Competition experiments revealed that Mn(II) inhibited Cd(II) uptake competitively with an apparent Ki of 140 nM in parental cells, but not in Cd-rB5 cells. The mutual inhibition of the uptake of Cd(II) and Mn(II) was also observed in other mammalian cells such as HeLa, PC12 and Caco-2 cells. The optimal pH for the uptake of both Cd(II) and Mn(II) was 7.4, suggesting the existence of a novel transporter for these metals distinct from already reported divalent metal transporter (DMT1) having an optimal pH at 5.5. These results demonstrate that Cd(II) utilizes a high-affinity Mn(II) transport pathway for the entry into mammalian cells, and that the suppression of this pathway resulted in a reduced accumulation of Cd in Cd-rB5 cells.

1340 INTERLEUKIN-6 AS A CO-STIMULATOR FOR METALLOTHIONEIN INDUCTION BY CADMIUM.

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Some cytokines as well as metals have capability of inducing metallothionein (MT) in various tissues. Previously we found that IL-6 played a pivotal role in the induction of MT in the remaining liver after partial hepatectomy. Using IL-6 GKO mice we have studied the possible association of IL-6 in induction of MT by Cd. Cd was injected sc at a dose of 0.5, 1.0 or 2.0 mg/kg, 2 times with a 24 h interval. At 24 h after the last injection, liver and blood were collected. Tissue MT and serum IL-6 were determined by using MT RIA and ELISA, respectively. Liver damage was estimated by GPT and GOT activities. We found that Cd induced MT in the liver and kidney in both IL-6 GKO and B6J129Sv (wild-type control) mice. Nevertheless, Cd treatment at dose of 2.0 mg/kg resulted in 1.8 and 1.4 times larger amounts of liver and kidney MT levels, respectively, in B6J129Sv mice compared to IL-6 GKO mice. Cd treatment at doses of 1.0 and 2.0 mg/kg tended to increase serum IL-6 level in the wild-type mice. GOT and GPT activities were increased in the serum of both strains of mice treated at 2.0 mg Cd/kg. We speculate that an increase in serum IL-6 level acts as a co-stimulator for the induction of MT in the liver and kidney in B6J129Sv mice. (This work was supported in part by STA Fellowship to A. Molotkov.)

1341 CHRONIC CADMIUM TOXICOSIS IN PRIMATES: RENAL TUBULAR ATROPHY AND OSTEOMALACIC OSTEOPENIA INDUCED IN OVARIECTOMIZED CYNOMOLGUS MONKEYS.

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To develop an experimental model of chronic cadmium toxicosis in primates, repeated intravenous injections (2 - 3 times per week for 13 - 15 months) of 0 (n=4), 1.0 (3), and 2.5 mg/kg (2) cadmium chloride (Cd) was performed in ovariectomized cynomolgus monkeys. Cd treatment induced normocytic-normochromic anemia, and distinctive kidney and bone lesions. In regard to the kidney, histopathological examination revealed interstitial fibrosis, dilatation and degeneration of tubules, and atrophy of tubular epithelium in the renal cortex. The renal tubular dysfunction appeared to be produced from the early stage of the treatment period (4 or 5th month and later) as indicated by increases of urinary enzyme activities such as ALP, gamma-GT, LAP, LDH, and NAG. In regard to the bone, mineral densities (by DXA method) of the lumbar vertebrae and femur showed 26 - 27 or 20 - 22% decrease. Histomorphometrically, the osteoid volume against the bone volume of the lumbar vertebrae and femur cortex were 12 - 15 or 5 - 12 times higher than those of the control specimens. Moreover, decreases in bone volume of the trabeculae in the lumbar vertebrae, femur, and sternum were also obvious. A significant decrease in the plasma inorganic phosphorus level was almost simultaneous with leakages of tubular marker enzymes in urine. Moreover, a decrease in the plasma calcium level and an increase in the urine deoxypyridinoline level appeared later. In conclusion, chronic cadmium toxicosis is reproducible in primates by repeated intravenous injections of Cd following ovariectomy, and is characterized by renal anemia, tubular atrophy with interstitial fibrosis in the renal cortex, and osteomalacic osteopenia, which closely resemble human itai-itai disease.

1342 THE DEVELOPMENT OF THE CADMIUM DIETARY EXPOSURE MODEL (CDEM).

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The Cadmium Dietary Exposure Model (CDEM), utilizes national survey data on food cadmium concentrations and food consumption patterns to estimate dietary intakes in the U.S. population. The CDEM has been linked to a modification of the cadmium biokinetic model of Kjellström and Nordberg (KNM) to derive estimates of kidney and urinary cadmium that reflect U.S. dietary cadmium intake and related variability. Variability in dietary cadmium intake was propagated through the KNM using a Monte Carlo approach. The model predicts a mean peak kidney cadmium burden of approximately 3.5 mg and a 5th-95th percentile range of 2.2-5.1 mg in males. The corresponding peak renal cortex cadmium concentration in males is 15 (10-22, 5th-95th %) mg/g wet cortex. Predicted kidney cadmium levels in females were higher than males: 5.1 (3.3-7.6) mg total kidney; 29 (19-43) mg/g wet cortex. Predicted urinary cadmium in males and females agreed with empirical estimates based on the NHANES III, with females predicted and observed to excrete approximately twice the amount of cadmium in urine than males. The predicted 95th percentile values for peak kidney cadmium burden are approximately 60% of the peak kidney burden predicted for a chronic intake at the U.S. EPA Reference Dose of 1 mg/kg-day (8.2 mg). (Supported in part by EPA Cooperative Agreement CR822761 and EPA Contract 68-C6-0024. Statements in this report do not reflect opinions or policies of the U.S.EPA.)

1343 CONTRASTING ROLE OF NA+ IONS IN MODULATING CU+2 VERSUS CD+2 INDUCED HEPATOCYTE TOXICITY.

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Cu^{+2} induced hepatocyte cytotoxicity involved membrane lipid peroxidation and "ROS" formation and was prevented by antioxidants. No increase in cellular Na^+ was observed although cytotoxicity was markedly enhanced by Na^+ omission from the media. Omission of Na^+ from the incubation medium however greatly increased Ca^{+2} accumulation in Cu^{+2} treated hepatocytes. This was partially reverted upon transferring the cells to a Na^+ containing medium Removing media Ca^{+2} with EGTA also prevented Cu^{+2} induced hepatocyte cytotoxicity. This suggests that a Na^+-dependent Ca^{+2} transporter is involved in controlling excessive accumulation of Ca^{+2} caused by the Cu^{+2} induced membrane lipid peroxidation. On the otherhand Cd^{+2} induced hepatocyte cytotoxicity involved mitochondrial toxicity and ATP depletion but not lipid peroxidation. Omission of Na^+ or Cl^- from the medium ameliorated Cd^{+2} induced hepatocyte toxicity. Furthermore the Na^+/H^+ exchange inhibitor amiloride or the Cl^- channel blocker glycine also prevented Cd^{+2} induced hepatocyte toxicity. Cd^{+2} but not Cu^{+2} treated hepatocytes were also much more suseptible to a hypotonic media. This suggests that Cd^{+2} cytotoxicity could be attributed to disruption of cell volume regulation mechanisms. The increased osmotic load caused by uncontrolled accumulation of intracellular Na^+ likely resulted from the activation of Na^+/H^+ exchanger and the Na^+/HCO_3^- cotransporter by acidosis caused by the ATP depletion induced by Cd^{+2}.

1344 PROTECTIVE EFFECTS OF ZINC ON RAT HEPATIC STELLATE CELLS TO CADMIUM.

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Zinc (Zn) is an essential trace element that protects against hepatotoxicity produced by a variety of chemicals, among them Cadmium (Cd). The mechanism of Zn protection has been attributed to the following: 1-stabilization of lysosomal, mitochondrial and microsomal membranes; 2-inhibition of P-450-dependent mono-oxygenase activity; 3-stabilization of cellular thiol and activation of glutathione-associated enzymes and 4-sequestration of reactive moieties, free radicals and Cd by the Zn-induced metallothionein. The aim of the present study was to evaluate the protective effect of Zn on Cd toxicity in a rat hepatic stellate cell line (CFSC-2G). Cells were pretreated during 24 h with 60μM $ZnCl_2$, and then 3 or 5 μM $CdCl_2$ were added for another 24 h. MTT assay showed that mitochondrial function diminished 15 and 30% with 3 and 5 μM respectively, and this damage was reversed with Zn pretreatment. Lipid peroxidation damage increased approximately 103% with both Cd concentrations. This effect was not observe in Zn pretreated cells. Catalase activity was inhibited 21% in Cd-treated cells. Zn-pretreated cells presented catalase activity values similar to the control ones. Glutathione (GSH) content was diminished in 33% and 42.8% with 3 and 5 μM $CdCl_2$ respectively while no change, compared to control cells, was observed in Zn-pretreated hepatic stellate cells. No change in glutathione reductase activity was observed in any treatment. Metallothionein-I (MT) induction was determined. MT-I mRNA was presented in all treatments. However, Cd treated cells presented higher MT-I mRNA content than Zn pretreated cells. In conclusion, the present study suggests that Zn reduces Cd toxicity in rat hepatic stellate cells by controlling lipid peroxidative damage and GSH levels rather than by inducing MT-I.

1345 EFFECTS OF CADMIUM ON THE EXPRESSION OF CYCLIN D1 AND CYCLIN DEPENDENT KINASE (CDK4), TUMOR SUPPRESSOR GENES P27 AND P53, AND ONCOGENE C-MYC IN *IN VITRO* TWO STAGE TRANSFORMATION PROCESS.

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In vitro two stage transformation of BALB/3T3 cells is a well-known neoplastic process similarly with *in vivo* two stage carcinogenesis process. To determine the effects of cadmium in *in vitro* two stage transformation and elucidate cellular/molecular mechanisms of two stage transformation, we transformed BALB/3T3 mouse embryo cells with MNNG as a initiator and $CdCl_2$ as a promoter at high transformation frequency, and detected the alterations of the protein and/or mRNA expression levels of cyclin D1, cyclin dependent kinase 4 (CDK4), PCNA, tumor suppressor gene p27 and p53, and oncogene c-myc. Whole cell protein and total RNA were extracted from Control, MNNG, and MNNG+$CdCl_2$ (forming morphologic transformation focus at high frequency). Western blot analysis revealed that: [1] the expression levels of cyclin D1, CDK4, and PCNA were higher in MNNG+$CdCl_2$ group than in the other two groups.; [2] wild-type p53 protein was strongly expressed in MNNG group, but not expressed in MNNG+$CdCl_2$ group; [3] In MNNG+$CdCl_2$ group, the expression level of p27 was reduced significantly compared to Control and MNNG groups; [4] c-myc protein was highly expressed in MNNG+$CdCl_2$ group among three groups. In addition, quantitative RT-PCR and Northern blot showed that: [1] cyclin D1 and PCNA mRNA expression levels were highest in MNNG+$CdCl_2$ group; [2] mutant-p53 mRNA was apparently expressed in MNNG+$CdCl_2$ group, while slightly expressed in MNNG group; [4] p27 mRNA expression pattern was quite similar with that of protein. These results indicates that transformed cells express higher levels of cyclin D1, CDK4, and PCNA than nontransformed cells, while tumor suppressor genes p53 and p27 were strongly inhibited and oncogene c-myc was highly activated in transformed cells. Our data also elucidates that cadmium enhances the transformation process of MNNG-treated cells by inhibiting the tumor suppressor gene and accelerating the proliferation of initiated cells.

1346 THE EFFECT OF DIOXIN ON VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AS ASSAYED BY TWO PCR-BASED METHODS.

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Cardiovascular abnormalities are among the earliest and most striking teratogenic effects of TCDD (tetrachlorodibenzo-p-dioxin). Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen crucial in normal cardiovascular development. However, its role in abnormal development in animals exposed to environmental contaminants has not been determined. We examined whether VEGF expression in fish embryos is influenced by exposure to TCDD. Pre-gastrula zebrafish were injected with TCDD in triolein or triolein alone (control). Embryos from each treatment group were harvested at 12 and 24 hours post fertilization (hpf), and total RNA was isolated for use in two RT-PCR methods. Semi-quantitative RT-PCR involves normalizing signals from a target gene(s) to those of beta-actin. In competitive RT-PCR, recombinant DNAs with binding sites for gene-specific primers are used as internal standards, allowing quantification of the number of copies of a given sequence per unit RNA. Repetitions of semi-quantitative RT-PCR yielded similar patterns but large variation in the magnitude of observed differences. The data show no change in VEGF or CYP1A mRNA at 12hpf, but suggest slight induction of both genes by triolein and further induction by TCDD when expression is assayed at 24hpf. Competitive RT-PCR revealed a different scenario, indicating an increase in VEGF expression associated with triolein injection, but no TCDD-specific response by VEGF. At present, the discrepancies in the data make it difficult to draw firm conclusions as to the effect of TCDD on embryonic VEGF expression.

1347 TCDD CHANGES GROWTH FACTOR AND MATRIX PROTEIN EXPRESSION IN FETAL RAT VAGINAL TRACT.

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TCDD exposure induces reproductive and developmental effects. In rats, *in utero* exposure causes abnormalities in the female rat reproductive system, including reduced fecundity and a vaginal thread. The vaginal thread is manifested prenatally and altered morphology is detected as early as GD18. Fusion of the Mullerian ducts (MD) in the lower vaginal tract does not progress in treated fetuses, resulting in a residual mesenchyme surrounded by epithelial cells. The etiology of this defect may include disruptions in growth factor-mediated cellular proliferation, differentiation, and/or migration, as well as basement membrane remodeling. In this study, the expression of epidermal growth factor receptor (EGFR), transforming growth factor β-3 (TGF-β3), aryl hydrocarbon receptor (AHR), and laminin was examined immunohistochemically. Time-pregnant Long Evans rats (Charles River, Raleigh, NC) received on GD15 a single, oral dose of 1 microgram TCDD/kg or vehicle (5 ml corn oil/kg). Dams were sacrificed on GD 18 or 19 and reproductive tracts of female fetuses were dissected. Tissues were fixed in 3% paraformaldehyde, dehydrated and embedded in paraffin. Sections were prepared with control and treated tissues placed on each slide. Antibodies to EGFR, TGF-β3, and AHR (Santa Cruz Biotechnology) and laminin (Sigma) were incubated with sections overnight and detected with the Vector ABC Kit (Vector Labs, Burlingame, CA). TCDD elevated TGF-β3 in reproductive tract epithelium and mesenchyme. AHR expression increased in the MD luminal epithelium, but EGFR appeared to be unaffected by TCDD exposure. Laminin in basement membrane of Wolfian duct and urethra was expressed more strongly in TCDD-treated fetuses. It is not clear which cellular processes are required for fusion of MD in the lower reproductive tract of rodents, however the effects of TCDD suggest that growth factors and matrix remodeling may be important in removal of cells from between the ducts and fusion of the ductal epithelia. (Disclaimer: This abstract does not necessarily reflect EPA policy.)

1348 EARLY MORPHOGENETIC EVENTS ARE ALTERED IN THE FORMATION OF THE LOWER REPRODUCTIVE TRACT IN FEMALE RAT FETUSES GESTATIONALLY EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Exposure to TCDD and dioxin-like compounds results in a wide variety of effects in experimental animals, including a wasting syndrome, immunosuppression, thymic atrophy, chloracne, teratogenicity, carcinogenicity, as well as other toxic and biochemical effects. In rats, a single oral dose of 1.0μg TCDD/kg on gestation day (GD) 8 or GD15 caused structural and functional abnormalities in the female rat reproductive system, including reduced fecundity and the presence of a vaginal thread. The vaginal thread in TCDD-exposed rats consists of mesenchyme surrounded by epithelial cells and is clearly visible in histological sections as early as PND2. The objective of this study was to investigate TCDD-induced alterations during embryogenesis to better understand vaginal thread formation. Time-pregnant Long Evans rats received a single, oral dose of 1.0μg TCDD/kg on GD15. Dams were sacrificed on GD17, 18, 19, and 21 and the reproductive tracts from female fetuses were examined histologically. Subtle differences in the spatial orientation of the Mullerian ducts (MD) were present as early as GD17. Abnormalities in Mullerian duct fusion (a process critical to vaginal morphogenesis) were detected as early as GD18 and these effects constitute the origin of the vaginal thread. Furthermore, on GD19 the length of unfused MD was significantly greater in TCDD-treated fetuses than in controls. This information may help elucidate the mechanism of action of TCDD-induced effects on vaginal development in the rat. (This abstract does not necessarily reflect EPA policy. CHH supported by EPA, CT 902908.)

1349 TCDD DOSE RESPONSIVENESS AND EARLY GESTATIONAL EXPRESSION OF A TCDD RESPONSIVE LACZ REPORTER TRANSGENE IN MICE.

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The Aryl-hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that mediates the toxicity of halogenated aromatic hydrocarbons including 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD). These compounds are potent reproductive and developmental toxicants due to their ability to modulate gene expression and cellular differentiation and proliferation. The determination of the tissue-specific transcriptional activity of the AhR complex *in vivo* will allow the identification of tissues where the AhR may have some functional role that is susceptible to disruption by exposure to TCDD. A transgenic mouse model has been developed in our lab that can delineate the temporal and spatial context of active AhR in a cell- and region-specific manner. This model has previously identified several target tissues during mid gestation (gestational day [GD] 13-16) including the genital tubercle and paws. Longer TCDD exposures (48 and 72h) showed a similar pattern of expression. Embryos exposed to TCDD (30μg/kg) for 24h beginning on GD 6 to 12 have also been evaluated. Results indicate that the transgene is expressed in individual cells particularly in the developing ear. A dose response study was completed to evaluate responsiveness of the transgene at lower doses. Mice were exposed to 30, 15, 3, and 1.5μg/kg TCDD for 24h. Analysis of transgene induction was completed on an organ-by-organ basis. A 2.6 fold induction above vehicle was noted in the liver at the lowest TCDD dose and a 2.2 fold induction in the lung at the 3μg/kg dose. These results indicate that the model is capable of detecting low dose activation of the reporter, thus allowing the sensitive detection of developmental targets of TCDD. (Supported in part by NIEHS Grant ES09430, Center Grant ES01247, and Training Grant ES07026.)

1350 POLYBROMINATED BIPHENYL EXPOSURE *IN UTERO*: POSSIBLE EFFECTS ON BEHAVIOR IN THE MOUSE.

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In Southern Michigan in 1973, livestock were heavily contaminated with polybrominated biphenyls (PBBs) due to accidental ingestion of the flame retardant "Firemaster"; humans eating these livestock suffered numerous health problems-somnolence and amnesia, mental confusion, leukocytopenia, chloracne [sentinel sign of aryl hydrocarbon receptor (AHR)-mediated toxicity in dioxin-exposed workers], and low-birth-weight and lowered I.Q. in children exposed to PBBs *in utero*. To investigate any correlation between neurobehavioral toxicity caused by PBBs and the involvement of the AHR, we are comparing 3,4,5,3',4',5'- (coplanar, cHBB) with 2,4,6,2',4',6'-hexabromobiphenyl (non-coplanar, ncHBB) treatment of pregnant C57BL/6J (B6, high-affinity-AHR) inbred mice and B6.D2-Ahr^d (low-affinity-AHR) congenic mice. In utero treatment was given at gestational day 5, and the offspring were tested 60 days post partum for locomotor activity plus four behavioral tests: the delayed spatial alternation (DSA) T-maze, latent-learning, Morris water maze, and step-down passive-avoidance (SDPA) tasks. There were no striking differences among groups in the DSA T-maze or SDPA endeavors. In contrast, using a water-finding task we found that cHBB-treated B6 mice show a trend in a deficit in latent learning-compared with that in cHBB-treated B6.D2-Ahr^d mice or either control group. cHBB-treated B6 mice also weighed significantly less than cHBB-treated B6.D2-Ahr^d mice or either control group. These data are consistent with the possibility that the AHR might be involved in the onset of cognitive deficits seen following *in utero* exposure to cHBB. Dopamine is known to play a key role in cognitive and emotional states, including latent learning. Although the underlying molecular mechanisms of these behaviors have not been determined, we postulate that CNS developmental expression of the AHR may predispose those areas of the CNS where the AHR is expressed to a greater risk of cHBB toxicity-perhaps by local disruption of dopamine signaling. (Supported in part by NIH Grant P30 ES06096.)

1351 EFFECTS OF DIETARY *p,p'*-DDE ADMINISTERED MATERNALLY ON REPRODUCTIVE DEVELOPMENT OF ADULT MALE RATS.

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To test the hypothesis that exposure to endocrine-active compounds during development results in permanent alterations in sexual development, we examined sexual behavioral responses and luteinizing hormone (LH) secretion patterns in adult male rats following their exposure to the environmental antiandrogen *p,p'*-DDE at early developmental stages. Twenty-eight day-old female rats were fed NIH-07 diet containing 0, 2.5, 25, or 250 ppm DDE for eight weeks before breeding with untreated males and during pregnancy and lactation. Male offspring were placed on standard NIH-07 diet at weaning. LH levels in serum samples collected from four-month-old male rats were 4.56 ± .41 (n=14), 3.59 ± .32 (n=14), 4.25 ± .59 (n=13), and 1.93 ± .77 (n=14) ng/mL for the 0, 2.5, 25, and 250 ppm DDE groups, respectively ($p<0.01$, with 2.5 and 250 ppm groups being significantly different from the 0 ppm group). When additional male rats were evaluated for masculine sexual behavior at approximately five months of age, no change was found in the number of mounts, number of intromissions, latency of ejaculation, and postejaculatory interval for the DDE-treated rats. However, the 2.5 ppm group showed significantly prolonged latency of mount and latency of intromission ($p< 0.05$). All male rats were then castrated and estrogen-plus-progesterone (E+P) conditioned before being tested for the potential of displaying E+P elicited feminine sexual behavior. In the 0, 2.5, 25, and 250 ppm groups, 8 (n=13), 42 (n=12), 20 (n=10), and 55% (n=11) of the respective males showing various degree of lordosis when mounted by untreated stud males. In addition, when canulated and stimulated with an E+P injection regime, the rats in the 250 ppm group exhibited a time-dependent elevation in the circulating LH level. The results suggest a substantial degree of variability in the evaluation of adult rat sexual behavior. Further research and analysis are needed to ascertain whether neonatal DDE can exert long-term effects on adult sexual behavior. Such effects, particularly when associated with low-dose DDE exposures, need to be assessed for potential human relevance.

1352 DEVELOPMENTAL EFFECTS OF AROCLOR* 1242 ON MALE OFFSPRING AFTER GESTATIONAL AND LACTATIONAL EXPOSURE.

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Developmental effects of PCBs on male reproduction in rodents have been studied previously in several laboratories, however, with inconsistent results. The effects on testicular weights and fertility depend on the test congener or mixture, the dosage, the developmental stage during exposure, and the age of the animals when examined. We have previously reported an increase in testis weight and a decrease in sperm fertilizing ability in 3,3',4,4'-tetrachlorobiphenyl treated young males. The effect of Aroclor 1254 on sperm fertilizing ability after neonatal exposure, however, was not observed until 45 weeks of

age. The objective of this study is to examine the effects of gestational and lactational exposure of Aroclor 1242 (0, 10, 25, 50 and 100 mg/kg/2 days) on testicular development and sperm quality in 16-week old male mice. Sperm concentration and quality were examined using *in vitro* fertilization and computer-assisted sperm motion analysis. Testicular gene expression in mice from 25mg/kg treatment group was examined using a commercial cDNA array. There was no effect on maternal body weight, fecundity, litter size, litter weight, sex ratio or 21-day survival index. There was a dose-dependent increase in liver weights in 21 day old male offspring ($p<0.04$), but no observable change in testis and thymus weights at 3 weeks of age. There was, however, an increase in anogenital distance in the male offspring in the 25 mg/kg treatment group on day 21 ($p<0.002$). At 16 weeks of age, there were no detectable changes in sperm count or fertilizing ability. There were no changes in liver or thymus weight, however, there was a dose-dependent trend of increased testes weight. cDNA array analysis did not reveal any changes in gene expression. (Sponsored by the Michigan Great Lakes Protection Fund.)

1353 INSULIN-LIKE GROWTH FACTOR EXPRESSION IN MURINE EMBRYOS EXPOSED *IN VITRO* TO *o,p'*-DDT.

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During pregnancy, insulin-like growth factors (Igf) are synthesized by both mother and embryo and are thought essential for implantation and fetal development. Because the appearance of Igf ligands and receptors coincides with the expression of estrogen receptors in preimplantation embryos, it is possible that the events are closely linked and subject to perturbation by environmental estrogens that may accumulate in fluids of the reproductive tract. The goal of this study was to determine the effects of an estrogenic pesticide o,p'-DDT on the expression of Igfs in murine embryos at the 2-cell, 8-cell and blastocyst stages using immunocytochemistry. Pronuclear embryos were obtained from superovulated CD-1 mice and cultured for 24, 72 and 114 h in 25 microliter drops of Earle's balanced salt solution (EMG) under oil, containing 0.1% ethanol (control) or 0.1 microg/ml o,p'-DDT. Development of embryos to stage was scored before the embryos were fixed in paraformaldehyde. Embryos were incubated overnight with 6 microg/ml polyclonal anti-Igf-1R, -Igf-II or –actin, rinsed, and incubated 1h with FITC-labeled anti-IgG of appropriate specificity. DNA was stained with propidium iodide before the embryos were placed in mounting medium and examined with a Nikon Optiphot-microscope. Fluorescence intensity was scored for reactions blocked with the peptide of interest and statistically compared to results for unblocked reactions. Three immunocytochemistry experiments were completed in which all three stages of development were analyzed. A total of 193 stage-appropriate exposed embryos were compared to 194 control embryos. ANOVA was used to analyze the data. The model included the variables: pesticide, blocked vs unblocked reactions, antibody and stage of development. In comparison to control treatment, o,p'-DDT significantly reduced embryo development to the 2-cell, 8-cell and blastocyst stages ($p=0.0167$). Overall, antibody activity was reduced by pre-incubation with the relevant protein ($p=0.0001$). Immunocytochemistry data suggest that *in vitro* exposure to o,p'-DDT did not significantly alter expression of Igf-1R, Igf-II or actin proteins for embryos at the 2-cell, 8-cell or blastocyst stages ($p=0.1174$). Further research will include RT-PCR for more quantitative comparisons of growth factor expression. (Financial support was provided by the WD Connor Trust and MMRF.)

1354 RABBIT ORAL DEVELOPMENTAL TOXICITY STUDIES WITH TWO PERFLUORINATED COMPOUNDS.

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Rabbit developmental toxicology studies were conducted on two perfluoronated compounds – perfluorooctanesulfonate (PFOS) and 2-(N-ethyl perfluoro-octanesulfonamido)-ethyl alcohol (N-EtFOSE). Dosages of 0, 0.1, 1.0, 2.5 and 3.75 mg/kg/day in 0.5% Tween 80 were used for both studies. Although no compound related deaths occurred, maternal toxicity (reduced body weight gain and feed consumption values) was present at the higher dosage levels of both studies. On the basis of these data, the maternal no-observable-effect-level (NOEL) of N-EtFOSE and PFOS was 0.1 mg/kg/day (the 1.0 mg/kg/day and higher dosages caused statistically significant reductions in body weight gains or weight losses and the 2.5 and 3.75 mg/kg/day dosages also significantly reduced absolute and relative feed consumption values). The developmental NOEL was 1.0 mg/kg/day for both compounds (the 2.5 and 3.75 mg/kg/day dosages of N-EtFOSE caused increased incidences of late resorptions and abortions and the 2.5 and 3.75 mg/kg/day dosages of PFOS caused reductions in fetal body weights and delays in ossification). Detailed fetal external gross, soft tissue and skeletal examinations failed to reveal any malformations related to treatment. It was concluded that these perfluoronated compounds were not selective developmental toxicants in rabbits.

1355 EMBRYOTOXICITY OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5*H*)-FURANONE (MX) AND FOUR HALOACETIC ACIDS IN MICROMASS *IN VITRO* TEST.

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3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) and other halocompounds, such as trichloroacetic acid (TCA), dichloroacetic acid (DCA), tribromoacetic acid (TBA) and dibromoacetic acid (DBA) are drinking water disinfection by-products (DBPs). They are created when water with naturally occuring organic compounds and bromine is chlorinated or ozonated. These compounds are known or suspected to cause cancer and reproductive effects at high concentrations. The embryotoxic potential of MX, TCA, DCA, TBA and DBA was evaluated using rat limb bud (LB) micromass test. Following dissociation of LB tissues with trypsin, single-cell suspensions were prepared in Ham's F-12 culture medium containing 5% fetal calf serum (pH 7.0-7.4). The cell suspensions were filtered and adjusted to give 2×10^7 cells per ml. Aliquots of 5 µl were delivered to 96-well tissue plates and incubated for 2 hours at 37°C in a humidified air containing 5% CO_2. Eight concentrations of MX, TCA, DCA, TBA or DBA were added in a final volume of 300 µl culture medium per well. At the end of the 5-day culture period, cells were stained with Neutral Red and Alcian Blue for determination of concentrations that produced a 50% inhibition of cell proliferation (IP_{50}) and differentiation (ID_{50}) The embryotoxic potential of MX was high in the micromass test and decreased in the following order: MX >> DBA> >TBA >DCA >TCA. The IP_{50}-and ID_{50}-values for MX were 7.1 µg/ml and 6.5 µg/ml, respectively.

1356 EVALUATING THE ROLE OF GAVAGE TREATMENT OF TRICHLOROETHYLENE, TRICHLOROACETIC ACID, AND DICHLOROACETIC ACID ON THE DEVELOPING SPRAGUE-DAWLEY RAT FETUS.

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Trichloroethylene (TCE) and its metabolites are commonly found in finished drinking water and as groundwater contaminants in many regions of the United States. Therefore, it is important that the health effects of TCE and its metabolites be assessed, both epidemiologically and experimentally. Epidemiological studies have shown a correlation between drinking water that contains halogenated hydrocarbons and an increased incidence of congenital cardiac malformations in children born to mothers residing in these areas. Follow-up studies in avian and rodent models have also shown an increased incidence of cardiac malformations at varying dose levels of these compounds. However issues have been raised with these studies regarding data gaps, the lack of a dose-response relationship, and the use of different evaluation techniques. The objective of this study was to validate previous reports of cardiac teratogenicity following maternal administration of TCE, trichloroacetic acid (TCA), and dichloroacetic acid (DCA) to Sprague-Dawley rats via gavage on gestation days six through fifteen. All-trans retinoic acid (RA) was used as a positive control. Maternal and fetal examinations were performed on gestation day twenty-one. Fetal hearts were examined in situ, removed, and formalin-fixed for later microscopic/dissection examination. The mean uterine weights of the TCA group were significantly lower than the vehicle control group. Fetal body weights in the TCA and DCA groups were significantly decreased. A significant number of external malformations were observed in the DCA-treated fetuses. No significant differences in cardiac malformation incidence were observed in the TCE-, TCA-, or DCA-treated groups. Expected incidence levels of both maternal and fetal lesions were observed in the retinoic acid positive control group.

1357 ALTERED GENE EXPRESSION IN THE DEVELOPING MALE REPRODUCTIVE TRACT FOLLOWING *IN UTERO* EXPOSURE TO DI-*N*-BUTYLPHTHALATE.

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Developmental exposure to di-*n*-butyl phthalate (DBP), a ubiquitous plasticizer and solvent, alters male rodent reproductive function and sexual differentiation. Although the reproductive effects are similar to that of flutamide, a classical androgen receptor antagonist, DBP does not directly interact with the androgen receptor. The objective of this study was to explore the potential mechanisms of DBP-induced reproductive malformations by examining the expression of critical regulatory genes during reproductive development and determining their association with cellular proliferation and differentiation. Pregnant Sprague-Dawley rats were administered 500mg/kg/day of DBP or 50mg/kg/day of flutamide in corn oil by gavage from gestation days (gd) 12 to 21, the period of male reproductive tract development. Steady-state levels of androgen receptor (AR), steroidogenic factor (SF-1), epidermal growth factor (EGF), and its cognate receptor EGF-R mRNAs were analyzed by semi-quantitative RT-PCR using RNA from treated and control fetal testes. At gd 21, SF-1 gene expression was decreased by 20% in DBP treated testes, while a similar increase occurred with flutamide exposure. AR message and protein levels increased by 20% in DBP treated testes, while flutamide exposure resulted in a similar increase in message levels but did not translate into a similar increase in the level of protein. EGF mRNA was unaltered by DBP exposure but its cognate receptor mRNA was elevated by 40%. EGF-R mRNA levels were increased by nearly 20% by flutamide exposure. While the average change in the level of gene expression among treatment dams was not statistically significant, the greatest degree of change in mRNA levels occurred in fetuses most severely affected by DBP exposure as assessed by decreased anogenital distance. The rat Clontech Atlas cDNA array is currently being used to identify more global changes in gene expression with DBP exposure throughout the time course of reproductive development. These studies demonstrate that the gene expression profile associated with DBP exposure differs from that of flutamide and occurs by a yet to be defined mechanism.

1358 NONYLPHENOLS DIFFERENTIALLY AFFECT γ-GLUTAMYL TRANSPEPTIDASE IN TESTIS AND MALE ACCESSORY ORGANS IN RATS DURING DEVELOPMENT.

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We have previously shown that administration of nonylphenols (NPs) to neonatal male rat pups impair their subsequent reproductive development. Gamma-gluctamyl-transpeptidase (GTP), present in the testis and seminal vesicle, exhibits developmental maturation in these tissues. In this study, we examined the effect of NPs on GTP activities in the testis and male accessory organs to see whether NP perturbs GTP development in these tissues. Newborn male pups were given NPs at (8.0 mg/Kg body wt.) or vehicle (dimethylsulfoxide) for 15 days. They were sacrificed at 25 days of age. NP treated pups showed decrease level of GTP in their epididymus. GTP activity in the testis, seminal vesicle or prostate was minimally affected. To study the kinetic parameters, NPs were also given as a single injection to pups at ~22 days of age (8.0 mg/Kg body wt.) and sacrificed at 24 and 48 hours post-injection. GTP activity was not affected in the testis, seminal vesicle and prostate at both time points. The epididymus, however, showed a reduction in GTP activity at 24 hours post-treatment and decreased further at 48 hours. The degree of decrease in GTP activity was dose dependent and was detectable at NP concentration of 6.0 mg/Kg body wt. Though the exact function of GTP on reproductive development is not clearly understood, GTP knock out mice are found to be infertile. The adverse effects of NPs on male reproductive development might be at least in part through its action on GTP development in some of these male accessory tissues.

1359 EXPOSURE TO 2,4,5-TRICHLOROPHENYL-4'-NITROPHENYL ETHER INHIBITS DEVELOPMENT OF OTOLITHS IN CD-1 MICE.

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Certain nitrodiphenyl ethers, including the herbicide nitrofen (2,4-dichlorophenyl 4'-nitrophenyl ether), are potent developmental toxicants that not only cause perinatal mortality, but also induce subtle malformations in surviving offspring. These malformations, including diaphragmatic hernias and small or absent Harderian glands, are more readily detected postnatally than in prenatal screens. We have also observed that a small percentage of pups which survive prenatal exposure to the nitrofen analog, 245-TCN (2,4,5-trichlorophenyl 4'-nitrophenyl ether), engage in compulsive circling when disturbed. In earlier studies, 245-TCN was the only one of 30 diphenyl ethers to cause circling, and did so only at doses that induced a high frequency of perinatal mortality. Because circling may result from defective development of otoliths, we collected term fetuses exposed to either nitrofen or 245-TCN and processed them for examination of otoliths. When pregnant mice were exposed to 20 mg/kg/day 245-TCN on gestation days (GD) 6-15 (day of semen plug=GD 0), some offspring in each litter lacked one or more otoliths, and most otoliths were decreased in size even when present. Single doses of 50 mg/kg on GD 8, 9, 10 or 11 also decreased otolith size. Preliminary data from such single day dosing studies demonstrate that the highest incidence of otolith damage occurs on or after GD 11. This is in contrast to other diphenyl ether-induced malformations, for which the most sensitive day is GD 8 (in mice). Single-day dosing on GD 8, 9, 10 or 11,with up to 700 mg/kg technical nitrofen, (a dose causing nearly 100% perinatal mortality on GD 8), did not significantly decrease otolith size.

1360 DEVELOPMENTAL NEUROTOXIC EFFECTS OF 2,2',4,4',5-PENTABROMODIPHENYL ETHER (PBDE 99) IN THE NEONATAL MOUSE.

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Sponsor: K. M. Crofton.

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additatives. In a recent study we have seen that neonatal exposure to some brominated flame-retardants can cause permanent aberrations in spontaneous behavior that seem to worsen with age. In view of an increasing amount of PBDEs in mothers milk and in the environment, the present study was undertaken to investigate whether there is a critical and limited phase for induction of persistent neurotoxic effects of PBDE 99. Mice were exposed neonatally on day 3, 10 or 19, to 8 mg PBDE 99/kg body weight. Uptake and retention of [^{14}C]PBDE was studied in the mouse brain after exposure to 1.5 MBq [^{14}C]PBDE 99/kg body weight, on postnatal day 3, 10 or 19. Spontaneous behavior was observed in 4-month-old mice. Mice exposed to PBDE 99 on day 3 or 10 showed significantly impaired spontaneous motor behavior. Neonatal mice, exposed to [^{14}C]PBDE 99 on postnatal day 3, 10 or 19, were sacrificed 24 h or 7 days post-treatment and the amount of radioactivity in the brain was measured. [^{14}C]PBDE 99 was detected in the brain but no significant difference was evident between the age categories after 24 h. On day 7, [^{14}C]PBDE 99 was still present in the brain, though radioactivity had decreased significantly in all age categories. Nicotine-induced behavior in adult mice has also been tested after neonatal exposure to PBDE 99. The nicotine-induced behavior test revealed a hypoactive response in contrast to the hyperactive response shown in the control animals, indicating effects on the cholinergic system. In conclusion, the behavioral disturbances observed in adult mice following neonatal exposure to PBDE 99 are induced during a defined period of neonatal life and the adult response to nicotine is significantly changed.

1361 EFFECT OF ETHANE DIMETHANESULPHONATE (EDS) AND MOLINATE ON THE DEVELOPMENT OF THE PRENATAL MURINE REPRODUCTIVE TRACT.

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Endocrine disrupting compounds that disturb Leydig cell steroidogenesis may reduce testosterone biosynthesis and subsequently result in dysgenesis of the male reproductive tract. The effect of ethane dimethanesulphonate (EDS) and molinate on reproductive tract development in CD-1 mice exposed *in utero* was investigated based on reported reproductive damage in adult male rats. EDS is a model compound specifically cytotoxic to the Leydig cells and molinate is a thiocarbamate herbicide that is reported to inhibit esterase activity and thus prevent the assembly of cholesterol esters, a necessary precursor to testosterone biosynthesis. Timed pregnant CD-1 dams were dosed i.p. with 0, 25 or 50 mg/kg/d EDS, or 0, 50 or 100 mg/kg/d molinate on gestation days 11 thru 16. On gestation day 18, mouse fetuses were removed via caesarian section; anogenital distance (AGD) was measured and fetuses were examined for reproductive tract malformations. No reproductive tract malformations were

detected but a significant dose-dependent decrease in AGD was observed with both treatments in both male and female pups. AGD is an androgen-dependent endpoint and, thus, the results of these gestational exposures suggest that the disruption of fetal Leydig cell steroidogenesis yields reduced testosterone levels. Current studies are underway to examine testis histology and to measure whole body testosterone throughout the dosing regimen via radioimmunoassay. Potentially, this mechanism could result in a delay in puberty and decreased fertility. (This abstract does not necessarily reflect EPA policy DKT; Training Agreement T901915.)

1362 EARLY EMBRYONIC LOSS INDUCED BY DIBUTYLTIN DICHLORIDE (DBTCl) IN RATS.

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Dibutyltins are extensively used as heat and light stabilizers for poly(vinyl chloride) plastic products. The present study was conducted to evaluate the adverse effects of dibutyltin on initiation and maintenance of pregnancy after administration during early pregnancy in rats. Following successful mating, female rats were given DBTCl by gastric intubation at 0,3.8,7.6, or 15.2 mg/kg on days 0-3 or days 4-7 of pregnancy (sperm day = day 0 of pregnancy). Female rats were sacrificed on day 20 of pregnancy and pregnancy outcome was determined. In females successfully mated, DBTCl caused complete failure in implantation, which was evidenced by total absence of any implantation site, and the pregnancy rate was significantly decreased after administration of DBTCl on days 0-3 at 7.6 and 15.2 mg/kg. The incidence of postimplantation embryonic loss per litter in the groups give DBTCl on days 0-3 were comparable to those in the control group. No decrease in the pregnancy rate was found after administration of DBTCl on days 4-7 of pregnancy. The incidence of postimplantation embryonic loss per litter was significantly increased after administration of DBTCl on days 4-7 at 3.8 mg/kg and above. It could be concluded that DBTCl causes pre-and postimplantation embryonic loss when administered during early pregnancy.

1363 ALTERATION OF PALATAL CYCLIC AMP SIGNALING COMPONENTS BY SECALONIC ACID D.

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Cyclic AMP (cAMP), known to play an important role during normal palate development, relays its signals via protein kinase A (PKA) and the transcription factors such as cAMP response element binding protein (CREB) and modulator (CREM). Secalonic acid D (SAD), a cleft-palate-causing mycotoxin, is known to affect palatal cAMP levels. In the present study, the effect of SAD on the downstream components of cAMP-PKA pathway was investigated. Western blots of palate samples collected from control and SAD-treated mice showed that, despite no effects on CREB, level of phosphorylated CREB (pCREB) increased soon after SAD treatment (GD 12) but was reduced below that of controls later (GD 14). Electrophoretic mobility shift assays using nuclear extracts from the control and SAD treated tissues and [32P] labeled consensus oligonucleotide of CRE (cAMP response element) revealed similar level of the complex formed between CRE and CREB/CREM. Interestingly, the gel supershift assays with the antibodies against CREB and CREM, showed that the proportion of CREM binding to CRE increased whereas that of CREB decreased at the earlier time point following SAD treatment. This correlated well with an increase in CREM protein following SAD treatment. These results suggest that early increase in pCREB by SAD leads to induction of CREM which results in increased CREM binding to CRE. Since CREM is a repressor protein, these results indicate that SAD may downregulate genes involved in cell proliferation. (Supported by NIH grant # DE/OD 11822.)

1364 EFFECTS OF LINURON ON ANDROGEN-DEPENDENT REPRODUCTIVE DEVELOPMENT IN THE MALE SPRAGUE-DAWLEY RAT.

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Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) is an herbicide known to block androgen action in the male rat. Studies were undertaken to characterize the ability of linuron to activate transcription through the androgen receptor (AR) *in vitro* and to determine whether *in utero* exposure to linuron alters androgen-dependent male rat reproductive development. In the *in vitro* HepG2 system, linuron did not stimulate transcriptional activity of the AR in the absence of dihydrotestosterone (DHT). However, linuron competitively antagonized DHT-induced transcriptional activity of the AR in a dose-responsive manner with an IC_{50} of 15μM. Pregnant rats were given linuron by gavage at 0, 12.5, 25, or 50 mg/kg/day (n=11/group) from gestation day 12 to 21. Anogenital distance, birth weight, and pup weight gain were unaffected. Male thoracic areola and/or nipple retention was observed in 11/58 rats (5/10 litters), 19/57 rats (6/10 litters), 27/69 rats (9/11 litters), and 32/44 rats (8/8 litters) in the 0, 12.5, 25, and 50 mg/kg/day dose groups, respectively. Prenatal linuron exposure did not affect preputial separation, testicular descent, or gross morphology of the external genitalia. Reproductive organ weights and hormone levels were unaltered in the adult male offspring. However, reproductive tract lesions were observed in all linuron-exposed groups. Atrophic testes in adult offspring were seen in 2/56 rats (2/10 litters), 8/69 rats (4/11 litters), and 5/44 rats (3/8 litters), while atrophic epididymides occurred in 1/56 rats (1/10 litters), 8/69 rats (4/11 litters), and 2/44 rats (1/8 litters), in the 12.5, 25, and 50 mg/kg/day dose groups, respectively. Partial or absent epididymides and vasa deferentia were observed in 3/44 rats (2/8 litters) only in the 50 mg/kg/day dose group. The primary histologic lesion in grossly abnormal testes was germ cell degeneration with spermatogenic arrest. These data demonstrate that *in utero* exposure to linuron results in testicular toxicity and malformations of the epididymides and vasa deferentia, indicating that linuron selectively impairs testosterone-, rather than DHT-mediated, reproductive development. These findings are distinctly different from the effects induced by flutamide, an AR antagonist that shares structural similarities with linuron.

1365 DEVELOPMENTAL TOXICITY OF OCTYLTIN STABILIZER IN MICE.

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The Octyltin stabilizer ZK 30.434 is a mixture of 80% dioctyltin diisooctylthioglycolate (DOTTG) and 20% of monooctyltin triiosooctyl thioglycolate (MOTTG) which is used as stabilizer for rigid polyvinylchloride (PVC) materials. One of the application of such stabilized films is the packaging of foodstuffs. Exposure to humans occur via migration of DOTTG/MOTTG from PVC materials. The purpose of the study was to investigate the development toxicity of the mixture DOTTG/MOTTG and determine the no-observed adverse effect level (NOAEL) dose. For this purpose 2 developmental toxicity studies were conducted using different doses. In the first study pregnant mice were treated orally with 0, 20, 30 or 45mg DOTTG/MOTTG/kg b/w. In the second study the pregnant mice recieved 0, 67 and 100mg DOTTG/MOTTG/kg b/w. The control groups received oral daily doses of 5 ml Arachis/kg b/w. by gavage for the entire treatment period. All the animals were treated daily on days 6-17 of gestation and sacrificed on day 18 of gestation. The number of implantation sites and viable fetuses showed no substance-related effects. The resorption rates (resorption/implantation) were significantly increased and fetal weight significantly reduced in the 2 higher dose groups. External anomalies including bent forelimbs, cleft palate and exencephaly were found in fetuses treated with 100mg DOTTG/MOTTG/kg b/w. A significant increase in cleft palate, however, was also found in the fetuses of 67mg/kg dose group when compared to the control fetuses. Moreover, in the 2 higher doses a dose-dependent increase in skeletal anomalies was observed. A part from a significant increase in supernumerary lumbar ribs observed at the dose level of 20mg/kg b.w., no other developmental effects were reported on the fetuses treated with low doses of DOTTG/MOTTG. It can be concluded that DOTTG/MOTTG is embryo-fetotoxic and induces developmental effects. The highest dose used (100mg/kg) is maternally toxic.

1366 DEVELOPMENTAL TOXICITY IN RATS TREATED ORALLY WITH 2-(2-IODOETHYL)-1,3-PROPANEDIOL DIACETATE.

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The transport of new chemicals around the European Union must be preceded by their registration with the Competent Authorities, a process which involves both hazard and risk assessment. In order to comply with this Notification of New Substances directive, the research chemical 2-(2-iodoethyl)-1,3-propanediol diacetate (CAS RN 127047-77-2) was examined in, among other tests, a 28-day rat oral toxicity study at doses of 15, 150 and

350 mg/kg/day. The male rats receiving 350 mg/kg/day of this material exhibited specific organ toxicity in the form of decreased testis weight and moderate seminiferous tubular atrophy. A dose of 150 mg/kg/day had no effect on the testes. A reproductive toxicity study was therefore undertaken to determine whether the tubule degeneration was of sufficient magnitude to impact on male fertility. To maximise the amount of information available from the additional study, an assessment of female fertility and general reproductive performance was performed and the offspring were examined for gross abnormalities. Male fertility was not influenced by oral treatment at doses up to 350 mg/kg/day, in spite of the re-appearance of a similar degree of degeneration of the seminiferous tubules; and female fertility was also unaffected at this dose. However, many of the offspring born from females treated at doses of 150 and 350 mg/kg/day died by Day 1 post partum and in 13 of the decedent offspring there was a treatment-related abnormality in the form of disruption of palatal fusion and mineralisation. The 13 affected offspring also showed delayed respiratory epithelial development. This abnormality was distinct from the typical cleft palate which occurs spontaneously and was in fact seen in three offspring from the control rats in the study. Based on these findings, 2-(2-iodoethyl)-1,3-propanediol diacetate was classified as a rat developmental toxin and, in the present absence of an understanding of the mechanism of this effect, a potential human developmental toxin.

1367 SUBCRONIC AND DEVELOPMENTAL TOXICITY STUDIES OF *N*-BUTYL PROPIONATE VAPOR IN RATS.

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Two inhalation studies ere conducted to evaluate the possible subchronic and developmental toxic effects of n-butyl propionate (NBP). In the subchronic study, Sprague-Dawley rats (15/sex)were exposed to 0, 250, 750, or 1500 ppm vapor for 6hr/day, 5 days/wk for 13 wks. Five of the rats/sex/group were held after the final exposure for an 8 week recovery period. Clinical signs, body weights, food consumption, hematology and clinical chemistry evaluations were conducted. At the end of exposure and recovery periods, necropsies were preformed, organs weighed, and tissues processed for microscopic examination. Exposure did not produce deaths or adversely affect clinical signs, hematology, clinical chemistries, organ weights, or the histology of major visceral organs. The only systemic toxic effects were decreases in body weight, body weight gain, and feed consumption in the 1500 ppm group rats. Morphologic changes were limited to the nasal cavity as evidenced by a concentration-related increased incidence and severity of olfactory epithelium degeneration in rats of the 750 and 1500 ppm groups. Both the systemic and nasal cavity efects appeared reversible after exposure ceased. In the developmental toxicity study, pregnant Sprague-Dawley rats (24/group) were exposed to 0, 500, 1000, or 2000 ppm vapor for 6 hr/day on gestation days (gd) 6-15 and euthanized on gd 20. All exposed dams exhibited reductions in body weight, body weight gain, and feed consumption. Intrauterine growth and survival were equivalent across all groups and there were no treatment-related developmental or teratogenic effects. The NOAELs determined for NBP were 250 ppm for subchronic toxicity (based on the olfactory epithelium degeneration) and 2000 ppm for developmental toxicity (no developmental effects at highest exposure level tested). Under the conditions of the latter study, a NOAEL was not determined for maternal toxicity.

1368 DEVELOPMENT TOXICITY OF 3-NITROBENZOIC ACID (NBA) IN WISTAR RATS.

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Pregant Wistar rats were exposed to NBA by whole body inhalation at concentrations of 0, 0.4, 1.0 or 2.9 mg/m3 for 4 hr/day 7 days/week throughout gestation. In pregnant dams exposed to NBA at the concentration of 2.9 mg/m3, a significant increase in the level of aspartate aminotransferase and alanine aminotransferase in blood and a significant increase of the liver lipid peroxidation were observed. Relative liver weights of pregnant dams were significantly decreased at concentrations of 1.0 and 2.9 mg/m3. NBA treatment significantly increased embryolethality at the concentrations of 1.0 and 2.9 mg/m3. The number of metacarpal bones and the number of ribs were significantly decreased at the same concentrations. Upper and lower incisor eruption was significantly delayed in the 2.9 mg/m3 experimental offspring group. A non-significant delay in eye opening and hair appearance were observed also at the maximal tested concentration. A dose-dependent retardation of the body weight was more evident in female progeny. Offspring prenatally exposed to NBA at the concentrations of 1 and 2.9 showed significant changes in behavior in the open field test. The relative weights of the pups' liver, heart, lungs and adrenal gland were significantly decreased in the 1.0 and 2.9 mg/m3 experimental groups. Thus, the LOELs (Lowest Observed Effect Level) and NOELs (No Observed Effect Level) of NBA were 1 and 0.4 mg/m3 both for the maternal and developmental toxicity, respectively.

1369 ASSESSMENT OF THE TERATOGENICITY OF TRIVALENT AND HEXAVALENT CHROMIUM COMPOUNDS IN FEMALE RABBITS.

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The exposure of pregnant female rabbits to trivalent (chromium chloride) or hexavalent (potassium dichromate) chromium compounds in drinking water (500ppm) during the organogensis period (6-18th day of gestation) revealed embryotoxic - and fetotoxic effects.Both trivalent and hexavalent compounds induced dwarfism, kinky and short tail and a significant reduction in the number of implantation sites and in the number of viable fetuses. The number of females with resorption was significantly increased in the hexavalent-exposed group.Visceral abnormalities in the form of lung hypoplasia, heart hypertrophy, intrathoracic hemorhage and dilated nares and brain lateral ventricles were found in the fetuses of both chromium (III, VI) treated mothers. Skeletal anomalies (reduced number of sternal and caudal bones) were also recorded in both chromium groups. Furthermore, reduced ossification in parietal and inerparietal bones was significantly increased in the hexavalent chromium exposed females. Chromium levels in blood, placenta and fetuses significantly increased in both chromium groups. In conclusion, the exposure of pregnant rabbits to trivalent and hexavalent chromium compounds would have adverse effects on the embryonic and fetal development.

1370 BIOLOGICALLY-BASED DOSE-RESPONSE MODEL FOR METHYL MERCURY DEVELOPMENTAL TOXICITY INCORPORATING NOVEL *IN VIVO* CELL CYCLING DATA.

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We are developing a biologically-based dose response model that describes the developmental toxicity of methyl mercury (MeHg) in the developing embryonic rat brain. Key components of the model include biologically-derived rate constants for proliferation of neuroepithelial cells. We are using 5-bromo-2'-deoxyuridine (BrdU) DNA labeling *in vivo* and flow cytometry to obtain these parameters. We found that in the untreated animal, the fraction of cycling midbrain cells declines from 73 (±4) percent on gestational day (gd) 12 to 10 (±4) percent on gd 16. The estimated cell cycle time increases from 16 hours on gd 12 to 20 hours on gd 16, while the estimated S phase duration increases from 8 hours on gd 12 to 10 hours on gd 16. Similar values were obtained *in vitro* for rat midbrain cells cultured from gd 12 to gd 14 (Ponce et al., 1994). We are now evaluating the effects of MeHg on cell cycle kinetics *in vivo*. Preliminary data indicate that dosing on gd12 to achieve a gd14 brain concentration of approximately 3 μg/g, results in a significant increase in the fraction of BrdU labeled cells (labeling time=1.5 hr), suggestive of a slowing of S-phase progression. We are now collecting data on the effect of MeHg on the overall fraction of cycling cells and the cell cycle time. This data will be incorporated into our model and compared to fetal midbrain cell number data being collected in parallel. This will allow us to describe the effect of MeHg on cell cycle progression *in vivo* and its effects on neural development. We will also assess the utility of *in vitro*-derived data for modeling, thus improving our knowledge of the uncertainty associated with *in vitro* to *in vivo* extrapolation. (Supported by NIEHS T32ESO-7032, USEPA CR825173, USEPA R825358 and USEPA/NIEHS R826886.)

1371 DEVELOPMENTAL TOXICITY OF LEAD IN SMOOTH MUSCLE.

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Exposure to heavy metals has been shown to permanently alter the growth state of smooth muscle cells. Prior studies in our lab have characterized an *in vitro* model of normal smooth muscle differentiation based upon differential isoactin gene expression. In an effort to determine what effect lead exposure

has on normal intestinal smooth muscle cell (ISMC) differentiation, primary cultures of ISMC's were grown in the presence of lead at a concentration corresponding to 13.75 micrograms/deciliter blood lead level. Morphologic analysis showed that prolonged lead exposure has the potential to disorganize/disrupt the highly characterized "hills and valley" phenotype routinely observed in cultured ISMCs. Northern blot analysis using the γ-smooth muscle, α-smooth muscle, β-cytoplasmic, and γ-cytoplasmic isoactin-specific cDNAs indicated that cultured ISMCs grown in the presence of lead displayed dramatic alterations in isoactin gene expression versus control cells. Lead-exposed ISMC's possessed a γ-smooth muscle:alpha smooth muscle isoactin ratio of 0.19±0.08. This smooth muscle isoactin ratio does not correspond to any of the previously characterized ISMC phenotype-specific smooth muscle isoactin ratios. Rather, lead-exposed ISMCs display a unique smooth muscle isoactin ratio suggesting a possible altered cellular phenotype that is intermediate to those previously described. These studies demonstrate that lead exposure has a profound effect of ISMC differentiation *in vitro* providing a basis for future studies designed to identify the complex transcriptional endpoints that are susceptible to heavy metal-induced developmental toxicity.

1372 TERATOGENICITY SCREENING OF ANTISENSE PHOSPHORODIAMIDATE MORPHOLINO OLIGOMERS IN ZEBRAFISH EMBRYO MODEL.

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Antisense technology holds great promise for therapeutic applications. Phosphorodiamidate morpholino oligomers (PMOs) are a new class of antisense agents that inhibit gene expression by hybrid arrest of translation. We examined the teratogenic potential of antisense PMOs in zebrafish embryogenesis model. AVI-4126, a 20-mer PMO targeted to c-myc, was used for these studies. Zebrafish have become a popular model for studies of vertebrate development and toxicology. They grow rapidly, reach sexual maturity in approximately three months and produce transparent embryos that complete development in 96 hr. Blastula stage zebrafish embryos were exposed to different concentrations (0.1-100 μM) of AVI-4126 and were morphologically evaluated for any developmental abnormality and subsequent lethality. Uptake of PMO in embryos was studied by fluorescent microscopy and histology. We observed fluorescence in a wide variety of embryonic tissues including muscle and cartilage structures. The zebrafish embryos (up to N=60 per dose group) treated with AVI-4126 during blastula stage of development did not show any teratogenic effect at any dose. Information obtained from such studies will be useful for further assessment of teratogenic potential of these antisense agents in more complicated whole animal models.

1373 A DEVELOPMENTAL NEUROTOXICITY STUDY OF PROPYLTHIOURACIL IN RATS.

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In humans, hypothyroidism causes irreversible mental retardation and various neuromotor disabilities. Propylthiouracil (PTU) administration to pregnant rats is a commonly used experimental model of human congenital hypothyroidism. The objective of this study was to evaluate the sensitivity of the experimental design described by U.S. EPA OPPTS Guideline 870.6300 (Developmental Neurotoxicity) to predict the adverse effects in rats of a known human developmental neurotoxicant. Propylthiouracil was administered at dosage levels of 3.8, 19 and 38 mg/kg/day by oral gavage once daily from gestation day 6 through lactation day 10. Offspring were evaluated by use of an observational battery and evaluated for motor activity, acoustic startle response and learning and memory in the Biel water maze. Live litter size was reduced in the 38 mg/kg/day group. Pup viability from birth to PND 4 was reduced in the 19 and 38 mg/kg/day groups. Pup weights were reduced by 10% at birth for all dosage levels, and remained reduced for the duration of the study. The observational battery demonstrated delays in pupillary response, startle reactivity, mobility and neuromuscular development on postanal day (PND) 20. Neuromuscular measures continued to be impaired at the PND 35 and 45 observations. Motor activity was not affected. Acoustic startle response was reduced at all dosage levels when evaluated on PND 22. When evaluated on PND 60, an augmented acoustic startle response was noted at all dosage levels. Latency to escape the Biel maze was increased for all dosage levels at the PND 22 evaluation; however, swimming ability was markedly reduced for all treatment groups. When assessed at PND 62 swimming ability was comparable between all groups. However, time to escape the Biel maze was increased for males and females at all dosage levels by approximately 60%; there was no dose response relationship. The experimental design used in this study is sensitive to the effects of PTU, a known developmental neurotoxicant.

1374 BRAIN MORPHOMETRY IN A DEVELOPMENTAL NEUROTOXICITY STUDY OF PROPYLTHIOURACIL.

A. E. Radovsky, J. T. Yarrington, G. D. Cappon, K. S. Regan and R. E. Wilson. *WIL Research Laboratories, Inc., Ashland, OH.* Sponsor: D. G. Stump.

Morphometric evaluation of the brains of postnatal day 11 rat pups from an oral gavage study of the developmental neurotoxicity of propylthiouracil in Sprague Dawley Crl:CD®(SD) IGS BR rats was done in accordance with OPPTS 870.6300. Linear measurements were made of 35 regions from 5 levels of the brain of 6 pups/sex from the vehicle (0.5% methylcellulose) control group and from the high dose group whose mothers had been given 38 mg/kg/day of propylthiouracil from gestation day 6 through lactation day 10, inclusively. Four coronal sections were made that included the cerebral cortex, basal ganglia, hippocampus, and the thalamus and midbrain. These levels corresponded to Plates 11, 14, 21, and 26; and the midsagittally-sectioned cerebellum and pons corresponded to Plate 44, of The Rat Brain by Paxinos & Watson (1982). Systematic computer-assisted (PAX-It, MIS, Inc., Franklin Park, IL) measurements were made from images acquired with an Optronics model S6067 digital camera at total magnifications of 10X, 16X, 25X, or 40X. Measurements included the lengths and widths of the most rostral hemisphere, the thalamus, and the cerebellum; the widths of the caudoputamen, fornix, and midbrain; and the thickness of the entire hippocampal formation; the thickness of the cortex at 12 defined sites; the hippocampal formation (height and length of the dentate hilus and of layers of pyramidal neurons); and specific cell layers (the external and internal granular cell layers of lobule 9 of the cerebellum; and the marginal zone and the most superficial neuronal layer of the cingulate cortex at the midline). Measurements that had more than 20% standard deviation in the control group were considered unreliable. A t-test gave a p-value of less than .05 for measurement means of the decreased thickness of the frontoparietal cortex in the brains of exposed male and female rat pups; the increased distance between the layers of pyramidal neurons of the hippocampus in exposed male pups; and the increased height of the dentate hilus and decreased thicknesses of the base of cerebellar lobule 9 and the pons in exposed female pups in comparison to the measurement means of controls. A subset of the 35 measurements was selected as sensitive and reliable for brain morphometry in developmental neurotoxicity studies.

1375 VALIDATION OF TEST PROCEDURES FOR A DEVELOPMENTAL NEUROTOXICITY SCREEN USING METHIMAZOLE.

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The U.S. EPA guideline for a developmental neurotoxicity study (OPPTS 870.6300) includes a standardized test battery (e.g., developmental landmarks, automated motor activity, auditory startle habituation, tests of associative learning and memory, and neuropathology, including brain measurements) that are administered at specific ages. This guideline requires that labs validate their test procedures by establishing test norms for the appropriate age groups, demonstrating competence in evaluating effects in neonatal animals perinatally exposed to chemicals, and providing positive control data that demonstrate the sensitivity of the procedures being used. The antithyroid drug methimazole has been recommended for use as a positive control for such a developmental test battery (Comer and Norton, 1982). This paper discusses the results of a study with methimazole that was conducted in accordance with these guideline requirements and discusses how those results contribute toward satisfying the aforementioned requirements. In this study, methimazole was administered in the drinking water (0 or 0.1 mg/ml) to pregnant female Wistar rats (25/dose group) on gestation day 16 through lactation day 10 (Comer and Norton, 1982). The offspring were then tested in accordance with OPPTS 870.6300, with additional measurements of circulating [T3 and T4] and thyroid morphology. This treatment produced a number of effects in male and female offspring, including an average 16% and 84% decrease in blood [T3] and [T4], respectively, a modest decrease in body weight, developmental delays, including delayed preputial separation and vaginal patency, effects on motor activity and startle response amplitude, and reductions in brain measurements. These results support the use of this treatment in validating the test procedures in this developmental test battery.

1376 EFFECTS OF 5-AZA-2-DEOXYCYTIDINE (D-AZA) ON REPRODUCTIVE CAPACITY AND POST-NATAL DEVELOPMENT OF CD-1 MICE.

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It has been reported that d-AZA induces DNA hypomethylation and can therefore alter gene expression. The embryonic developmental toxicity of d-AZA may be mediated by this mechanism. In addition, body weight gain and post-natal development is gene dependant. It has been shown that here is a correlation between reproductive ability and body weight. The objective of this study was to evaluate the effects of 5-aza-2'-deoxycytidine (d-AZA) on reproductive capacity and post-natal development of CD-1 mice. Pregnant CD-1 mice were administered 1.0 mg/kg of d-AZA at gestation day (GD) 10. Controls were left untreated. The mice were allowed to give birth. The body weights of the resulting F1 control and treated pups were recorded at three different times points (3, 5 and 6 months of age). To evaluate the reproductive capacity, 5 month old treated F1 males and females were mated with normal females and males (Groups 1 and 2, respectively). The control group consisted of mated control males and females (Group 3). Mating pairs were housed separately for four days and the presence of plugs were recorded (plug day designated as GD 0). At gestational day 17, pregnant females were killed. Dam, uterine, and litter weights were recorded. Fecundity and teratological data were also recorded. Our data suggest that body weight differences between treated and control parents are statistically significant and were more pronounced with increased age ($p>0.01$). There was a significant difference ($p>0.01$) in number of plugs and pregnancy rates between each group compared to controls. Ten percent of group 1 females had plugs, but no pregnancies were observed. 100% of group 2 females had plugs resulting in a 75% of pregnancy rate. Forty-five percent of group 3 females presented plugs resulting in a 18.18% pregnancy rate. The reproductive data obtained suggests that reproductive capacity of d-AZA treated males is more adversely affected than that of treated females. Detailed reproductive analyses are ongoing to better characterize the male reproductive toxicity of d-AZA.

1377 TOMUDEX-INDUCED MAMMALIAN DEVELOPMENTAL TOXICITY IS PREVENTED BY CO-TREATMENT WITH FOLINIC ACID.

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The thymidylate synthase (TS) inhibitor, Tomudex (TM), is a structural analogue of folate. TS inhibition has been shown to result in impaired embryonic development. We evaluated the developmental toxicity of TM in a rodent whole embryo culture (WEC) system and tested the ability of folinic acid to rescue embryos from TM toxicity. Gestational day (GD) 9.5 rat embryos were cultured for 48 hours in the presence of 0, 50, 100, 150, 250, 350 or 500 ng TM/4ml culture medium(CM). Following culture, embryos were evaluated for growth and development. Embryos exposed to ≥150 ng TM had a concentration dependent increase in incidence of open neural tubes, rotational defects, eye defects and abnormal caudal appendages, and a concentration dependent decrease in developmental score. Significant embryo lethality was observed at concentrations ≥ 350 ng TM /4ml CM. Additional embryos were cultured with 0-1100 ng TM/4ml CM and co-treated with 10 μg folinic acid/4ml CM. At the end of 48 hours, embryos were evaluated as above. All TM exposed embryos co-treated with folinic acid developed similarly to control embryos. Folinic acid co-treatment completely prevented the occurrence of malformations resulting from exposure to TM. Folinic acid alone (10 μg folinic acid /4ml CM) had no impact on embryonic development. Preliminary studies have been conducted on GD 9.5 embryos cultured with 0-950 ng TM/4ml CM for 2 hours and then transferred to CM with no TM. At the end of 48 hours of culture, TM exposed embryos were evaluated and found to be indistinguishable from control embryos suggesting that embryos can recover from short term exposure to as much as 950 ng TM/4ml CM. These data suggest that TM is developmentally toxic to the midgestational embryo and that folinic acid can competitively inhibit TM toxicity.

1378 DEVELOPMENTAL TOXICITY OF AMINOPTERIN IN *DROSOPHILA*.

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To further characterize the *Drosophila*-based prescreen to detect developmental toxicants, aminopterin (4-aminofolic acid [APN]; CAS No. 54-62-6), was studied. APN, a documented developmental toxicant and teratogen, was formerly used clinically as an antineoplastic agent. APN inhibits dihydrofolate reductase preventing the formation of folinic acid and stopping one carbon metabolism needed for the synthesis of DNA in rapidly dividing cells. Initially, 7 APN concentrations ranging from 4 - 80 μg/vial were evaluated. In a second experiment, 6 concentrations ranging from 0.01 - 2 μg/vial were utilized to determine if a threshold of developmental toxicity could be observed. Each experiment included a concurrent control. *Drosophila* were exposed throughout development (egg through third instar larva) in culture vials to medium containing APN. Each vial contained 1g of powdered medium and 5ml of distilled deionized water or a solution of test chemical in water. A mated, untreated, Oregon-R wild-type female (Mid-American *Drosophila* Stock Center, Bowling Green State University, Ohio) 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 controls using chi-square. The incidence of wing blade notches was significantly increased (all $p< 0.001$) at 2 μg/vial, 17/138; 4 μg/vial, 20/170; 10 μg/vial, 25/96; 14.1 μg/vial, 9/81; 20 μg/vial 13/82; 28.3 μg/vial 9/43; 40 μg/vial 18/61; and at 80 μg/vial, 10/25. One wing blade notch was observed among 414 control flies. Bristle defects were not increased by APN. Mortality of the offspring was increased and the first day of emergence was delayed at the two highest APN concentrations. The results with APN parallel the developmental toxicity reported in mammals and replicate and expand published *Drosophila* data. These findings provide additional support for increased utilization of this assay as a prescreen for the detection of developmental toxicants.

1379 AMPHETAMINE-STIMULATED DOPAMINE (DA) RELEASE IN F_1 AND F_2 MALE RATS EXPOSED TO THE ESTROGENIC COMPOUND, GENISTEIN.

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Developmental steroid hormone treatment causes adverse effects; however, the potential effects of developmental exposure to endocrine disruptors such as the phytoestrogen genistein are relatively unknown. Because estradiol affects striatal DA release, it is plausible that genistein might have similar effects. Here, Sprague-Dawley rats (F_0 generation) were exposed to 0, 100, or 500 ppm genistein in a soy-free diet. Genistein treatment continued throughout the F_1 and F_2 generations. Between postnatal days (PND) 100-175, striatal microdialysis samples (DA, DOPAC, HVA, and 5-HIAA) were collected from awake males of the F_1 (n=10 for 0 ppm; n=9 for 500 ppm) and F_2 (n=7 for 0 ppm; n=4 for 100 ppm; n=7 for 500 ppm) generations for 2 hr at 20 min intervals. After the baseline period, 2 mg/kg d-amphetamine was injected ip and sample collection continued for the subsequent 3.7 hr. There were no statistically significant effects of genistein treatment on any measure. Results indicated that all rats responded similarly to amphetamine with increased DA levels, decreased DOPAC and HVA levels and only minor changes in 5-HIAA levels. These results would indicate that developmental/chronic genistein exposure has few effects on striatal DA release. (Supported by Interagency Agreement #224-93-001 between the FDA and the NIEHS.)

1380 TERATOGENIC EFFECTS OF VALPROIC ACID IN THE FETAX SYSTEM: SIMILARITIES TO EFFECTS OBSERVED IN HUMANS.

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The vast number of new chemicals and pharmaceuticals which requiring safety evaluation and teratogenicity testing has lead to renewed interest from regulatory and industrial communities in the development and licensing of alternative *in vitro* teratogenesis assays. The FETAX assay has a number of advantages to offer in this respect. It is a high-throughput, rapid assay system (96h), allowing large numbers of samples to be screened for morphological, histological and biochemical effects. The assay in-corporates all the major stages of embryonic development. Since development occurs ex utero very specific interventions are possible. The relevance of the assay with regard to the detection of compounds teratogenic to mammals, specifically humans, may, however, be questioned due to species differences. Therefore, further investigation of this testing system requires using several known mammalian teratogens and non-teratogens in addition to the comparison of the results with published human epidemio-logical studies. One good candidate for such an investiga-

tion is valproic acid (VPA), a well characterized human teratogen inducing spina bifida as well as craniofacial and cardiac malformations in humans and laboratory species following *in utero* exposure of the embryos. Following standard range-finding experiments, stage 8-11 X enopus laevis embryos were selected and exposed to mM concentrations of VPA (corresponding to serum therapeutic VPA levels in epilepsy treatment) for 96h, i.e., in accordance with the ASTM standard static renewal protocol. Embryos were fixed and mortality, malformation and growth inhibition were assessed. Dose-response curves for mortality and malformation were steep. LC50 and LC100 values of 0.27 mM and 0.4 mM respectively were noted and VPA-exposed embryos displayed a specific and reproducible craniofacial narrowing (EC50 0.15 mM). Embryo death at concentrations of *0.3 mM was preceded by axial malformations and pronounced cardiac edema. Embryos surviving exposure to 0.5 mM VPA displayed severe notochord anomalies, cardiac edema and microcephaly, which closely mimic the malformations characteristic in human valproate syndrome.

1381 ASSESSMENT OF THE EFFECTS OF PEGVISOMANT ADMINISTERED SUBCUTANEOUSLY ON EMBRYO-FETAL DEVELOPMENT, FERTILITY AND IGF-I CONCENTRATIONS IN RABBITS.

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Pegvisomant is an injectable recombinant human growth hormone receptor antagonist for treatment of acromegaly. Pegvisomant reversibly binds to the human growth hormone receptor leading to a block of signal transduction and to a reduction in circulating insulin-like growth factor I (IGF-I). In a preliminary study, nonpregnant rabbits were administered subcutaneous (sc) injections of 3 mg/kg/day pegvisomant for three consecutive days. Two hours following the third dose, the mean serum IGF-I concentration was reduced 55% relative to the control group value. In a dose range-finding study in pregnant rabbits administered sc injections of pegvisomant at 0.3, 1, 3 and 10 mg/kg/day from gestation days (GD) 7-20, the mean serum IGF-I concentration in the 10 mg/kg/day group was reduced by 48% compared to the control group value two hours following dosing on GD 20, and mean serum growth hormone concentrations were increased 71 and 830% in the 3 and 10 mg/kg/day groups, respectively. These results demonstrated that pegvisomant is able to produce a pharmacologic effect in rabbits at 3 and 10 mg/kg/day. In a definitive embryo-fetal development study in rabbits administered pegvisomant sc at 1, 3 and 10 mg/kg/day from GD 7-20, no maternal or developmental toxicity was observed. In a study of the effects of pegvisomant on early embryonic development in rabbits following sc injection at 1, 3 and 10 mg/kg/day from GD 0-7, postimplantation loss was increased in the 10 mg/kg/day group (33.0% compared to 21.3% in the control group). No other embryo toxicity and no maternal toxicity were observed. Because the therapeutic intent of pegvisomant is to return IGF-I concentrations to normal in individuals with elevated IGF-I concentrations, the failure to sustain pregnancy in the 10 mg/kg/day group at a dose that has been demonstrated to reduce rabbit IGF-I concentrations below normal may not be indicative of results in humans under therapeutic conditions. To our knowledge, this is the first report of IGF-I concentrations in pregnant rabbits.

1382 STREPTOZOCIN TREATED DAMS:F_1 GESTATIONAL THROUGH POST-LACTATIONAL EXPOSURE TO CADMIUM CHLORIDE.

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Depending on the route and duration of exposure, cadmium can induce toxicity in several tissues including the lung, liver, kidney and gonads. Mature female nulliparous rats were exposed to $CdCl_2$ in drinking water two weeks after a single IP injection of streptozocin (SZ), and bred to non-treated males. Cadmium exposure in the F_1 pups, continued through post-lactation to postnatal day (PND) 45. At PND 45 F_1 animals were euthanized, plasma and selected organs were collected weighed and quickly frozen or placed in formalin for histological analysis. Cadmium chloride at 200μM significantly reduced the body weight and spleen weight of pups from SZ treated dams. At 100 μM $CdCl_2$ increased the heart weight and spleen while it decreased weight of the pancreas of pups of SZ treated dams. The lung, kidney, and testis weights of pups in this treatment group were similar to the control. Only the weight of the pancreas of pups from SZ-alone treated dams was found to be significantly less than the control. The data indicate that gestational, lactational and post-lactational exposure to the diabetogenic streptozocin and cadmium results in signs of general toxicity at PND 45. Further evaluation is required to determine the long-term reproductive and developmental implication at the cellular and molecular level.

1383 GENETIC DIFFERENCES IN SENSITIVITY TO ETHANOL TERATOGENESIS IN ZEBRA FISH.

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Fetal Alcohol Syndrome (FAS) is a constellation of congenital anomalies seen in some newborns of alcoholic women that is characterized by prenatal and postnatal failure-to-thrive, central nervous system disorders, and a distinctive set of patterning defects that affect the cardiovascular system, facial structures and limbs. Data from clinical twin studies and animal models argue strongly for a robust genetic component to FAS. The long-range goal of this project is to elucidate the molecular mechanisms by which ethanol perturbs embryonic and fetal development and to identify genes that play a role in sensitivity to ethanol-induced teratogenesis. We first examined ethanol teratogenesis by exposing eight genetically-isolated lines of zebrafish to a series of ethanol concentrations. Indeed, these lines displayed striking variability in their sensitivity to the embryolethal effects of ethanol, the LD50 ranging between 17 and 240 mM. We also analyzed ethanol-induced growth retardation effects by measuring embryo length 6 days post-fertilization; ethanol delayed the growth in both the sensitive and resistant lines, but with a greater reduction of mean length in the sensitive lines. These data are consistent with a genetic basis for ethanol sensitivity. These preliminary results also suggest that the zebrafish, which develops outside the mother, might offer an excellent model system for studying directly the contribution of fetal genes to ethanol-induced teratogenesis. The zebrafish vertebrate model system has proven to be very powerful for the purpose of identifying genes that play a role in specific physiological and developmental events. Comparing ethanol-sensitive and -resistant zebrafish will elucidate the genetic and molecular mechanisms behind the sensitivity of vertebrate embryos to alcohol toxicity, and may apply directly to alcohol sensitivity in humans. (Supported in part by NIH Grants RO1 ES07058 and P30 ES06096.)

1384 COMPARISON OF AUDITORY STARTLE REFLEX AND MOTOR ACTIVITY IN RATS DOSED WITH AMPHETAMINE AND CHLORPROMAZINE.

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As part of the Environmental Protection Agency's developmental neurotoxicity study, Health Effects Test Guidelines OPPTS 870.6300, positive control data are required to demonstrate the sensitivity of the procedure being used. Two of the tests required by this guideline are assessment of motor activity and auditory startle reflex. As part of a positive control validation study, groups of 4 male and 4 female Sprague Dawley rats were injected intraperitoneally (1 ml/kg) with amphetamine (1 mg/kg) or chlorpromazine (6mg/kg). An additional group was dosed with saline (1 ml/kg) to serve as controls. Approximately 30-60 minutes post-dose, each animal's activity was monitored in an Opto-Varimex motor activity box over a fifteen minute testing period. Results showed an increase in animal activity for amphetamine and a decrease for chlorpromazine as expected. The study was repeated with the exception that animals were placed in sound-attenuated chambers to measure habituation of the startle reflex response. Animals were acclimated for 5 minutes and then presented with 20 msec, 120 dB burst of noise at 8 second intervals for 50 trials. The peak amplitude of the response and latency to startle was averaged over 5 blocks of 10 trials. A decrease was noted in the startle amplitude for chlorpromazine, but the latency to startle for chlorpromazine and amphetamine and the startle response for amphetamine were similar to the saline control group. Amphetamine and Chlorpromazine would appear to be suitable positive control agents for motor activity testing, but not appropriate for positive control testing in the startle reflex system.

1385 CHARACTERIZATION OF URINARY METABOLITES OF [1, 2, 3-^{13}C] ACRYLAMIDE IN MALE F-344 RATS FOLLOWING DERMAL APPLICATION OR IP INJECTION.

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Acrylamide(AM) is used in the manufacture of polymers and is a known neurological toxicant. Glycidamide (GA), an epoxide metabolite of AM, is a known mutagen. In this study the metabolism of AM was evaluated following intraperitoneal (ip) and dermal routes of administration. Male F344 rats were administered a 47 mg/kg ip dose or a 138 mg/kg dermal application of [1, 2, 3-^{13}C]AM. Urine was collected for 24 hr. Signals in the ^{13}C -NMR spectra of urine were assigned to previously identified AM-derived metabolites. Assignments included metabolites derived from direct AM conjugation with glutathione (AM-GSH)and metabolites derived following conversion of AM to GA. Following ip administration of [^{13}C]AM to F344 rats, ~62% of the dose was excreted in the urine. AM-GSH were excreted in the highest concentration (~69% of excreted metabolites). Similar results were previously obtained in our laboratory following a 50 mg/kg gavage administration to F344 rats. In both the ip and gavage studies, the remainder of the excreted metabolites (~30%) were derived following conversion of AM to GA. A small fraction of the applied dose was excreted in the urine (~1.5%) following dermal application of [^{13}C]AM, indicating that AM did not rapidly penetrate the skin. For dermal application, the AM-GSH deried metabolites accounted for ~52% of the excreted urinary metabolites, while GA-derived metabolites accounted for the remainder (48%). These data are important for development and validation of physiological-based pharmacokinetic models for AM. (This study was funded by the Acrylamide Monomer Producers Association.)

1386 GESTATIONAL THROUGH POST-LACTATIONAL EXPOSURE OF AMMONIUM PERCHLORATE TO DEER MICE (*PEROMYSCUS MANICULATUS*).

E. H. Roots, K. A. Thuett, B. A. Gentles, R. J. Kendall and E. E. Smith. *Texas Tech University, Lubbock, TX.*

Ammonium Perchlorate (AP) has recently been recognized as a pervasive and lingering water contaminant. AP is known to inhibit iodide uptake in the thyroid. AP's biological effects were evaluated in deer mice, a sentinel wildlife species. Ten mated pairs per dose group were exposed to AP in drinking water (1nM, 1microM, 1mM and control), starting at cohabitation and continuing through post-lactation. The first litters (F1a) were examined for differences in body weight, water consumption, survival indices, time of cohabitation to parturition and litter size. Body weights were observed at postnatal day (PND) 1, 5 and every 5 days thereafter up to PND 70. Body weights on PND 1 were not significantly different. PND 5 were found to differ significantly between the 1mM and control groups. The 1mM treatment group was significantly different from the control for all observation days excluding PND 15. It is interesting to note that body weights appeared dose-dependent. The F1a body weights indicate that growth and development may be affected by AP exposure. There was no significant difference for water consumption or survival indices for postnatal days 1, 5, 10 and 20. The mean number of days for cohabitation to parturition showed a dose-dependent change (control=30 days; treatment groups ranged from 31-38). In addition, while there was 100% conception in the control group, there was a 10% reduction in the treatment groups. These preliminary data indicate that AP dosage does appear to affect impregnation or ability to maintain pregnancy. However, litter size did not show significance among dose groups. Furthermore, in preliminary behavioral observations, it appears that treatment groups exposed to higher concentrations of AP are less active. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the 311 HSW/IERA or the U.S. Government.

1387 DEVELOPMENTAL TOXICITY OF AMMONIUM PERCHLORATE ADMINISTERED ORALLY IN DRINKING WATER TO DEER MICE (*PEROMYSCUS MANICULATUS*).

K. A. Thuett, E. H. Roots, B. A. Gentles, R. J. Kendall and E. E. Smith. *Texas Tech University, Lubbock, TX.*

Ammonium perchlorate (AP) is manufactured for use as an oxidizer in solid fuel propellant for rockets, missiles, and fireworks. Environmental concerns include contamination of surface and ground water, and its ability to affect the thyroid gland, which may affect growth and development. In this study, male and female deer mice (10 pair per treatment group) were given continual access to control, 1nM, 1microM, or 1mM AP dissolved in drinking water, from the first day of cohabitation. Body weights of second litter ($F2_b$) pups were recorded beginning on day 1 and taken every 5 days thereafter. Pups were then weaned and necropsied at post-natal day (PND) 21. Gonads, kidneys, adrenals, liver, and heart were collected and evaluated for indication of general toxicity. Selected organs were fixed and processed for histopathology, which is in progress. Body weights and weights for ovaries, testes, and adrenals showed no significant difference between treatment groups. Kidney ($p=0.0001$), liver ($p=0.0001$), and heart ($p=0.0020$) weights, all showed significant differences between treatment groups. Mean kidney weight (0.1389 g) of the 1mM group was higher than that of the control (0.1209 g), while the 1microM group weight (0.0995 g) was lower than controls. The mean liver weight (0.4775 g) of the 1mM group was higher than that of the control (0.3964 g), while the 1microM group weight (0.3112 g,) was lower than controls. The same pattern occurred for the heart with the 1mM group weight (0.0713 g) being higher, and the 1microM group weight (0.0562 g) being lower than control (0.0704 g). The lack of significance in body weight, in contrast to the significance seen in organ weights, indicates that AP effects may be due to organ-specific developmental toxicity. Based on the preliminary data, further developmental toxicological characterization of *in utero* and lactational exposure to AP is needed to fully determine a dose-dependent response. (This research was supported in part by the U.S. Department of Defense, through the Strategic Environmental Research and Development Program (SERDP) under a Cooperative Agreement with the USAF, Institute for Environment, Safety, and Occupational Health, Brooks AFB, TX.)

1388 POSTNATALLY ADMINISTERED α-DIFLUOROMETHYLORNITHINE (DFMO) HAS MINIMAL EFFECTS ON SPATIAL LEARNING AND MEMORY IN ADULT RATS.

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Postnatal treatment with α-difluoromethylornithine (DFMO), a potent inhibitor of ornithine decarboxylase, has been shown to stunt growth measures such as body and brain weight, eye opening, and fur growth in rats and delay development of complex integrated behaviors such as swimming ontogeny when given from birth to weaning (20-21 days). Shorter courses of treatment (e.g., PND 5-10) affect growth measures to a lesser degree (cerebellar stunting and delay of eye opening) but do not alter various reflex, motor coordination or spontaneous activity indices measured up to postnatal day 28 (Cada et al., in press). In the present study, 29 Sprague-Dawley litters (4 female, 4 male each) were injected subcutaneously with 500 mg/kg DFMO or saline vehicle daily on postnatal days 5-12. Measures of spatial learning and memory were conducted in adulthood: complex maze for water reward on postnatal days 71-75 and Morris water maze on postnatal days 93-96. DFMO treatment did not significantly affect learning or retention in the complex maze. Number of rewards earned increased across the five days of testing in both treated and control groups. In the Morris water maze, males were more accurate and swam faster irrespective of treatment group. DFMO treatment did not affect measures of accuracy, speed, distance or proximity. Improvement across days was seen on latency, path length, and proximity measures for both groups. These results indicate that multiple measures of spatial learning and memory are not affected by early postnatal DFMO treatment.

1389 EVALUATION OF RAT EPIDIDYMAL SPERM MOTION PARAMETERS GENERATED BY THE HAMILTON-THORNE SPERM ANALYZER (IVOS) FOR USE IN TOXICOLOGICAL ASSESSMENT.

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To determine which parameters provide utility in toxicological assessment for male reproductive function, a collaborative working group evaluated epididymal sperm motion using the Hamilton-Thorne Sperm analyzer (IVOS). Male rats were treated with 6 known toxicants, adriamycin (ADM), α-chlorohydrin (ACH), ethylene glycol monoethyl ether (EGEE), 2,5-hexandione (2,5-HD), ornidazole (OZ) and sulfasalazine (SASP). The males were evaluated for body weight, testicular and epididymal weight, epididymal sperm count, sperm motion and fertility outcome. The sperm motion parameters included percentage of motile sperm (total motility), percentage of progressively motile sperm (progressive motility), curvilinear velocity (VCL), average path

velocity (VAP), straight line velocity (VSL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity (LIN) and straightness (STR). Decreases in testicular and epididymal weights were observed in the ADM, EGEE and OZ studies. Impaired fertility of the males treated with ACH, EGEE, SASP and OZ was indicated by decreases in pregnancy index, number of implantation sites and/or number of live fetuses. For all toxicants, abnormal epididymal sperm motion was detected at the lowest observed effect level by the IVOS parameters. Progressive motility was consistently reduced and the most sensitive parameter for all toxicants. Velocity parameters (VAP, VSL, VCL) responded sensitively to abnormal sperm motion for all toxicants except EGEE. In conclusion, among the parameters generated by the IVOS, progressive motility and velocities are considered to be sensitive and valuable parameters for detecting adverse effects on sperm motion and should be considered for inclusion in analyses of sperm function.

1390 MATERNAL IMMUNOSTIMULATION AND PREVENTION OF TERATOGENESIS.

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Activation of the maternal immune system during early gestation via exogenous, non-specific immunostimulants has been reported to protect against certain fetal defects (i.e., resorption, cleft palate, digit and tail anomalies). The mechanism(s) behind such protection remain unclear. In this study, female C57Bl/6 and ICR mice were pre-treated with immunostimulants from two weeks prior to mating up to day 3 of gestation. Urethane, a known teratogen and immunotoxicant, was then administered at various time points during gestation. The maternal thymus and spleen were evaluated for changes in organ weight, cellularity, leukocyte subpopulation differentials, apoptosis, and cell proliferation. These data were then compared to fetal outcome in respective litters. Immune stimulation did not affect the maternal thymic effects of urethane. Further, no changes in maternal thymic or splenic T-cell profiles were detected which correlated with reduced teratology in litters from immunostimulated mothers. Adrenal weights were unchanged for all treatment groups suggesting no significant corticosteroid involvement. Immunostimulation increased the percentage of macrophages in the maternal spleen. (Supported by NIH RO1-ES 09642-01.)

1391 GLUCOSE-DEPENDENT EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS IN EMBRYONIC MOUSE HEART.

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Glucose is critical to the organogenesis-stage embryo and its excess or deficiency interferes with normal development. The early embryonic heart depends on glucose and glycolysis and is particularly sensitive to glucose availability. Elevated glucose produces increased synthesis of extracellular matrix (ECM) proteins in adult cells, including renal and endothelial, and may contribute to diabetic complications. The relationship between glycemic exposure and ECM proteins was therefore examined in the embryonic heart. CD-1 mouse embryos were exposed on gd 9.5 (plug = gd 0.5) for 24 hr in whole-embryo culture (WEC) to 40 mg/dl glucose (hypoglycemia), 150 mg/dl glucose (normoglycemia), or 600 mg/dl glucose (hyperglycemia). Embryos were fixed, embedded in paraffin, and sectioned at 5μm. Sections were evaluated by immunohistochemistry (IHC) using a rat monoclocal antibody for laminin (Lam) and a goat polyclonal antibody for fibronectin (FN). Hearts were isolated from embryos and pooled, sonicated, and 5μg resolved and evaluated for FN by SDS-PAGE/Western analysis. Results of IHC studies demonstrated strong expression of both Lam and FN in the embryonic heart, which appeared to increase with increasing level of glycemic exposure. Similarly, FN level, as detected by SDS-PAGE/Western analysis, was low in hearts after embryonic exposure to hypoglycemia, moderate after normoglycemia, and highest after hyperglycemia. Thus, glucose excess appears to increase ECM protein expression in embryonic heart as in other tissues. The underlying mechanism is still under investigation.

1392 A STUDY OF VEHICLES FOR DOSING CULTURED RODENT EMBRYOS WITH NON-AQUEOUS SOLUBLE COMPOUNDS.

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Rodent whole embryo culture is a valuable approach for studying compound-induced teratogenicity during early organogenesis. Finding vehicles for non aqueous-soluble compounds has been problematic due to developmental toxicity associated with many solvents, including dimethylsulfoxide (DMSO) and ethanol. The purpose of this study was to identify alternative vehicles for non-water soluble compounds. We tested carrier solutions containing bovine serum albumin (BSA) and glycerol as well as the solvents, formamide, dimethylformamide, DMSO and two fractions of DMSO. Based upon the fact that DMSO has a higher freezing point than its degradation products, parent DMSO was fractionated from these products by freezing point differences. Our results indicate that at dose concentrations of 0.08% and 0.04%, respectively, the BSA carrier solution and the solvents, formamide, dimethylformamide and low freezing point fraction of DMSO (lofp-DMSO) exhibited no significant toxicity when compared to untreated cultured rat embryos. All-trans retinoic acid (RA), a non-aqueous soluble compound and potent teratogen, was diluted in the various solutions and a dose response study was undertaken in embryo culture to determine the delivery efficiency of the vehicle. Varying degrees of RA embryopathy were observed at different dose ranges when RA was diluted in the different vehicles. Although RA completely solubilized in DMSO and DMF, RA solubilized in DMSO produced a higher incidence of embryolethality at 1uM and a greater severity in malformations at the 0.1μM dose, indicating that DMSO may enhance teratogenicity. RA suspended in the glycerol carrier solution produced malformations at the 1 and 10 μM dose, whereas when suspended in the BSA carrier solution, RA produced malformations only at the high dose. These results suggest that the carrier solutions had varying influences on RA toxicity. Based upon these studies, we suggest that dimethylformamide and formamide, at 0.04% dose concentrations, are non-embryo-toxic and may serve as good alternative vehicles for the delivery of non-water soluble compounds in rodent whole embryo culture.

1393 THE EFFECT OF TIME OF CESAREAN SECTION ON FETAL BODY WEIGHTS IN RATS ON DEVELOPMENTAL TOXICITY STUDIES.

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Cesarean sections in rat developmental toxicity studies are generally performed on Gestation Day 20 and, depending on the facility, can be performed between 0800-1700 hrs. The effects of litter size on individual fetal weights has been well documented, however, the effects of time of cesarean section on individual fetal body weights have not been appropriately examined. In order to examine time related effects on fetal body weights at cesarean section, forty-two time-mated Sprague-Dawley IGS rats were received on Gestation Day (GD) 2 and cesarean section were performed on GD 20. On GD 20, female rats received a cesarean section during three intervals: 0800-0900 hrs. (Group I), 1130-1230 hrs. (Group II) and 1500-1600 hrs. (Group III). Mean litter size was unaffected by the time of cesarean section. Mean litter sizes for Groups I, II, and III were 12.8, 11.7, and 12.6, respectively. A significant increase in fetal body weight for Groups II and III was observed when compared to Group I. The mean fetal body weights for the combined sexes (male and female) for Groups I, II, and III were 3.8, 4.2 and 4.5 grams, respectively. Fetuses in this study exhibited an increase in body weight of approximately 0.1 grams per hour on Gestation Day 20. In addition, there was a greater incidence of decreased ossification of the 5 and/or 6 sternebra (e) unossified in fetuses when cesarean sections were performed between 0800-0900 hrs on Gestation Day 20. Based on these results, it is imperative that cesarean sections be performed at the same time each day, in as short a period of time as possible. More importantly, equal numbers of dams from each group should be cesarean sectioned during the same time interval to reduce variations in fetal body weights.

1394 COMPUTATIONAL EVALUATION OF HAZARDOUS AIR POLLUTANTS FOR DEVELOPMENTAL TOXICITY USING A STRUCTURE ACTIVITY APPROACH.

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Birth defects cause significant infant mortality and morbidity, resulting in great emotional and economic burden in the United States as well as in the rest of the world. In the United States alone, during 1993, developmental abnormalities were the underlying cause of death for 21.3% of infants less than one year of age. There is concern that exposure to air pollutants may effect the integrity of human health within industrialized countries. The United States Environmental Protection Agency's list of Hazardous Air Pollutants were screened against Structure Activity Relationship models derived from a machine learning algorithm for predictions regarding risk of human developmental toxicity. Multiple random sampling was employed in order to derive 10 structurally based models which were utilized to screen individual and representative mixture Hazardous Air Pollutants. The results of the computational screen were pooled in the form of a cumulative index (indicating high, moderate, and low potential) and evaluated according to their level of confidence. Air pollutants such as 2-acetylaminofluorene, 4-aminobiphenyl, biphenyl, 4-nitrobiphenyl, 3,3'-dimethylbenzidine, and 3,3'-dichlorobiphenyl have been identified by the structural models to pose a high risk to the developing human fetus. An additional screen against a mutagenicity data base has identified additional compounds that may be suspect due to their genotoxic risk.

1395 APPLICATION OF CLONAL GROWTH MODELS TO CANCER RISK ASSESSMENT.

R. B. Conolly and F. Miller. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

The 2-stage clonal growth model, a mechanistically-motivated model for cancer dose-response assessment, offers significant theoretical advantages over alternatives such as the linearized multistage model (LMS) or the low dose linear extrapolation approach proposed under the US EPA's new Guidelines for Carcinogen Risk Assessment. These advantages include a model structure that incorporates rates of cellular death, division and mutation in a manner consistent with the generally accepted pathway by which critical mutations accumulate and clinically detectable tumors are formed. The main focus of the symposium will be on a new cancer risk assessment for formaldehyde based on a clonal growth model. The historical development of quantitative models for cancer risk assessment, including the multistage and LMS models and the 2-stage clonal growth model will be presented. The key technical features of the new formaldehyde assessment, including advanced dosimetry modeling, identification of target cell populations, and incorporation of mode of action information will be covered. Finally, the issues that regulatory agencies such as the US EPA must consider in deciding when a new cancer risk assessment, such as the formaldehyde assessment, is appropriate for use in regulatory activities will be discussed.

1396 MULTISTAGE CANCER MODELS AND QUANTITATIVE ASSESSMENT.

S. H. Moolgavkar. *Fred Hutchinson Cancer Research Center Biostatistics, Seattle, WA.* Sponsor: R. B. Conolly.

A historical survey of stochastic models of carcinogenesis will be given and the use of these models for risk assessment discussed. The first multistage models were proposed in the 1950s to explain the regularity of the age-specific incidence curves of common human carcinomas. The current default procedure for quantitative risk assessment is the linearized multistage procedure, which is based on an early model proposed by Armitage and Doll. This procedure makes a number of approximations that will be discussed in non-technical terms. These approximations are not valid when tumor probabilities are high as in experimental studies. The Armitage-Doll model did not consider cell proliferation kinetics. Models explicitly incorporating cell proliferation kinetics were introduced in the 1970s. These models, the best known of which is the two-stage clonal expansion model, can be used to investigate the effects of both genotoxic and non-genotoxic carcinogens. The use of these clonal growth models for risk assessment will be discussed and illustrated by means of an example. This example will set the stage for the discussion of the formaldehyde risk assessment to follow.

1397 REGIONAL DOSIMETRY OF FORMALDEHYDE IN RAT AND RHESUS MONKEY NASAL PASSAGES AND HUMAN RESPIRATORY TRACT.

J. S. Kimbell, R. Subramaniam, P. M. Schlosser, E. A. Gross, R. B. Conolly and K. T. Morgan. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

Rates of cell death, division, and mutation are determinants of tumor incidence and are affected by formaldehyde in a dose-dependent manner. Exposure of tissue lining the respiratory tract to inhaled formaldehyde varies by location and these site-specific patterns vary among species. Formaldehyde-induced lesions observed at the carina of the rhesus monkey lower respiratory tract (LRT) suggested that both the upper respiratory tract (URT) and LRT might be at risk in humans. Regional uptake in rat and monkey nasal passages and in the entire human respiratory tract was needed for a new human health cancer risk assessment using a clonal growth model. Three-dimensional computational fluid dynamics (CFD) models of inspiratory nasal airflow and uptake in the rat, rhesus monkey, and human, and a typical-path model of uptake in the human URT and LRT incorporating oronasal and cyclic breathing were developed. Local formaldehyde flux (mass/area/time) was estimated for activity patterns of breathing rates in humans using both CFD and typical-path models. The amount of surface area affected by specific ranges of flux (flux intervals or "bins") was estimated. The rat nasal surface area was partitioned into 20 flux bins while 45 bins were used for the entire human respiratory tract. Sensitivity analyses showed that cancer risk predicted by the clonal growth model was sensitive to the number of flux bins but did not change when more than 20 or 45 bins were used for the rat nose and human respiratory tract, respectively. The use of anatomically-accurate CFD models and a human typical-path model accounts for interspecies differences in inspiratory airflow and formaldehyde uptake patterns and thus contributes to a reduction in uncertainty in cancer risk estimates. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1398 NEED FOR IDENTIFICATION OF TARGET CELL POPULATIONS WHEN DEVELOPING CLONAL GROWTH MODELS OF CARCINOGENICITY.

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Independent of the number of mutational stages included in a clonal growth model of a carcinogenic process, identifying the target cells of interest is a critical aspect of model development. Most investigators determine the cell division rate (α) based on the fraction of cells labeled (LI) in the organ of interest over the time interval (t) during which the cells were exposed to the label. This approach assumes that all the cells are capable of entering into the initiated pool and does not take into account the fact that only a fraction (f) of the cells are not terminally differentiated. Thus, f affects the calculated cell division rate since $\alpha=(2t)^{-1} \ln(1-LI/f)^{-1}$, where ln stands for natural logarithm. More accurate knowledge of the number of cells at risk means that the statistical estimation of other parameter values will also be more accurate. Since the fraction of terminally differentiated cells can vary between animals and humans, incorporating data on the number of cells at risk will tend to increase the accuracy of a clonal growth model and decrease the level of uncertainty associated with risk estimates derived from the model. Determining the fraction of cells at risk is not necessarily a straightforward task. If the clonal growth model has dose-response information for specific locations, such as for particular anatomical regions in the nose or airway generations in the lower respiratory tract built into it, then this level of resolution must also be determined for the cells at risk. In addition, anatomical and morphometric data must typically be synthesized from a number of sources to calculate the fraction or number of cells at risk. The kind of data and types of calculations needed to account for cells at risk in a clonal growth model of carcinogenesis will be illustrated for formaldehyde carcinogenicity in the respiratory tracts of rats and humans.

1399 FORMALDEHYDE CANCER DOSE-RESPONSE ASSESSMENT USING A 2-STAGE CLONAL GROWTH MODEL.

R. B. Conolly. *Chemical Industry Institute of Toxicology, Research Triangle Park, NC.*

Two modes of action, direct mutagenicity and cytotoxicity/regenerative cellular proliferation (RCP), have been implicated in the carcinogenicity of

formaldehyde in rat nasal epithelium. The biologically-motivated, two-stage clonal growth model readily incorporates data on these modes of action and thereby facilitates reduction of uncertainty in cancer risk assessment through increased used of relevant mechanistic data. Dose-response data on DNA-protein cross-links (DPX), representing direct mutagenicity, and on RCP were used to define effects of formaldehyde on parameters of the clonal growth model. The dose-response for DPX is linear in the low dose region, below about 1 ppm, and supralinear at higher concentrations. The dose-response for RCP is U-shaped, below control at 0.7 and 2 ppm and above control at 6, 10 and 15 ppm. However, since the U-shape was not significantly different from a hockey stick-shaped curve, cancer risk predictions were developed using both dose-response shapes for RCP. With U-shaped RCP, no additional human cancer risk was predicted below 1 ppm. This result, obtained in the presence of the low-dose linear, directly mutagenic effect mediated by DPX, illustrates the strong influence of cell kinetics in clonal growth models. Using hockey stick-shaped RCP, 40-year occupational exposure starting at age 18, 8 hr/day, 5 days week, to 0.1 or 1.0 ppm with a continuous 80 year lifetime background environmental exposure level of 4 ppb, human additional risks were predicted to be $2x10^{-8}$ and $1x10^{-5}$, respectively. The model predicts considerably lower risks than previous formaldehyde cancer risk assessments, which is consistent with the greater use of conservative default assumptions in the previous assessments and the use of more mechanistic data in the current assessment. This work also illustrates the pivotal role of dose response data for RCP in cancer risk assessments using clonal growth models.

1400 RISK ASSESSMENT WITH MECHANISTIC MODELS: ISSUES FOR ACCEPTANCE IN REGULATORY APPLICATIONS.

A. M. Jarabek. *National Center for Enviromental Assessment, Research Triangle Park, NC.* Sponsor: F. J. Miller.

To keep contemporary with the evolving science of toxicology, various U.S. EPA guidance expands the role of mechanistic data in risk assessment. Emphasis is placed on evaluating the mode of action (MOA) for a chemical, defined as its influence on molecular, cellular and physiological functions. "Precursor lesions" may be more accessible data to obtain, as well as more proximate to the relevant exposure than historical outcomes such as tumors, but establishing their biologic linkage requires integration of data based on different levels of organization (e.g., cellular to population) and incorporation of all relevant mechanistic data on dosimetry, toxicant-target interactions, and responses. Effective application of these principles requires new approaches that are outside routine testing paradigms and aimed at elucidating MOA through hypothesis-generated research and modeling. Refining future risk assessment lies in providing a framework to formalize an understanding of the reduced uncertainty due to the increase in accuracy of the MOA description, versus historical approaches that are presumed to be protective (conservative) when essential MOA elements are unknown. Attributes of the formaldehyde clonal growth model approach will be contrasted with historical default assumptions to illustrate challenging issues between development of MOA data and its acceptance in risk assessment applications.

1401 MOLECULAR APPROACHES TO STUDIES OF GLUTATHIONE METABOLISM AND FUNCTION.

C. V. Smith. *Ohio State University, Columbus, OH.*

Glutathione (γ-glutamylcysteinylglycine; GSH) is a critical participant in many biochemical and physiological processes, serving as a key metabolic intermediate and cofactor, and functioning in cellular protection mechanisms against many chemically reactive intermediates and xenobiotics that would otherwise alkylate or oxidize critical biological molecules. In part because of these multiple primary functions in cellular protective mechanisms, GSH has served as something of a toxicological Rosetta stone, providing important clues to mechanisms of metabolism of many xenobiotic and endogenous substances and insights to the mechanisms of action of many toxicants and disease processes. Modulations of GSH status and function, principally through the use of pharmacological agents or through dietary manipulations, have been utilized extensively in studies of tissue damage. Although much has been learned from such investigations, the pharmacological agents employed frequently manifest multiple effects, the contributions to outcome assessments of which have often been overlooked or underestimated. More recently, the tools of modern molecular biology have made available new experimental models that avoid the confounding secondary effects of pharmacological agents and are well suited for testing important fundamental hypotheses regarding GSH function. The major objective of the symposium is to present and discuss recent advances in the development of new experimental models of GSH function made possible by modern molecular biological approaches, with emphasis on the application of these experimental models to testing toxicologically important hypotheses.

1402 GLUTHIONE AND APOPTOSIS IN γ-GLUTAMYL TRANSPEPTIDASE-DEFICIENT MICE.

D. J. Reed and Y. Will. *Oregon State University, Corvallis, OR.*

Metabolic conversion of glutathione (GSH), glutathione disulfide (GSSG), and glutathione conjugates to constituent amino acids is initiated by γ-glutamyl transpeptidase (GGT). GGT is in high concentration in the brush border of the kidney, with lesser amounts in the biliary tree and elsewhere. Inhibition or elimination of this enzyme results in glutathionuria and levels of GSH increasing from micro- to millimolar in urine. GGT-deficient (GGT-KO) mice are severely limited in growth and life span due to the lack of ability to maintain GSH homeostasis and a chronic tissue-specific GSH deficiency. Although the cause of death of these mice 10-12 weeks after birth remains uncertain, they are useful models to elucidate aspects of the role of GSH in mechanisms of cell death. Thymocytes treated with dexamethasone (DEX) undergo a step-wise dysregulation of mitochondrial function involving mitochondrial permeability transition (MPT). Depolarization and uncoupling of MPT have been discussed as the "point of no return" in apoptosis and are strongly influenced by thiol status. We characterized the GSH status of splenocytes and thymocytes obtained from GGT-KO mice and found a significant decrease in cells obtained from GGT-KO when compared to cells obtained from GGT-WT. Since little is known about the role of mitochondrial GSH in splenocytes, we further elucidated how mitochondrial and cytosolic GSH relate to apoptotic events. Thymocytes were subjected to cytofluorometric analysis to examine apoptotic events. Thymocytes obtained from GGT-KO mice were more susceptible to apoptosis than were thymocytes obtained from wildtype mice. Supplementation of GGT-KO mice with the GSH precursors, N-acetylcysteine (NAC) and 2-oxo-4-thiazolidine-carboxylic acid (OTC) showed complete reversal of increased susceptibility. These and other findings with the GGT-KO mice will be discussed in terms of the usefulness of this model to address questions about the role of GSH and GGT in the toxicity of various chemicals.

1403 THE EFFECTS OF OVEREXPRESSION OF GLUTAMATE-CYSTEINE LIGASE ON CELL SURVIVAL, CELL GROWTH AND APOPTOSIS.

T. J. Kavanagh. *University of Washington, Seattle, WA.*

The tripeptide thiol glutathione (GSH) is important in free radical scavenging, xenobiotic conjugation and maintenance of cellular redox status. Glutamate-cysteine ligase (GLCL; also known as γ-glutamylcysteine synthetase) is the rate limiting enzyme in GSH synthesis. GLCL is composed of catalytic (GLCLC) and regulatory (GLCLR) subunits. We transfected the mouse cDNAs for these subunits driven by a metallothionein promoter into Hepa-1 cells, and derived lines which stably overexpress GLCLC and GLCLR alone or in combination. Cells which highly overexpress GLCLC+GLCLR have slightly higher GSH levels in the presence of Zn when compared to controls, are resistant to oxidants, and to TNF+ActD-induced GSH depletion, loss of mitochondrial membrane potential, and apoptosis. These findings imply that upregulation of GLCL activity contributes to resistance to oxidative stress and drug-induced apoptosis in cancer cells.

1404 GLUTATHIONE PEROXIDASE-1 REGULATION AND FUNCTION.

M. J. Kelner. *UC San Diego, San Diego, CA.*

The cytosolic selenium-dependent glutathione peroxidase (GPX1) is one of the most extensively characterized selenoproteins. The enzyme catalyzes the conversion of hydrogen peroxide and hydroperoxides to water and the corresponding alcohols, respectively, using reduced GSH as a hydrogen donor. The post-transcriptional regulation of GPX1 *in vitro* by selenium has been well defined. Most multicellular organisms, however, are not deficient in selenium. This implies that redox-controlled transcriptional regulation of GPX1 may be more physiologically relevant in humans. Studies in our laboratory have identified several regulatory mechanisms and elements in the human GPX1 gene. [1] There exist in the 5'-nontranslated region (promoter) of the human GPX1 gene both positive and negative regulatory elements. There exists at least one positive or oxidative responsive regulatory element (ORE) capable of tran-

scriptionally responding to stress induced by paraquat, peroxides and some agents not traditionally considered oxidizing agents. The consensus sequence of this element is being defined and its homology to similar oxidative responsive elements in other glutathione peroxidases determined. [2] Negative regulatory elements (NREs), capable of binding to nuclear proteins, were also identified in the GPX1 gene. Sequences similar to these two NREs have been identified in other antioxidant enzymes, suggesting a common regulatory mechanism. [3] The ability of the 5'-untranslated region in GPX1 mRNA to specifically bind to a regulatory protein has been documented and 3 nucleotides critical for protein/mRNA binding identified. [4] Null or "knock-out" GPX1 mice have been generated to further investigate the tissue-specific distribution of GPX1. These findings will be discussed in terms of the role of GPX1 in protecting cells against general oxidative damage from a variety of toxins.

1405 GLUTATHIONE REDUCTASE MODULATION BY MODER MOLECULAR METHODS.

C. V. Smith. *Ohio State University, Columbus, OH.*

Glutathione disulfide (GSSG) is produced when glutathione (GSH) reduces hydroperoxides and other oxidants. Glutathione reductase (GR) catalyzes the NADPH-dependent reduction of GSSG back to GSH, thus coupling the antioxidative capabilities of GSH to the substantial eductive capacity afforded by cellular NADPH. A very few humans have been identified with unusually low GR activities, apparently resulting from production of an unstable protein. Much of the toxicological relevance of GR is attributed from studies of the effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), which irreversibly inhibits GR *in vitro* and *in vivo*. However, BCNU also alkylates and crosslinks DNA and large and variable fractions of the effects of BCNU on cytotoxicities are more closely correlated with the effects on DNA than on inhibition of GR. We have used a cDNA for human GR to transfect Chinese hamster ovary (CHO) cells. By transfection with hGR in the antisense orientation we have generated a stable CHO-derived cell line that exhibits 50% of the normal cell GR activity and is more susceptible to oxidant injury. Transfection of CHO cells with hGR in the sense orientation has been used to generate cells with increased GR activities and resistance to oxidant challenge, especially if the transgene product is directed to the mitochondria by attachment of a functional mitochondrial targeting signal 5' to the hGR cDNA. This transgene construct has been used with an adenoviral transfection system to enhance GR activities and augment oxidant resistance in human-derived H441 cells. GR functions as an antiparallel (head-to-tail) homodimer with two active sites per dimer, each site formed with critical residues from each monomer. We have generated several dominant negative mutant constructs that attenuate the activity of the human gene product, but are less effective in inhibition of endogenous CHO cell GR activities, despite the high interspecies identities of the GR primary sequence. The most remarkable observation thus far has been the substantial enhancement of cellular resistance to oxidant injury provided by increased GR activities in the mitochondrial compartment. (Supported by GM44263 from the National Institutes of Health.)

1406 HUMAN IMMUNOTOXICITY: EXAMPLES & STRATEGIES FOR DETERMINIG RISK: INTRODUCTION.

M. J. K. Selgrade. *USEPA NHEERL, Research Triangle Park, NC.*

Laboratory rodent studies indicate that a number of chemicals are immunotoxic. Human data has been more difficult to obtain. In some cases parallel effects have been demonstrated in humans and rodents following exposure to immunosuppressive chemicals. However, for many chemicals, different endpoints have been assessed in humans and rodents making comparisons across species difficult. Rodent studies often assess antigen-driven responses, whereas human studies usually assess less invasive, static indicators of immune competence. Examples of human studies that assessed antigen-driven responses will be presented in this workshop as well as attempts to relate immune suppression to enhanced disease susceptibility in humans. Because data from human *in vivo* exposures is difficult to obtain, studies on *in vitro* exposure of human cells will be described as a tool for assessing human immunoxicity. Evidence for a common mechanism underlying rodent and human effects will also be presented. Human data has been more readily available for protein allergens. The use of this data along with animal data to understand how magnitude, frequency, and duration of exposure affect risk of sensitization will be described. The workshop will conclude with a discussion of the best approaches and designs for human immonotoxicity studies, use of animal data in assessing human risk, and research needed to improve assessment of immunotoxicity in humans.

1407 IMMUNOLOGIC EFFECTS OF POLYOCHLORINATED BIPHENYL (PCB) AND DIOXIN EXPOSURE IN DUTCH TODDLERS.

N. Weisglas-Kuperus. *Sophia Children's Hospital, Rotterdam, Netherlands.*
Sponsor: M. J. Selgrade.

Prenatal exposure to PCBs and dioxins is associated with changes in the T-cell lymphocyte populations in healthy Dutch infants. We investigated whether these changes persisted into later childhood and whether this exposure was associated with the prevalence of infectious or allergic diseases and humoral immunity in toddlers. 207 healthy mother-infant pairs were studied. Prenatal exposure to PCBs and dioxins was estimated by the sum of PCBs 118, 138, 153 and 180 (SPCB) in maternal and cord plasma and in breast-fed infants by the dioxin, planar and mono-ortho PCB toxic equivalent (TEQ) levels in milk. At 42 months of age, body burden was estimated by the SPCB in plasma. Prevalence of infectious and allergic diseases was assessed by parent questionnaire. Anti-body levels after mumps, measles & rubella vaccination were assessed. In some cases lymphocyte phenotypic markers were analyzed. In toddlers prenatal PCB exposure was associated with increased T cell numbers and lower antibody levels. Adjusted for confounders (gender, parity, maternal education breast feeding, parental occupation, smoking by parents, family history of atopy, day care or nursery attendance), prenatal PCB exposure was associated with less shortness of breath with wheeze. Current PCB body burden was associated with a higher prevalence of recurrent middle ear infections and chickenpox and a lower prevalence of allergic reactions.

1408 THE HEALTH CONSEQUENCES OF STRESS INDUCED IMMUNE MODULATION.

R. Glaser and J. Kiecolt-Glaser. *The Ohio State University, Columbus, OH.*
Sponsor: M. J. Selgrade.

In a series of studies, we examined the impact of academic stress on different aspects of the cellular immune response in medical students. The results of these studies showed significant down-regulation/dysregulation of several aspects of the cellular immune response. In order to explore whether these statistically significant differences were biologically significant, we performed a study in which medical students were inoculated with a recombinant Hepatitis-B (Hep-B) vaccine. Each vaccination was administered to coincide with the third day of a three day examination series. Students who reported greater social support and lower anxiety and stress had higher antibody levels to the vaccine and a more vigorous T-cell response at the end of the third inoculation (6 months). In a second series of studies with caregivers of Alzheimer's Disease (AD) patients, similar down-regulation of several aspects of the cellular immune response was observed in caregivers as compared to well-matched control subjects. Once again, we explored the possibility that these changes were significant enough to effect how a person responds to a vaccine; we used the influenza virus vaccine. AD caregivers showed a poorer antibody response and virus-specific T-cell response following vaccination with the flu vaccine as compared to the control subjects. The differences in antibody and T-cell responses to these two virus vaccines provide a demonstration of how psychological stress may be able to alter a person's immune response to a vaccine and therefore risk for infection. We believe that the data obtained in these studies provide a clue for how stress can affect how a person would respond to infection with a live virus.

1409 MECHANISMS OF HUMAN IMMUNOTOXICITY INDUCED BY POLYCYCLIC AROMATIC HYDROCARBONS (PAHs): LESSONS FROM MURINE *IN VITRO/INVIVO* AND HUMAN *IN VITRO* STUDIES.

S. W. Burchiel. *University of New Mexico, Albuquerque, NM.*

Polycyclic aromatic hydrocarbons (PAHs), such as 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP), are well known immunotoxicants in animals and likely in humans. Human B and T lymphocytes appear to be sensitive to DMBA, resulting in altered antigen receptor signaling and disruption of Ca^{2+} homeostasis. Prolonged elevation of Ca^{2+} in lymphocytes induces apoptosis. There appear to be several mechanisms responsible for elevation of Ca^{2+} in B and T cells. PAHs produce a somewhat

nonspecific and rapid mobilization of Ca^{2+} from intracellular stores via an IP3-dependent and protein tyrosine kinase (PTK)-associated pathway. In human lymphocytes, the src and src-like PTKs that are rapidly (5-10 min) activated by PAHs include Lck, Fyn, and Zap-70 in T cells; Lyn and Syk are activated in human B cells. These kinases are also activated following prolonged (18 hr) exposures to BaP, through a mechanism that requires P4501A or 1B metabolism. Extracellular Ca^{2+} is required for these prolonged effects. Alpha-naphthoflavone (ANF) was found to prevent the inhibition of T cell proliferation produced by BaP, as well as to prevent B cell apoptosis, and Ca^{2+} elevation. BaP metabolites appear to produce immunotoxicity by both oxidative (N-acetylcysteine, NAC-sensitive) and electrophilic mechanisms. Since the mechanism (s) of immunotoxicity appear to be similar in mice and humans, and there seems to be good agreement between *in vivo* and *in vitro* studies, PAHs may be excellent candidates for developing models for mechanism-based risk assessment. (Supported by ES-05495.)

1410 ALLERGY TO ENZYMES: USE OF PRE-CLINICAL AND CLINICAL DATA TO ASSESS THE RISK TO MAN.

K. Sarlo. *Procter & Gamble Company, Cincinnati, OH.*

Detergent enzymes are well known occupational allergens. Exposure guidelines and safe handling practices have minimized sensitizations (allergic antibody) and greatly reduced the incidence of allergy and asthma among the detergent workforce. After many years of medical and environmental monitoring, we can conclude that allergic symptoms are elicited mainly by high, peak exposures to enzyme dust. Sensitizations develop also via exposure to lower levels of enzyme dust. The prevalence of sensitization to protease in our detergent manufacturing facilities is between 11%-16%. There have been no asthma cases in the past 7 years. Allergy to enzymes among consumers of detergent products is a rare event. In 1970, there were a few cases of enzyme allergy after exposure to high levels (~200 ng/m3) of enzyme in a dusty detergent product. Current laundry exposures are lower with no sensitizations. Effect and no-effect exposure conditions are known for enzymes used or misused in laundry products and personal cleansing applications. Exposure to protease levels of 11 ng/m3 (range: 3-29 ng/m3) in a shower via use of an enzyme-containing beauty bar led to 6% sensitizations in a 6-month clinical trial. In comparison, exposure to less than 0.1 ng/m3 via hand laundry or personal cleansing with an enzyme synthetic laundry bar did not sensitize several hundred atopic Filipino study subjects after 2 years of use. Via retrospective and prospective clinical evaluations of exposed populations, we are beginning to understand how magnitude, frequency and duration of exposure relate to sensitization to enzymes. The use of animal models to assess the relative allergenic potential of enzymes has been used to establish exposure guidelines for the detergent industry. Animal models can also be used to understand how different "patterns" of exposure relate to the induction of allergy. Taken together, the pre-clinical/clinical data on enzyme allergy will allow us to identify thresholds for sensitization and to accurately calculate risk of developing sensitization to enzymes among exposed populations.

1411 CURRENT STATUS OF MODEL DEVELOPMENT FOR PHOTOBIOLOGY RISK ASSESSMENT WITH RELEVANCE TO HUMANS.

G. F. Gerberick. *Procter & Gamble Company, Cincinnati, OH.*

Acute and chronic exposure to ultraviolet radiation (UVR) produces damage to human skin. Such damage is a human health concern and believed to play a role in several diseases including development of human skin cancers. Chemicals—including drugs—which absorb UVR may directly affect acute and chronic photobiological responses. More controversial is the view that some pharmacological/toxicological effects of a chemical may increase human health risk by exacerbating UVR-induced damage indirectly viz a viz a secondary mechanism. Efforts are ongoing to develop and/or refine acute and chronic phototoxicological models, and both *in vitro* and *in vivo* models have been used successfully to assess human risk of direct phototoxicological effects of chemicals. The conduct of clinical studies measuring well-defined photobiological endpoints represents an important alternative. The methods and endpoints of human clinical photobiological studies will be presented. The overall issue of public health and chemical safety with regard to phototoxicological concerns will be discussed by US FDA representatives.

1412 EFFECTS OF ULTRAVIOLET (UV) LIGHT ON BIOLOGICAL SYSTEMS.

I. E. Kochevar. *Harvard, Boston, MA.* Sponsor: G. F. Gerberick.

Humans are exposed to solar UVB (290-320 nm) and UVA (320-400 nm) light which has both deleterious and beneficial effects. For photobiological responses to occur, the UV light must be absorbed by molecules that are termed "chromophores". Endogenous tissue chromophores include DNA, proteins, urocanic acid, porphyrins, nicotinamide adenine dinucleotide (NADH) and flavins. Exogenous chromophores may be present in drugs or topically applied preparations. Biological responses to UV light vary with the wavelength of the light since each chromophore has a distinct absorption spectrum and because the penetration depth of light into tissue varies with the wavelength. For example, 10% of 300 nm radiation reaches approximately 15 μm into fair human skin whereas 10% of 365 nm radiation penetrates about 150 μm. Absorption of UV light by molecules produces very short lived excited state molecules which may undergo chemical reactions with cellular molecules that initiate cellular responses. Other processes of excited state molecules, such as production of heat or light (fluorescence) do not cause biological responses. The chemical reactions include formation of reactive oxygen species, conversion of 7-dehydrocholesterol to cholesterol, and production of dimers between thymines in DNA. The cellular responses, which include initiation of new gene expression, mutagenesis, and apoptosis, lead to observable responses in skin such as erythema, melanogenesis, hyperproliferation of the epidermis and tumorigenesis.

1413 PHOTOTOXICOLOGICAL RISK ASSESSMENT: LIMITATIONS OF CURRENT MODELS.

J. F. Nash. *Procter & Gamble Company, Cincinnati, OH.* Sponsor: G. F. Gerberick.

Determination of the phototoxicological potential of a chemical or product is a specialized subset of the human safety assessment. The decision to initiate such an assessment is based on the principle that the compound of interest absorbs ultraviolet (UV) light in the solar spectrum, i.e., 290-760 nm. Because many molecules absorb UV in this range, such information provides little insight about the potential biological consequences of the absorbed UV energy. To this end, the *in vitro* 3T3 Neutral Red Uptake (NRU) phototoxicity-screening assay may be used to determine the relative toxicological potential of a chromophore. The 3T3 NRU screening assay has undergone a rigorous validation demonstrating the sensitivity and specificity of the results. As with other *in vitro* assays, system incompatibility and establishment of a no observed effect level (NOEL) or dose threshold are potential limitations. There are animal models to evaluate phototoxicity as well as the photoallergenicity potential of a chemical. Importantly, the animal models have been evaluated with established human positive and negative controls. In this regard, human assays of phototoxicity and photoallergenicity exist and are used primarily as confirmatory tests or for establishing a NOEL. Up to this point, the tests address the acute phototoxicological potential of a chemical. The long-term phototoxicological concern, mainly photocarcinogenicity, may be evaluated using modifications of genotoxic and rodent carcinogenicity assays. Specifically, the photogenotoxic potential of a compound may be evaluated using photomutagenic, photoclastogenic and/or photo-comet assays. These approaches are adaptations of existing genotoxic models with the addition of UV light. The *in vivo* model most commonly used to evaluate photocarcinogenicity is the SKH1 (hr/hr) albino hairless mouse. For both photogenotoxicity and photocarcinogenicity, the absence of known human positive controls is problematic. Moreover, the use of the hairless mouse to investigate secondary mechanisms of photocarcinogenicity is complex and of unknown relevance in evaluating human health risk.

1414 THE USFDA/NIEHS PHOTOTOXICOLOGY RESEARCH CENTER: UNDERSTANDING AND DEVELOPING ANIMAL MODELS FOR PHOTOCARCINOGENESIS TESTING.

P. C. Howard[1], R. L. Sams[1], B. J. Miller[1], W. G. Wamer[2] and J. Z. Beer[3]. [1]*NCTR/FDA, Jefferson, AR.* [2]*Center for Food Safety and Applied Nutrition, FDA, Washington, DC and* [3]*Center for Devices and Radiological Health, FDA, Washington, DC.* Sponsor: G. F. Gerberick.

The Phototoxicology Center was developed in response to the need for mechanistic and tumorigenicity testing of products of interest to the FDA and

NIEHS. Mice are exposed to simulated solar light using a 6.5 kWatt xenon arc solar simulator, or any combination of fluorescent tube generated light (e.g. UVA + UVB). Mechanistic studies on the rates of cell proliferation and apoptosis, DNA damage formation, and expression of mutations in specific cell types will be used to understand the effects of compounds with or without light on skin cells and tumor development. Tumor studies will be conducted using the SKH-1 hairless mouse. Future utilization of this facility will include evaluating the tumorigenic response of new rodent models (e.g. TgAC transgenic mice) to simulated solar light, and comparison to the SKH-1 mouse. Our current studies are focused on the effects of alpha-hydroxy acids on induction of skin tumors in SKH-1 mice with simulated solar light.

1415 REGULATORY CONSIDERATIONS ON PHOTOBIOLOGY RISKS.

A. Jacobs. *FDA/CDER, Rockville, MD.*

The U.S. Food and Drug Administration is charged with evaluating the safety of drug products, including photosafety. At present, with a few exceptions, only drugs applied to sun-exposed areas of the skin are assessed for photosensitivity (i.e., photoirritation and photoallergy) or for the potential to enhance ultraviolet (UV)-associated skin cancer. However, some systemically administered drugs, such as fluoroquinolone antibiotics, have been shown to be photosensitizing. Regulatory photobiology questions for dermatologic products include when and how to test, risk/benefit and approvability, and appropriate risk communication. For example, a new UV filter, which is intended for protection of skin from sunlight, should always be tested for possible adverse effects due to concurrent sunlight exposure. For some photosensitizing drug products, a warning to avoid exposure to sunlight may be sufficient and additional phototesting may not be necessary. Considerations for phototesting of nonphotosensitizing drugs include duration of use, whether the skin receives a large exposure, effects on the skin, and pharmacological activity.

1416 PHOTOCARCINOGENICITY OF DRUGS—HUMAN EXPERIENCE.

R. S. Stern. *Beth Israel Deaconess Medical Center, Boston, MA.* Sponsor: G. F. Gerberick.

Photocarcinogenicity of Drugs—Human Experience.A number of commonly used medications including quinolone antibiotics, psoralens and various tetracycline derivatives are photosensitizers. These chemicals enhance the erythema response to sunlight and sunlight exposure. Drugs might increase the risk of skin cancer by one or more of a variety of mechanisms including direct genotoxic effects, effects on the immune system, increasing penetration of UV light, and stimulating cellular proliferation. The effect of such exposures on cancer risk has been best quantified in humans for oral psoralen photochemotherapy (PUVA). The experience of a cohort of 1380 patients followed for more than 20 years who received PUVA therapy for the treatment of psoriasis documents that long-term high dose exposure to PUVA greatly increases the risk of squamous cell carcinoma. After 15 years, the risk of melanoma is also increased among high dose patients. PUVA therapy is immunosuppressive in the skin, induces psoralen DNA adducts, is genotoxic and mutagenic. Substantially increased risk is only observed after many purposeful exposures to ultraviolet and this drug. These data suggest that at least some photosensitizing chemicals can substantially increase the risk of skin cancer in humans, but with this drug when used in adults, long-term multiple exposures appear to be necessary for a clinical meaningful increase of risk. The photocarcinogenic risk of drugs is likely to be a function of their photobiologic effects, frequency of use, and perhaps age of the user.

1417 AUGMENTED HUMORAL IMMUNE FUNCTION IN METALLOTHIONEIN-NULL MICE: POSSIBLE INTERACTIONS WITH METALLOTHIONEIN AND THE SIGNAL TRANSDUCTION PATHWAY.

K. C. Crowthers, V. Kline, C. Giardina and M. A. Lynes. *University of Connecticut, Storrs, CT.*

Infection, inflammation, autoimmune disease, and neoplasia are all associated with stressful conditions that can result in increased metallothionein (MT) synthesis. MT is a small, thiol-rich protein which can act as a reservoir of essential heavy metals, scavenge free radicals, and provide a homeostatic mechanism that regulates damage caused by toxic heavy metals. In previous work, we have shown that exogenous MT can suppress the developing humoral immune response. This finding suggested that endogenous MT might similarly alter immune function, and that there would be substantial changes in humoral immune function in mice that have a targeted deletion in the MT-1 and -2 genes. Here we show that MT-null (MTKO) animals display a significant enhancement in their ability to mount a T-cell dependent antibody response. This augmented *in vivo* response is paralleled by increases in the proliferation response to mitogenic stimulation and enhanced differentiation of antigen-specific B cells to plasma cells. Moreover, we have found that cells from MT-null animals display significantly elevated levels of NF-κB activity when compared to wildtype controls. Taken together, these results suggest that both metal- and stress-induced immune suppression may be mediated by increased expression of MT, as well as serve as a negative regulator of the immune response in unstressed conditons.

1418 SUSCEPTIBILITY TO ULTRAVIOLET B RADIATION-INDUCED SYSTEMIC IMMUNOSUPPRESSION IN FOUR DIFFERENT TRANSGENIC MOUSE STRAINS WITH SPECIFIC DEFECTS IN NUCLEOTIDE EXCISION REPAIR: XPA, XPC, TTD/XPD AND CSB NULL MICE.

J. Garssen[1], H. Van Steeg[1], F. De Gruijl[2], J. De Boer[1], B. Van Der Horst[3], H. Van Kranen[1], G. Weeda[3], J. Hoeijemakers[3] and H. Van Loveren[1]. [1]*National Institute Public Health and Environment, Bilthoven, Netherlands,* [2]*Utrecht University, Utrecht, Netherlands and* [3]*Erasmus University, Rotterdam, Netherlands.*

Exposure to ultraviolet light, especially wavelengths in the UVB band present in sunlight, can impair immune responses in animal and man. UVB exposure appears to inhibit most specifically Th1-mediated immune responses as both contact hypersensitivity (CHS) and delayed type hypersensitivity (DTH) are very sensitive to suppression by UVB irradiation. It is remarkable that this immunomodulation is not restricted to the exposed skin but is also found at (distant) sites, i.e., a systemically mediated immunosuppression can be induced. As UVB radiation is not able to penetrate much deeper than the viable cell layers of the epidermis, the immunosuppression is likely to be mediated by these exposed cells, their products or photoactivated factors present in the superficial layers. Various processes have been found to contribute to the UV-induced immunosuppression: e.g., urocanic acid isomerization, release of neuropeptides, and membrane damage through which signalling pathways (e.g. NF-kB) become activated. Amidst all these processess, DNA damage appears to play a paramount role in UV-induced suppression of many cellular immune responses. Enhanced (enzyme-mediated) clearance of this damage counteracts the immunosuppression dramatically. Conversely, persistence of the damage by a lack of DNA repair would plausibly lead to the enhancement of the immunosuppression. The aim of the present study is to determine what type of DNA damage, i.e., in which part of the genome, contributes most to the UV-induced immunosuppression and the acute skin reactions (such as edema and erythema, i.e. "sunburn"). To this end, 4 different strains of transgenic mice with specific defects in nucleotide excision repair (NER) were used: CSB mice with defective transcription coupled repair (TCR), XPC mice with defective global genome repair (GGR), and XPA and TTD/XPD mice with TCR and defective GGR. The immunomodulation was assessed in a DTH reaction against an infectious agent, Listeria monocytogenes, and a CHS reaction against picrylchloride. Our results indicate and confirm that UV-induced damage in the transcribed DNA strand clearly plays a major role in the acute sunburn reaction. However, the UV-induced immunosuppression is not evidently related to this DNA damage and the related sunburn reaction, nor specifically to the DNA damage in the non-transcribed DNA. The only genotype that appears to be extremely sensitive to the UV-induced immunosuppression is XPA-/-, the only one with a complete lack of both TCR and GGR. A slight increase in susceptibility to UV-induced suppression of CHS in TTD/XPD-/- mice suggests that persistence of pyrimidine (6-4)pyrimidone photoproducts in the transcribed strand may contribute most specifically to the immunosuppression.

1419 MECHANISM OF MACROPHAGE ACTIVATION BY ANGELAN ISOLATED FROM ANGELICA GIGAS NAKAI, TRADITIONAL ORIENTAL DRUGS.

H. M. Kim, Y. J. Jeon, K. S. Ahn and S. B. Han. *Kor Res Inst Biosci Biotech, Taejon, Republic of Korea.*

In our previous studies we showed that the primary target cell of angelan, a polysaccharide purified from Angelica gigas Nakai, is a macrophage (Han et

al., 1998). In the present study we examined the effect of angelan on iNOS, IL-1b, and TNF-a transcription in mouse macrophage line RAW 264.7. We show that angelan produces a marked induction of iNOS, IL-1b, and TNF-a transcription by RAW 264.7 cells. Since these gene transcriptions have been recently shown to be under the control of NF-κB/Rel family of transcription factors, we assessed the effect of angelan on NF-κB/Rel using a electrophoretic mobility shift assay. Treatment of RAW 264.7 cells with angelan produced strong induction of NF-κB/Rel binding. Treatment of RAW 264.7 cells with angelan slightly induced AP-1 binding activity, whereas Oct binding was not affected by angelan. Angelan stimulated macrophages to activate NF-κB/Rel, whereas neither B-cells nor T-cells were affected by the angelan. In conclusion, we demonstrate that the stimulation effect of angelan on macrophage is mediated by specific activation of NF-κB/Rel.

1420 EVIDENCE FOR A ROLE BY SMADS IN TGF-β1-INDUCED MODULATION OF T-CELL EFFECTOR FUNCTION.

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TGF-β1 is the most potent known immune suppressive factor. Elevated levels of TGF-β1 have been associated with xenobiotic-induced liver injury, e.g. carbon tetrachloride (CCl4) and acetaminophen. We have previously established that a profound TGF-β1-mediated, T-cell dependent transient immune suppression accompanies modest CCl4-induced liver injury. More recently, we have demonstrated that TGF-beta1 modulates interleukin-2 (IL-2) expression in CD3 and CD28-activated splenic T-cells. The mechanisms underlying TGF-β1-mediated suppression of T-cell function are currently unknown. The objective of the present studies was to test the hypothesis that TGF-β1-induced immune modulation of T-cell effector function is mediated, at least in part, through serine/threonine TGF-β receptor activation of Smad transcription factors. Towards this end, we have identified several CAGA Smad response elements within the 5' minimal essential promoter region of the IL-2 gene. Western blot analysis indicates that Smad3 nuclear expression increases in a concentration- and time-dependent manner following TGF-β1 exposure. The most robust increase in Smad3 nuclear expression was observed following low TGF-β1 exposure; this observation correlates with low concentration TGF-β1-mediated augmentation of IL-2 expression. Additionally, TGF-β1- and CD3 and CD28-induced protein binding at the proximal phorbal ester DNA response element in the IL-2 promoter is abrogated with mutation of the 5' and 3' flanking CAGA Smad response elements. In summary, these data support the hypothesis that Smads may play a mechanistic role in hepatoxicant-induced immune suppression. (This work was supported by NIEHS Grant P01P42ES04911-08C.)

1421 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ALTERS DENDRITIC CELL MATURATION AND INTERFERES WITH NF-κB/REL SIGNALING.

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TCDD, the prototypic ligand for the Ah receptor, causes a host of immunomodulatory phenomena, including suppression of cell-mediated and humoral immune responses. Dendritic cells (DC) are professional antigen presenting cells that induce and maintain the adaptive immune response. DC develop from stem cells found in bone marrow and become fully mature upon the uptake of antigen, exposure to cytokines such as TNFα, and following cell-cell interactions. Studies were undertaken to determine if TCDD disrupts the process of DC maturation. Bone marrow cells from C57Bl/6 mice were exposed to 10^{-9} M TCDD and cultured with a DC growth factor, GM-CSF, for 7 days inducing a population of cells to express characteristic DC markers including CD11c, CD80, CD86, major histocompatibility class II protein (MHC II), CD40 and Dec205. These cells were then treated with TNFα for two days and maturation was evaluated by the level of expression of MHC II and CD86 on $CD11c^+$ cells. TNFα treatment increased the expression of MHC II and CD86 in vehicle-treated cells; TCDD significantly attenuated this expression indicating a suppression of DC maturation. Maturation of DC is dependent on the NF-κB/Rel family of transcription factors. Since TCDD was shown to interfere with DC maturation, we investigated the effects of TCDD on NF-κB/Rel signaling in DC. DC 2.4 cells, a murine bone marrow derived DC cell line, were treated with 10^{-9}M TCDD for 24 hours prior to activation with TNFα. Translocation of NF-κB/Rel to the nucleus as visualized by immunoblotting was blocked in TCDD exposed cells after TNFα activation when compared to vehicle-treated cells. These findings suggest that exposure to TCDD alters maturation of DC which could be a consequence of TCDD altering NF-κB/Rel signaling. (Supported by NIH #ES00040.)

1422 THE EFFECTS OF PCBS AND ESTROGENS ON PHAGOCYTE INOS, COX-2, NRAMP, AND CYP1A EXPRESSION IN THE CHANNEL CATFISH MONOCYTE/MACROPHAGE CELL LINE 42TA.

C. D. Rice and Y. Xiang. *Clemson University, Pendleton, SC.* Sponsor: D. Schlenk.

Recent studies suggest that environmental estrogens may negatively impact the reproductive physiology of fish and wildlife, and possibly humans, while planar halogenated aromatic hydrocarbons (HAHs) may antagonize this effect. However, the interactive effects of estrogens and HAHs in mixtures on immunophysiology remain uncharacterized. In mammals, both HAHs and environmental estrogens modulate phagocyte-associated proinflammatory responses. The effects of environmental estrogens and HAHs on inflammatory responses in fish are unknown. We treated cytokine/LPS-stimulated 42TA cells, a channel catfish monocyte cell line, with 17b-estradiol (E2), nonylphenol (NP), PCB-126, PCB-104, and PCB-126 + NP, PCB-104 + NP, PCB-126 + E2, and PCB-126 + PCB-104. Cell lysates were probed by SDS-PAGE/western blotting for inducible prostaglandin endoperoxide H synthase-2 (cyclooxygenase-2; COX-2), nitric oxide synthase II (iNOS), natural resistance associated membrane protein (nRAMP), and CYP1A. The mAbs used for western blotting were developed from peptide sequences chosen upon cloning and sequencing each of the above fish phagocyte proteins. Nitric oxide synthase II and nRAMP were generally unaffected by treatment. However, E2 suppressed, while the weak estrogen NP enhanced COX-2 and CYP1A expression. In comparison, PCB-126 enhanced COX-2 and CYP1A, while PCB-104 mimicked the effect of E2 by suppressing COX-2 and CYP1A. Our results are similar to those from published studies using rodents. Therefore, we suggest that the channel catfish monocyte/macrophage cell line 42TA may be a good model for understanding the effects of xenobiotic mixtures containing both Ahr- and ER-active compounds. Current efforts are underway to determine if inducible CYP1A and COX-2 in 42TA cells have enzymatic activity. Although the relative roles of macrophage monooxidation reactions (CYP?) and peroxidiation reactions (COX-2?) in metabolizing certain xenobiotics to reactive metabolites are still unclear, the 42TA cell line may provide some answers.

1423 ROLE OF NF-κB IN THALIDOMIDE AND DEXAMETHASONE INDUCED TUMOR NECROSIS FACTOR α EXPRESSION.

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Thalidomide, withdrawn from use as a sedative during pregnancy due to its teratogenic effects, has been recognized recently as having immunomodulatory activity, specifically the inhibition of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6. We have demonstrated previously that culture with thalidomide inhibits TNF-α mRNA expression and protein production by mitogen-stimulated human peripheral blood mononuclear cells (PBMC). We have now investigated the influence of this drug on nuclear levels of NF-κB in mitogen-activated PBMC, using dexamethasone as positive control due to its well characterized and NF-κB mediated effects on TNF-α expression. PBMC were stimulated optimally with mitogen (phytohemagglutinin or phorbol myristate acetate and ionophore) in the presence of 10^{-1}-10^{-5} mM thalidomide or dexamethasone, concentrations which caused a dose-dependent inhibition of TNF-α production. Cells were harvested at 0 to 8 hours and levels of NF-κB measured in nuclear extracts by electrophoretic mobility shift assays using a radiolabeled DNA probe specific for NF-κB and analyzed by optical densitometry. Nuclear levels of NF-κB were unaffected by thalidomide at all concentrations tested, including those (10^{-1}-10^{-3} mM) which resulted in significant inhibition of TNF-α mRNA and protein expression. In concurrent experiments, dexamethasone was found to reduce NF-κB levels in a dose-dependent manner. TNF-α gene expression is controlled by at least three separate transcription factors that are involved in binding to the promoter region. These observations suggest that thalidomide does not act directly upon NF-κB and therefore inhibits TNF-α production through another independent mechanism.

1424 SUPPRESSIVE EFFECTS ON LYMPHOCYTES FUNCTION BY MICROCYSTIN COMPOUNDS.

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Microcystin and nodularin are cyclic peptides produced by cyanobacteria. In the present study, we studied immunosuppressive effect of microcystin and nodularin. Microcystin-LR and -YR form and nodularin produced a dose-dependent inhibition of *in vitro* polyclonal antibody response and lymphoproliferative response to LPS. Microcystin-YR and nodularin also decreased lymphoproliferative response to ConA, but microcystin-LR showed no significant effect. Intraperitoneal administration of nodularin produced an inhibition of primary humoral immune responses to the T-cell dependent antigen, sRBC. Microcystin-LR, -YR, and nodularin also reduced IL-2 mRNA expression in murine splenocytes and thymocytes. When IL-2 mRNA stability was evaluated using quantitative/competitive RT-PCR, nodularin treatment led to rapid decline of IL-2 mRNA levels if actinomycin D was treated to PMA plus ionomycin (Io)-stimulated splenocyte culture. To further characterize the mechanisms for the reduced IL-2 mRNA level, the binding activity of transcription factors for IL-2 gene expression was measured by electrophoretic mobility shift assay. Nodularin reduced the NF-AT binding activity in PMA/Io-induced splenocytes, but no significant effect was observed on AP-1, NF-κB, and Oct binding activity. These results suggested that immunosuppressive effect of microcystin compounds might be due to the decreased IL-2 gene expression which was mediated by the inhibition of NF-AT binding activity. (Supported by the grant from the Ministry of Science and Technology, 97-N1-05-03-A-01.)

1425 *IN VIVO* INDUCTION OF TRANSCRIPTION FACTORS BY LIPOPOLYSACCHARIDE (LPS) THAT CONTROL SPLENIC PROINFLAMMATORY CYTOKINE TRANSCRIPTION.

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The effect of *in vivo* LPS exposure on the binding of transcription factors responsible for controlling gene expression of the proinflammatory cytokines TNF-α, IL-6 and IL-1β was assessed. The kinetics of AP-1, NF-IL6, NF-κβ, CREB, AP-2α and SP1 binding was measured in spleens of mice exposed ip to LPS (4 mg/kg BW) over an 8 hr period. AP-1 binding activity was elevated to 34- to 54-fold between 0.5 and 4 hr in LPS-treated mice. LPS increased NF-IL6 binding 108-fold within 0.5 hr with full recovery being observed by 4 hr. NF-κβ binding was unaffected. In LPS -treated mice, CREB, AP-2α and SP1 binding increased slightly after 0.5 hr but decreased markedly below control values up to 8 hr. Specificities of the transcription factors were verified by competition assays with unlabeled consensus and mutant oligonucleotides. Supershift and Western blot analysis were used to identify the LPS-inducible DNA binding proteins for AP-1(c-Jun[KM-1], Jun B, Jun D, and c-Jun AP-1 at 30 min; c-Jun/AP-1 at 4 hr), NF-IL6(c/EBPβ), NF-κβ(P65, P50 and c-rel at 30 min; p50 at 4 hr) and CREB(ATF-2 and CREB-1 at 30 min; CREB 2 at 4 hr). Taken together, the results indicate that LPS can modulate *in vivo* binding activities of transcription factors specific for regulatory motifs in TNF-α, IL-6 and IL-1β promoter regions. (Supported by PHS Grants ES 03358 and ES 09521.)

1426 INDUCTION OF MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1) AND C-C CHEMOKINE RECEPTOR 2B (CCR2B) EXPRESSION IN THE LIVER DURING ACETAMINOPHEN-INDUCED HEPATOTOXICITY: ROLE OF TUMOR NECROSIS FACTOR-ALPHA (TNF-α).

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Macrophages have been shown to play a critical role in acetaminophen-induced hepatotoxicity. These cells release cytotoxic mediators such as nitric oxide and peroxynitrate which contribute to the pathogenic process. Macrophage accumulation at injured sites is mediated by chemotactic cytokines. One of the most active members of this cytokine family is MCP-1 which acts by binding to a highly specific receptor, CCR2b. We speculated that MCP-1 may be involved in the recruitment of macrophages into the liver during acetaminophen-induced hepatotoxicity. To test this theory, we analyzed MCP-1 and CCR2b expression in the liver. Male C57BL6/129Sv mice were injected with acetaminophen (300mg/kg, i.p.) or PBS control. Animals were sacrificed 1-24 hr later and liver tissue collected and processed for RT-PCR. Acetaminophen administration was associated with a time dependent induction of MCP-1 mRNA in the liver which was evident within 6 hr of treatment of the mice and reached a maximum by 24 hr. This was correlated with increased serum MCP-1. Acetaminophen treatment also induced CCR2b mRNA expression which reached maximum levels after 12-24 hr. TNFα has been reported to upregulate MCP-1 expression. To analyze the role TNFα in chemokine expression in the liver following acetaminophen administration, we used mice with a targeted deletion of the gene for the TNFα p55 receptor. MCP-1 and CCR2b mRNA expression were significantly reduced in p55-/- mice treated with acetaminophen, when compared to wild type controls. Serum MCP-1 levels were also decreased. These data suggest that TNFα regulates the expression of MCP-1 and CCR2b in the liver in this model of hepatotoxicity. Moreover, this chemokine may be involved in macrophage infiltration into the liver after acetaminophen administration. (Supported by NIH grant GM34310 and the Burroughs Wellcome Fund.)

1427 CANNABINOL ENHANCEMENT OF ANTI-CD3/CD28-INDUCED INTERLEUKIN-2 PRODUCTION IS MEDIATED THROUGH THE PKC/MAPK PATHWAY.

T. Jan and N. Kaminski. *Michigan State University, East Lansing, MI.*

One of the key features of cannabinoid-mediated biological effects on the immune system is the modulation of cytokine expression by T cells. Full activation of T cells requires dual stimulatory signals through the T cell receptor/CD3 complex and CD28. In the present studies, the effects of cannabinol on IL-2 production induced by soluble anti-CD3 and anti-CD28 mAb (CD3/CD28) in murine splenocytes was studied. CD3/CD28 induced only modest IL-2 production as compared to PMA/ionomycin. Although PMA alone was incapable of inducing IL-2, it synergized with ionomycin, CD3 or CD3/CD28 to dramatically enhance the production of IL-2 which was all significantly attenuated by PD098059, an inhibitor of MEK-1. These results suggest that PMA-activated PKC/MAPK pathway plays a key role in IL-2 regulation and that CD3/CD28 does not fully activate the MAPK pathway. Among the different stimuli examined, only CD3/CD28-induced IL-2 was markedly enhanced by cannabinol pretreatment, whereas the others were all suppressed. The cannabinol-mediated enhancement of IL-2 production was abrogated by staurosporine (a PKC inhibitor), but not by dibutyryl-cAMP, pertussis toxin or cannabinoid receptor antagonists (SR141716A and SR144528). Western blot analysis revealed that cannabinol up-regulated the MAPK signaling pathway as evidenced by the elevation of phosphorylated extracellular signal-regulated kinase-1 (ERK-1) and ERK-2 in the nucleus of cannabinol-pretreated and CD3/CD28-activated splenocytes. Alike IL-2 production, the increase in ERK-1 and ERK-2 was also abrogated by PD098059. Taken together, the present studies demonstrate that cannabinol enhances CD3/CD28-induced IL-2 production by up-regulating the PKC/MAPK pathway in murine splenocytes, which is not mediated through the cannabinoid receptor and cAMP pathway. A second important observation is that cannabinol can differentially modulate IL-2 production by primary T cells depending on the activation stimulus. (This work was supported by NIDA Grant DA07908.)

1428 DISSECTION OF THE EFFECTS OF PROPANIL ON T CELL SIGNALING PROCESSES USING DNA ARRAY TECHNOLOGY.

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Propanil (3',4'-dichloropropionanilide), a post-emergence amide class herbicide, is used extensively during the cultivation of rice and wheat. Previous work in our laboratory has demonstrated that mice exposed to propanil have decreased T cell-dependent responses, decreased NK activity, and thymic atrophy with a decrease in the $CD4^+CD8^+$ immature thymocyte population. Analysis of cytokine production *in vivo* demonstrated a decreased production of interleukin (IL)-2, IL-6, Granulocyte/ Macrophage-Colony stimulating factor and interferon-gamma two days after exposure to propanil. In vitro, the mouse T cell line, EL-4, when stimulated with PMA and ionomycin in the presence of propanil (50 μM or less) had decreased IL-2 secretion, mRNA levels and stability but normal IL-2 mRNA kinetics. Recently, we have begun to dissect the steps in the signal transduction pathway that leads to IL2 production using the human T cell line, Jurkat. Preliminary data suggest that expression of some immediate early genes of the mitogenic and CREB signal pathways, in particular egr-1, are decreased by exposure to propanil. In addition, a marked increase in mRNA levels of several stress-induced and apoptotic pathway proteins including hsf-1, hsp90, inducible-nitric oxide synthase (iNOS), ATF-2 and Bax was observed. Many other genes, e.g., c-*fos* and I-

kappaBalpha, showed no change in expression levels. Taken together, these results suggest that exposure to low doses of propanil affects a multitude of important signal transduction proteins involved in T cell activation and IL-2 production. (Supported by NIH grant ES07512.)

1429 MICROARRAY ANALYSIS OF GENE EXPRESSION PATTERNS INDUCED BY IRRITANT AND SENSITIZING CHEMICALS.

B. He, A. E. Munson and B. J. Meade. *National Institute for Occupational Safety and Health, Morgantown, WV.*

Chemical-induced dermatitis continues to be an important occupational health problem. Despite decades of investigation, the molecular mechanisms underlying chemical induced hypersensitivity and irritation remain unclear due to the complicated interplay between properties of different chemicals and the immune system. In this study gene expression patterns induced by Toluene Diisocyanate (TDI, IgE-inducing sensitizer), Oxazolone (OXA, cell-mediated hypersensitivity inducing sensitizer) or Nonanoic acid (non-sensitizing irritant) were investigated using gene microarrays. Female BALB/c mice were dermally exposed on the ears once daily for 4 consecutive days. On day 5 the lymph nodes draining the exposure sites were collected and used for mRNA extraction. For TDI and OXA exposures, the concentrations used induced similar quantities of mRNA in the draining lymph nodes. The extracted mRNAs were reverse transcribed into cDNAs and then *in vitro* transcribed into biotin-labeled cRNAs. The hybridization of labeled cRNAs to GeneChip Mu6500 oligonucleotide arrays (Affymetrix,CA) and scanning were conducted by Research Genetics, Inc. AL. Of the 6,500 genes on the arrays, there were 19 genes whose expression levels were significantly different between TDI- and nonanoic acid-treated samples, 18 genes between OXA and nonanoic acid samples and 33 genes between TDI and OXA samples. These include immune response-related genes, transcriptional factors, signal transducing molecules and Expressed Sequence Tags. Microarray analysis identified differentially expressed genes which can be further investigated by conventional methods. Candidate genes will be chosen for further evaluation following exposure to a panel of chemical sensitizers and irritants. Further studies will be conducted to define the functional roles of these genes.

1430 A STUDY ON AN EXPOSURE MARKER FOR SAFROLE.

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Safrole is a known animal carcinogen. It exists in sassafras oil, black pepper, ginger, and *Piper* betle inflorescence, etc. The latter is an unique ingredient of the Taiwanese betel quid. To assess the contribution of safrole in the induction of oral and liver cancer among the Taiwanese betel quid chewers, it is desirable to have an assessable biomarker for exposure. It has been shown that the main urinary metabolite of safrole is the glucuronide conjugate of 1,2-dihydroxy-4-allylbenzene (DHAB). DHAB standard was synthesized via a demethylation of eugenol. Its structure was confirmed by NMR and a ≥95% purity by NMR, TLC, and HPLC analyses. Urine sample preparation involved a 16-hour anaerobic hydrolysis with β-glucuronidase and extraction with ethylacetate. Quantitative analysis was accomplished by an isocratic HPLC on an ODC column with UV detection. The linearity of the standard curve (3.5 to 28 μg/mL) was good (r≥0.997), all relative errors were <±12%, and recovery was 70.2±6.7% (n=4). When male Wistar rats were given safrole in corn oil, at 0, 75, 150, and 300 mg/kg, both the first and second day urines showed a linear dose-response relationship of r=0.90 and 0.99, respectively. The method was then applied to 11 human subjects, 7 betel quid non-chewers and 4 chewers. The results showed that DHAB of the non-chewers ranged from 0.75 to 17.75 μg/mg creatinine (crn) with a median of 1.64. For the chewers, it was 1.47~12.16 with a median of 4.17 μg/mg crn, which was ~2.54 times of the non-chewer's. Thus, it is concluded that DHAB can be the biomarker of exposure to safrole. (Supported by grant DOH88-HR-802, ROC.)

1431 EFFECT OF GENETIC POLYMORPHISMS OF CYP2A6 AND 2E1 ON URINARY COTININE LEVELS IN JAPANESE.

T. Kawamoto. *University of Occupational and Environmental Health, Kitakyushu, Japan.* Sponsor: K. Toshihiro.

Among nicotine metabolites, urinary cotinine has been popularly used as an exposure biomarker for cigarette smoking. However, it is well known that there are individual differences in nicotine kinetics and metabolism. In order to clarify the effects of the genetic polymorphims of drug metabolizing enzymes on nicotine metabolism, urinary cotinine levels in Japanese students and office workers were studied in the relation with cytochrome P4501A1 (CYP1A1), CYP2A6, CYP2E1, aldehyde dehydrogenase 2 (ALDH2), N-acetyltransferase 2 (NAT2) and glutathione S-transferase M1 (GSTM1). As a result, cigarette consumption and homozygous CYP2A6 deletion significantly affect urinary cotinine levels. The genetic polymorpshim in 5'-flanking region of CYP2E1 can be also considered as a clue of individual differences in nicotine metabolism. In a smoking challenge test, cumulated urinary cotinine excretion in the homozygously CYP2A6 deleted individuals was about one seventh as compared to the controls that have CYP2A6*1 allele.

1432 HUMAN SKIN SENSITIZATION POTENTIAL, *IN VIVO*: GLUCOWATCH® BIOGRAPHER.

D. R. Wilson and S. J. Fermi. *Cygnus, Inc., Redwood City, CA.* Sponsor: W. G. Reifenrath.

The GlucoWatch® biographer, developed by Cygnus, Inc., is a frequent, automatic, and non-invasive glucose monitor intended to provide diabetics with the information needed to control their glucose levels. Sponsored by Cygnus, Hill Top Research, Inc. (Miamiville, OH) performed a clinical trial evaluating the potential of the active GlucoWatch® biographer to induce contact sensitization (allergic skin response) in 31 diabetic and 56 non-diabetic subjects via Repeat Insult Patch Test. The active test system delivered iontophoretic current (up to 0.32 mA/cm^2 for 6 minutes during each 20 minute glucose measurement cycle). When worn, the following components of the biographer contacted the skin surface at distinctly different regions; i.e., device edge, active glucose collection gel pad (with glucose oxidase), blank gel pad (without glucose oxidase), skin contact adhesive area, and sweat sensors. These skin regions were evaluated separately. There were nine 14-hour biographer applications in the induction phase, and (after a 13 to 16-day break) a single 8-hour application in the challenge. 99 subjects initially enrolled in the study; 12 subjects dropped out, (9 diabetics and 3 non-diabetics). None of the diabetics dropped out for reasons related to diabetes. There were no serious or unexpected adverse events during the study. No subject scored greater than a 2 (moderate) for erythema or a 2 (light) for edema during the Induction or Challenge Period. Conclusion: There was no indication of contact sensitization in either diabetic or non-diabetic subjects.

1433 BIOMARKERS OF INTERNAL DOSE IN WORKERS EXPOSED TO CHLORONITROBENZENE.

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Para-chloronitrobenzene (p-CNB) has multiple pharmaceutical and agrochemical applications. P-CNB acts as a haematotoxic agent following metabolic activation to the phenylhydroxylamine. The aim of this study was to determine the occupational exposure level, and the internal dose, of p-CNB in exposed factory workers, from the Peoples Republic of China. Eight hour time weighted average (TWA) workplace exposure levels (n=21) ranged from 1.604 - 78.26mg/m^3 (mean: 7.97mg/m^3, UK 8hr TWA limit=1mg/m^3). Urine samples were collected from workers for the determination of recent exposure. HPLC-UV analysis of spot urines from exposed workers (n=43) revealed three metabolites; p-chloroaniline (p-CA), 2-chloro-5-nitrophenol (CNP) and N-acetyl-S-(4-nitrophenyl)-L-cysteine (NANPC). P-CA was detected in 30 (mean: 0.470μ mol/mmol creatinine, range: 0.140-2.47μ mol/mmol creatinine); CNP was detected in 41 (0.620μ mol/mmol, 0.006-4.779μ mol/mmol) and NANPC was detected in 42 (0.819μ mol/mmol, 0.158-2.574μ mol/mmol) of the exposed samples. Metabolites were not detected in non-exposed controls (n=10), within the detection limits of the assay (0.167μ mol on column). Blood samples were collected for the determination of cumulative exposure dose. Haemoglobin (Hb) was hydrolysed in base. The released p-CA was derivatised with pentafluoropropionic anhydride and quantified by GC-MS using single ion monitoring and negative chemical ionisation. The mean adduct levels from exposed (n=43) and factory controls (n=17) were 8.09pmol/mg Hb (range: 0.15-17.06pmol/mg Hb) and 1.70pmol/mg Hb (0.14-8.22pmol/mg Hb) respectively. The presence of p-CA was confirmed by GC-MS electron impact ionisation. No significant correlation was found between external exposure and markers of internal dose, or between urinary metabolite and Hb adduct levels. The urinary metabolites provided markers of short-term exposure, reflecting the inter-day variability in external exposure dose. The presence of Hb adducts and absence of urinary

metabolites in factory controls confirmed exposure had occurred in these workers over the life-span of the erythrocytes. Therefore Hb adducts are markers of the integrated body burden following exposure over a longer time period. In conclusion, it is important to quantify both short and long-term markers of exposure to detect variability in exposure levels with time.

1434 BIOMARKERS OF OXIDATIVE STRESS STUDY I. ARE PLASMA ANTIOXIDANTS MARKERS OF CC14 POISONING?

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Antioxidants in the blood plasma of rats were measured as part of a comprehensive, multilaboratory validation study searching for non-invasive biomarkers of oxidative stress. For this initial study an animal model of CCl4 poisoning was studied. The time-(2, 7 and 16 h) and dose-(120 mg/kg and 1200 mg/kg, i.p.) dependent effects of CCl4 on plasma levels of alfa-tocopherol, ascorbic acid, glutathione (GSH and GSSG), uric acid and total antioxidant capacity were investigated to determine whether the oxidative effects of CCl4 would result in losses of antioxidants from plasma. The alfa-tocopherol concentration was decreased in CCl4-treated rats. However, due to concomitant decreases in cholesterol and triglycerides, it was impossible to dissociate oxidation of a-tocopherol from generalized lipid changes. Ascorbic acid levels were higher with treatment at the earliest time point; the ratio of GSH to GSSG generally declined, and uric acid remained unchanged. Total antioxidant capacity showed no significant change except for 16 h after the high dose, when it was increased. These results suggest that plasma changes due to liver malfunction and rupture of liver cells impede detection of any potential changes in the antioxidant status of the plasma.

1435 DETERMINING AN OCCUPATIONAL DERMAL EXPOSURE BIOMARKERS FOR ATRAZINE THROUGH MEASUREMENT OF METABOLITES IN HUMAN URINE BY HPLC ACCELERATOR MS.

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Metabolites of atrazine were measured in human urine after dermal exposure using HPLC to separate and identify metabolites and accelerator mass spectrometry (AMS) to quantify them. Ring labeled 14C-atrazine was applied for 24 h to the forearms of human volunteers at low (0.167 mg, 6.45 microCi) and high (1.98 mg, 24.7 microCi) doses. The effective radiation doses to the subjects were in the 1-30 microSv range, similar to that delivered in a dental X-ray. Urine was collected for seven days. The urine was centrifuged to remove solids and the supernatant was measured by liquid scintillation counting prior to injection on the HPLC to insure that <0.17 Bq (4.5 pCi) was injected on the column. Peaks were identified by co-elution with known standards. Elution fractions were collected in 1 min increments, half of each fraction was analyzed by AMS to obtain limits of quantitation of 14 amol. Mercapturate metabolites of atrazine and dealkylated atrazine dominated the early metabolic time points, accounting for approximately 90% of the 14C in the urine. No parent compound was detected. The excreted atrazine metabolites became more polar with increasing time, and an unidentified polar metabolite that was present in all samples became as prevalent as any of the known ring metabolites several days after the dose was delivered. The mercapturate metabolites of atrazine appear to be the best class of biomarkers of dermal exposure. Knowledge of metabolite dynamics is crucial to developing useful assays for monitoring atrazine exposure in agricultural workers. (Work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract W-7405-ENG-48.)

1436 PMN FUNCTION AS BIOMARKER OF OXIDANT EXPOSURE.

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Welders are exposed in their work to oxidizing air pollutants like ozone and nitric oxide, as well as to metal oxides. Peripheral blood neutrophils of welders and of an age and smoking habits matched group were tested for PMA-stimulated release of superoxide anions. We found that the stimulated release of superoxide anions from PMN isolated from welders was significantly lower (p=0.021) than that of the matched group. Smokers and older workers had a lower PMN function as compared to that of non- smokers and younger workers. In order to elucidate this effect, another set of experiments were carried out in which PMN from healthy volunteers were also tested for the stimulated release of superoxide anions after being incubated with plasma of welders and of a matched (age and smoking habits) control group. These experiments showed that stimulated superoxide production was inhibited ($p<0.05$) by: 1) incubation with plasma from welders as compared to that of a matched group; 2) plasma from elderly individuals as compared to that of younger ones; 3) plasma from smokers as compared to that of non-smokers. These findings indicate that age and exposure to oxidants and cigarettes smoke induce the appearance in plasma of factors which affect neutrophil function. Determination of these factors could potentially be used to detect the exposure level of individuals to environmental pollutants. There is a definite advantage of analyzing plasma instead of PMN from exposed individuals.

1437 PHYSICOCHEMISTRY AND PULMONARY TOXICOLOGICAL PROPERTIES OF ORIMULSION FLY ASH.

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Orimulsion® is a fuel source that could be an economical substitute for coal and heavy oil currently used in utility and other types of commercial boilers. The environmental and public health risks associated with the combustion of Orimulsion® are unknown. This study compares the physicochemical and acute pulmonary toxicological properties of Orimulsion fly ash (OFA-100 and OFA-400) derived from the combustion of two forms of Orimulsion with similar properties of fly ash obtained from the combustion of a conventional fuel source, residual oil #6 (ROFA #6). Fuels were burned in a Package Boiler Simulator under identical combustion conditions and oil fly ash particles (<2.5μm MMAD)were collected on filters. Collected OFA-100, OFA-400 and ROFA #6 were analyzed for: particle acidity; 1MHCl and water soluble metal (V, Ni, Fe, Cu, Zn, Pb, Cd); and sulfate content. Particle acidty, sulfate level, as well as acid and water soluble total metal content were comparatively similar for all oil fly ash samples examined. OFA-100 and OFA-400 filters had considerably less water soluble metals when compared to the ROFA #6 filter, with the exception of elevated Ni and V levels in the OFA-400 filter. Male, 60 - 70 day old, Sprague-Dawley rats were exposed by intratracheal instillation to water extracts of each oil fly ash filter. Animals were examined for biomarkers of injury by bronchoalveolar lavage at 24h post-exposure. The measurements of edema and inflammation for all oil fly ashes were comparable at low dose levels. However, OFA particles were found to elicit significantly greater inflammatory responses at higher doses when compared to ROFA #6. ROFA #6 particles were found to induce more pulmonary edema when compared to animals exposed to either OFA sample. These results demonstrate that OFA-100 and OFA-400 particles display similar physicochemical properties when compared to ROFA #6. However, OFA-100 and OFA-400 particles led to a pro-inflammatory response, whereas the ROFA particles caused more lung edema. (This abstract has been reviewed and does not necessarily reflect EPA policy or endorsement. Research was support by the USEPA-UNC-CH Training Agreement #CT 826513.)

1438 NITROUS OXIDE EXPOSURE ASSESSMENT IN OPERATING-ROOM PERSONNEL: METHIONINE SYNTHASE AND HOMOCYSTEINE MONITORING.

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Prolonged exposure to nitrous oxide may cause adverse effects on both the hematologic and nervous system in humans; the clinical symptoms resemble those observed in vitamin B12 deficiency. In patients receiving nitrous oxide anesthesia, a considerable inactivation of methionine synthase in mononuclear white blood cells, as well as an increase in plasma homocysteine were observed. The present research focuses on the relationship between the extent of occupational exposure to the anesthetic gas nitrous oxide (N2O) and the behavior of methionine synthase in lymphocytes and homocysteine in plasma Three groups of humans, 30 in each group were evaluated: medical doctors, mainly anesthetists, operating room nurses and non-exposed matched subjects. Homocysteine levels in plasma were determined by HPLC with a fluo-

rescent detector. The method is based on the pre-column derivatization of homocysteine with ABDF. The activity of methionine synthase (holo and apo-enzyme) in lymphocytes was determined by measuring the production of 14C- methionine from 14C- methionine tetrahydrofolate with and without addition of methyl cobalamine.The obtained results (mean±SD) were: Homocysteine (microg/ml): 1.18±0.32, 1.14±0.28 and 1.25±0.44 for M.D.'s, nurses and control group respectively. Methionine synthase holo-enzyme asp-mol/hr/million cells was 99.8±29.8, 76.3±9.7 and 100.4±22.3, while methionine synthase holo and apo-enzyme was 158.2±33.7, 153.1±60.7 and 150.0±29.9 for the same study groups respectively. One can see that there is no significant difference between the levels of homocysteine and of methionine synthase of the three groups considered. Thus, it appears that the levels of nitrous oxide in the operating rooms of the hospitals included in this study are sufficiently low and therefore do not affect the biochemical parameters evaluated.

1439 FUMONISIN B1-INDUCED LIVER TOXICITY, LIVER FREE SPHINGANINE, AND LIPID PEROXIDATION COMPARED IN SIX DIFFERENT STRAINS OF MALE MICE.

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Fumonisin B1(FB1) is a fungal toxin that inhibits ceramide synthase, a key enzyme in the de novo sphingolipid biosynthesis pathway. In Sprague Dawley and Fischer 344 rat, New Zealand white rabbit, and BALB/c and other mouse strains, disruption of sphingolipid metabolism has been hypothesized to be an important mechanism of action of FB1-induced liver toxicity. However, there is one report that liver toxicity in rodents is not related to ceramide synthase inhibition based on the fact that the IC50 for ceramide synthase inhibition in primary rat hepatocytes is ~0.1 micromolar, whereas, evidence of cytotoxicity first occurs at ~75 micromolar FB1. This retrospective study was conducted to determine the relationship between liver toxicity based on blind pathology scores from H&E stained liver sections, serum alanine aminotransferase (ALT), liver free sphinganine, and lipid peroxidation (TBARS) in liver from six mouse strains dosed subcutaneously with 2.25 mg FB1/kg body weight/day for 5 days. The results show that there is a close correlation between the liver toxicity scores and liver free sphinganine (an indicator of ceramide synthase inhibition), and between the serum ALT and free sphinganine. There was no increase in lipid peroxidation based on the total thiobarbituric acid positive substances. Thus, in male mice, when liver pathology is observed, there is a close correlation between the incidence and severity of the pathology, increased serum liver enzymes and free sphinganine indicative of disrupted sphingolipid metabolism. (Supported in part by NIH ES09403.)

1440 ASSESSING THE STABILITY OF HEMOGLOBIN ADDUCTS AFTER ADMINISTRATION OF BENZENE TO MALE F-344 RATS.

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Sponsor: J. A. Swenberg.

Hemoglobin (Hb) adducts have potential for use in estimating the blood dose of electrophilic benzene metabolites. To relate adduct formation with dose, knowledge of adduct stability is necessary. The constant, predetermined lifespan of the red blood cell leads to zero-order kinetics of adduct removal if Hb adducts are stable. For unstable adducts, mixed zero- and first-order kinetics describe the loss of adduct by simultaneous hemoglobin turnover and chemical instability. The stability of total radiobound adducts ([^{14}C]R-Hb) and cysteinyl adducts of benzene oxide ([$^{13}C_6$]BO-Hb) was investigated in Hb of male F344 rats following administration of a single oral dose of 400 mg [U-^{14}C/$^{13}C_6$] benzene/kg body weight. The decay trends of both [^{14}C]R-Hb and [$^{13}C_6$]BO-Hb were best described by a zero-order process, with lifetimes for [^{14}C]R-Hb and [$^{13}C_6$]BO-Hb (47 and 55 days, respectively) that were consistent with the lifetime of rat erythrocytes (45-65 days). Based on these findings, BO-Hb can be used in human populations to estimate the average daily dose over the ~120 day lifetime of the erythrocyte.

1441 ARSENIC AND LEAD EXPOSURE IN CHILDREN LIVING IN SMELTER AREAS OF MEXICO AND BOLIVIA.

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The objective of this study was to compare the levels of environmental contamination and children exposure in areas next to primary and small scale secondary smelters (S-smelters). The primary smelter was a copper metallurgical located in the community of Morales in San Luis Potosi, Mexico, and the S-smelters were located in the Bolivian site of El Alto Lima. In El Alto Lima and Morales, arsenic and lead contamination was found in soil and dust (concentrations in soil were above the guidelines recomended by the United States Environmental Protection Agency). In Morales, the arsenic concentration in airborne particulate was 0.56 µg/m3. Children in Morales and in El Alto Lima, had similar geometric means of arsenic in urine; and in El Alto Lima, an inverse correlation was found for age and urinary arsenic ($r = -0.28$, $p<0.01$). Also, an inverse correlation was registered for age and blood lead levels (PbB) in children living in El Alto Lima ($r = -0.29$, $p<0.01$). Children aged 5-7 years presented the highest PbB and 44% of them had levels higher than 10 µg/dl. In Morales, a direct correlation between age and PbB was found at this site, ($r=0.29$, $p<0.01$). In the age group of 7-9 years, 56% of the children had PbB higher than 10 µg/dl and 8% had levels above 20 µg/dl. It is evident that studies are needed in order to identify adverse health effects in children living in the smelter sites; nevertheless, it is more important to develop risk reduction programs. (This work was supported by a grant from the Consejo Nacional de Ciencia y Tecnología, Sistema de Investigación Miguel Hidalgo [RN-27/96].)

1442 FORMATION OF HEMOGLOBIN ADDUCTS IN FEMALE RATS AND MICE EXPOSED TO BUTADIENE.

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1,3-Butadiene (BD) is an important industrial chemical that is carcinogenic in mice and rats. The present study was conducted to compare the formation of N-terminal valine adducts, hydroxybutenylvaline (HBVal) and trihydroxybutylvaline (THBVal), in hemoglobin from female F344 rats and B6C3F1 mice exposed to 0 or 1250 ppm BD for 1, 5 or 10 days. Briefly, globin was extracted from washed red blood cells, subjected to a modified Edman degradation and washed on C_{18} columns. An aliquot was removed for HBVal-pentafluorophenylthiohydantion (PFPTH) quantitation while the remainder was acetylated for quantitation of THBVal-PFPTH. Synthesized external standards for both HBVal-PFPTH and THBVal-PFPTH were used after Edman degradation. Gas chromatography/tandem mass spectrometry (GC/MS-MS) was used to quantitate HBVal-PFPTH, while THBVal-PFPTH was measured by either GC/high resolution mass spectrometry (GC/HRMS) or GC/MS-MS. The use of GC/MS-MS has improved the sensitivity and resolution for THBVal-PFPTH over GC/HRMS. Results show a linear increase in the formation of N-terminal valine hemoglobin adducts. Female mice had HBVal values of 0.4, 1100, 4410, and 7360 pmol/g globin at 0 ppm exposure and 1250 ppm BD for 1, 5, or 10 days, respectively. The THBVal values in female mice were 540, 4200, 19800, and 35900 pmol/g globin at 0 ppm exposure and 1250 ppm BD for 1, 5, or 10 days, respectively. Female rats had HBVal levels at 0.04, 180, 830, 1390 pmol/g globin at 0 ppm exposure and 1250 ppm BD for 1, 5, or 10 days, respectively. The THBVal levels in these same rats were 200, 1227, 4720, and 8020 pmol/g globin at 0 ppm exposure and 1250 ppm BD for 1, 5, or 10 days, respectively. These data clearly show that hemoglobin adducts accumulate in a linear fashion in both rats and mice, that THBVal is found in greater amounts than HBVal, and that mice form more adducts than rats. (Supported in part by the Chemical Manufacturers Association.)

1443 PROTEOMIC ANALYSIS OF RENAL AND HEPATIC PROTEIN EXPRESSION IN RATS EXPOSED REPEATEDLY TO JET FUEL VAPOR.

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Male Sprague-Dawley rats were exposed by whole body inhalation to JP-8 jet fuel vapor (1000 mg/m3 + 10%) or room air control conditions for 6 hr/day, 5 days/wk for 6 consecutive weeks (180 hr). Following an 82-day recovery period, rats were sacrificed and tissues evaluated for altered expression of 500+ proteins. Tissues were examined for both total protein abundance and protein charge modification. Kidney and liver samples were solubilized and separated via large scale, high resolution two-dimensional electrophoresis (2-DE) and gel patterns scanned, digitized and processed for statistical analysis. Through the use of peptide mass fingerprinting, confirmed by sequence tag analysis, three altered proteins were identified and quantified. Numerically, but not statistically significant increases were found in total abundance of lamin A (NCBI accession no. 1346413) in the liver, and of 10-formyltetrahydrofolate dehydrogenase (10-FDH; #1346044) and glutathione-S-transferase (GST; #2393724) in the kidneys of vapor exposed subjects. Protein charge modification index (CMI) analysis indicated significant alterations ($p<0.001$) in expressed lamin A and 10-FDH.

1444 ANTIOXIDANT EFFECTS OF BLACK TEA IN SMOKERS AND NON-SMOKERS.

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While the chemopreventive effects of green tea in humans have been examined, less information is aviliable on black tea (the most commonly consumed form of tea in Europe and North America). Both black tea and green tea have been shown to reduce carcinogen-induced tumorigenesis and mutagenesis in *in vivo* and *in vitro* systems. This chemopreventive activity appears to be based, in part, on the antioxidant properties of tea. Oxidative stress has been implicated as a causative or participating factor in several human diseases including cancer, neurodegenerative diseases, and heart disease. Increased oxidative stress with resulting oxidative damage has been associated with certain lifestyle behaviors, including smoking. We have previously reported a decrease of oxidative stress in an Asia study population and a American study population after consuming green tea. The present investigation examined the effects of black consumption on selected oxidative stress endpoints in an American study group. Study participants (50 males and females; 25-35 years of age; smokers and non-smokers) were given black tea for 2 weeks or placebo for 2 weeks. Blood and urine were sampled after each treatment. Biomarkers of oxidative stress, including oh8dG and MDA in urine and antioxidants, were measured. The relative level of reactive oxygen species (ROS) were evaluated in individuals by determining urinary 2,3-DHBA 6 hours after administrated 1 gram of aspirin. The relationship of oxidative stress biomarkers, dietary intake and tea consumption was analyzed. The results showed that black tea consumption decreased oxidative stress in most individuals, (decreased levels of oh8dG, MDA, and 2,3-DHBA). Cigarette smoke for the most part increased oxidative damage (oh8dG and MDA). These results showed that similar to green tea, black tea also function as an antioxidant in humans.

1445 CRITICAL END - POINTS SELECTED IN A REPORT SYSTEM FOR AFLATOXIN-OCCUPATIONAL EXPOSED POPULATION IN THAILAND.

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In recent years, a wide range of structurally dissimilar xenobiotics appears to be involved in toxicant-initiated oxidative stress. Aflatoxin B1 has previously been shown to alter mitochondrial function by inhibiting the electron transport chain as well as by uncoupling of oxidative phosphorylation. It has been proposed that the involvement of cytokine released from macrophage may inhibit RNA polymerase action and play a role to modify phosphodiester bond in white blood cells of spleen. Recently, prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate have been studied by Philips and colleagues. However, Jakab et al. (1994) reported that respirable particle of dust in animal feed may inhibit alveolar macrophage phagocytosis. We investigated different aluminosilicate as well as zeolite (clinoptilolite) formulations normally used in Thailand as feed adsorbants for their bulk density and particle size distribution. These adsorbants' effects to reduce aflatoxicosis were examined. It is found that clinoptilolite which is a non-selective aflatoxin binder can modulate aflatoxicosis in rats but may present occupational hazard in users. Health surveillance program which provide oxidative-stress toxicants related information were set up for exposed workers. The critical end-points selected are general reproductive health of exposed workers which are routinely monitored in public health report system (congenital malformation of offsprings, fertility and disorder of thyroid gland).

1446 CYP1A1, CYP1A2 AND CYP1B1 MRNA EXPRESSION LEVELS IN HUMAN BLOOD USING A REAL-TIME REVERSE TRANSCRIPTION-PCR SYSTEM.

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Environmental pollutants such as dioxin and related compounds induce CYP1A1, CYP1A2 and CYP1B1 via aryl hydrocarbon receptor-mediated mechanism. EROD activity is a very useful marker for exposure level of those environmental pollutants particularly in experimental settings, but has a demerit of low detection sensitivity in the human subjects in epidemiological studies. To quantify the CYP1A1, CYP1A2 and CYP1B1 mRNA in blood, we have thus developed a real-time quantitative reverse transcription-PCR assay based on TaqMan fluorescence methodology. By applying as little as 1 ng of total RNA from the blood, we could determine CYP1A1, CYP1A2 and CYP1B1 mRNA in the blood from 71 volunteers living in two different areas. Expressed levels of CYP1A1, CYP1A2 and CYP1B1 genes varied among individual participates, the difference of which was thought to reflect in part the exposure levels of TCDD and other environmental pollutants. The quantification by TaqMan fluorescence method of CYP1A1, CYP1A2 and CYP1B1 genes expression level in blood would be a sensitive and accurate method to monitor environmental contamination of analyzing large numbers of samples in a minimized time.

1447 A NEW APPROACH FOR EVALUATION OF EXPOSURE TO ENVIRONMENTAL HAZARDOUS AGENTS.

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The conventional procedures for biological monitoring are not always suitable for the current public health requirements. In this study we propose a unique approach for exposure detection. We have developed a bioassay system based on the sensitivity of *in vitro* cell culture to toxic agents present in the circulation. We have used two systems: a) the animal model in which human Y79 retinoblastoma cells were exposed to serum samples of paralyzed ostriches; b) the human model in which these cells were exposed to sera of males, non smokers, industry workers dealing with production of cholinesterase inhibitors. The assay was performed by exposure of Y79 human retinoblastoma cells to 1.5% human serum or 2% ostrich serum for 3 days and cell viability was measured by the neutral red assay. In the ostrich system, the sera of paralyzed birds caused a statistically significant reduction of 63% in cell viability in comparison to samples of healthy ostriches. Heat-treatment did not reverse the cell killing effect of the specimens obtained from the paralyzed ostriches, indicating that complement was not involved in this phenomenon. In the human system, although the workers were well protected and asymptomatic, there was 32% reduction in cell viability in comparison to that caused by sera of office workers. The present findings may offer a unique approach for diagnosis of latent exposure to hazardous environmental chemicals and to a mixture of harmful agents.

1448 DNA ADDUCT FORMATION IN THE LUNGS OF FEMALE SPRAGUE-DAWLEY RATS TREATED WITH THE ENVIRONMENTAL CARCINOGEN 1-NITROPYRENE.

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DNA adducts are useful biomarkers of exposure and assist in determining the absorption, metabolic activation/detoxification pathways, and DNA repair mechanisms following exposure to genotoxic chemicals. 1-Nitropyrene (1-NP), a well-known tumorigen and carcinogen, is a widespread environmental contaminant from a number of sources the foremost being diesel exhaust. The principal metabolic activation pathway leading to mutation and tumor induction of 1-NP is nitroreduction. Three DNA adducts [*N*-(deoxyguanosin-8-yl)-1-aminopyrene, 6-(deoxyguanosin-N^2-yl)-1-aminopyrene, and 8-(deoxyguanosin-N^2-yl)-1-aminopyrene] have been identified from the nitroreduction of 1-NP and may be responsible for the mutagenicity and carcinogenicity of this nitropolycyclic aromatic hydrocarbon. Each of these adducts have been detected in mammary tissue, a tumor target tissue in rats treated with 1-NP. Since diesel exhaust is a lung carcinogen in rats, we investigated if 1-NP would form DNA adducts in the tissue. Female Sprague Dawley rats were treated with 1-NP by i.p. injection at doses of 25, 50, and 100 mg/kg. The lungs were removed at 12, 24, and 48 hrs and 7, 14, and 28 days after the treatment. Lung nuclei were prepared, DNA was isolated, and DNA adduct formation analyzed by ^{32}P-postlabeling/HPLC. At the 100 mg/kg dose, all three adducts were detected in the lungs at 48 hrs and 7 days. These data suggest that 1-NP is activated by nitroreduction in rat lung and may contribute to the tumorigenicity observed with diesel exhaust.

1449 THE USE OF AN ANDROGEN RECEPTOR-ACTIVATED LUCIFERASE EXPRESSION (AR-CALUX) ASSAY FOR SCREENING OF STEROID GROWTH PROMOTERS IN CATTLE.

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Steroid growth promoters (Gps) are substances that are administered to farm animals in order to enhance the efficiency of meat production. The use of these compounds is prohibited in the European Union (EU). However, GPs are frequently detected in random samples from butchers, stressing the need for reliable, cheap and fast detection methods. Therefore, an androgen receptor mediated chemical-activated luciferase expression (AR-CALUX) assay was developed. In this assay, androgen receptor agonistic potential is quantified by measuring luciferase activity, using a 96 well format. A dose-dependent increase in luciferase induction, up to three-fold, was found with 1 nM R1881, a potent synthetic androgen. Due to a high background signal we expect to find a higher fold induction after improving assay conditions. The natural androgen receptor (AR) ligand dihydrotestosterone as well as the synthetic androgens 17-β-trenbolone and 17-α-trenbolone were less potent inducers in accordance with their lower AR binding and activating potency. Progesterone also induced luciferase expression. However flutamide, an anti-androgen, was able to suppress this response indicating that the response induced by progesterone is AR-mediated. 17-β-Estradiol and dexamethasone did not induce luciferase expression, strongly suggesting that the assay is specific for androgen receptor agonists.

1450 ANALYSIS OF ENVIRONMENTAL AND BIOLOGICAL EXTRACTS FOR DIOXIN-LIKE ACTIVITY UTILIZING A LUCIFERASE REPORTER GENE BIOASSAY.

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Most of the biological and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds are mediated by binding to the aryl hydrocarbon receptor (AhR) and the subsequent interaction of the ligand-activated AhR with dioxin response elements (DREs). A murine hepatoma cell line (hepa 1c1c7) was stably transfected with the p2Dluc plasmid containing two DREs upstream from the luciferase gene. A 96-well plate format has been optimized for this reporter gene bioassay system to assess TCDD/AhR-responsive luciferase activity of dioxin-like chemicals and complex mixtures. The transfected cell line is exceptionally sensitive, detecting levels of TCDD as low as 1 pM (0.06 pg/200 µl/well). The bioassay also exhibits good specificity, with agents having the following relative potencies: TCDD > 2,3,4,7,8, pentachlorodibenzofuran (PeCDF) > PCB 126 > PCB 118 ~ benzo(a)pyrene > omeprazole > PCB 153. A similar maximal signal was obtained with TCDD, PeCDF, and PCB 126. In contrast, PCB 153 (1000 nM) did not show any significant response. This model is currently being utilized as a screening tool to assess the relative dioxin-like activity of defined chemical mixtures and complex mixtures of chemically analyzed extracts from Great Lakes sediments, human milk, and human serum. (Supported in part by ATSDR H75-ATH298338.)

1451 THE TK+/- MOUSE: AN IN VIVO MUTATION MODEL THAT DETECTS LOSS OF HETEROZYGOSITY.

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Loss of heterozygosity (LOH) is a major mechanism by which cells loose tumor suppressor gene function and become cancerous. Until recently, there were no *in vivo* reporter gene systems capable of detecting spontaneous and treatment-induced LOH. Using gene targeting, we have developed mice heterozygous for the endogenous thymidine kinase (Tk) gene in which the wild-type (WT) allele of the Tk gene serves as a target for mutation. The Tk+/- mouse is an *in vivo* analog of Tk+/- mouse lymphoma cells, which are widely used in mutagenicity studies and are highly sensitive to LOH mutation. Tk mutant lymphocytes from Tk+/- mice are identified by their ability to form clones in the presence of the toxic thymidine analog 5-bromodeoxyuridine (BU). Results indicate that the Tk gene of Tk+/- mice detects mutants containing loss of the entire WT Tk allele (LOH), as well as point mutation and intragenic deletion and insertion. We found that more than 50% of the Tk mutants from untreated Tk+/- mice are caused by LOH. Also, LOH occurs frequently in the Tk gene of mice treated with carcinogens that produce mainly point mutation in the endogenous X-linked Hprt gene or in transgenic shuttle-vector genes. The results indicate that Tk+/- mice detect a greater range of the mutations involved in carcinogenesis than do other *in vivo* mutation models. Thus, Tk+/- mice may be a useful tool for assessing risks associated with exposure to potential carcinogens. (Sponsored by the SOT Regulatory and Safety Evaluation Specialty Section.)

1452 CELLULAR AND SUBCELLULAR LOCALIZATION OF PERIPHERAL BENZODIAZEPINE RECEPTORS FOLLOWING TRIMETHYLTIN-INDUCED BRAIN INJURY.

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The peripheral benzodiazepine receptor (PBR) is currently used as a marker of inflammation and gliosis following brain injury. Previous reports have suggested that elevated PBR levels in injured brain tissue are specific to activated microglia and infiltrating macrophages. We have produced hippocampal lesions using the neurotoxicant trimethyltin (TMT) to determine the cellular and subcellular nature of the PBR response. Degenerating, argyrophilic pyramidal neurons were observed in the hippocampus at 2 and 14 days after TMT exposure (8 mg/kg, single injection). Reactive microglia were evident at both time points with a maximal response occurring at 14 days and subsiding by 6 weeks. Astrocytosis was observed at 14 days and 6 weeks after exposure but not at 2 days suggesting a delayed onset but protracted astrocytic response relative to microglia. Morphological evidence from PBR immunohistochemistry and [^{3}H]-PK11195 microautoradiography indicated that both microglia and astrocytes are capable of expressing high levels of PBR after TMT brain injury. This was confirmed by double labeling of either *Griffonia simplicifolia* isolectin B_4 binding, a microglia-specific marker, or glial fibrillary acidic protein (GFAP) immunohistochemistry, an astrocyte-specific marker with PBR fluorescence immunohistochemistry. These results demonstrate that PBR expression is increased after brain injury in both activated microglia and astrocytes. Our findings also suggest the first evidence for nuclear localization of PBR in glial cells. (Supported by ES07062 to TRG.)

1453 BERYLLIUM BLOOD LYMPHOCYTE PROLIFERATION TEST (BLPT): VARIABILITY AND POSITIVE PREDICTIVE VALUE.

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Lymphocyte proliferation assays are widely used as biomarkers for exposures to various metals including beryllium. Results are typically categorized as: abnormal (positive), normal (negative) or borderline. This study evaluated nearly 5000 blood lymphocyte proliferation test (BLPT) results collected since 1992 from three labs during periodic surveys at two beryllium plants in Tucson, AZ and Elmore, OH. Single split blood samples were sent to two independent laboratories. If both labs report negative BLPT-the person is considered not sensitized. Discrepancies between the two labs or uninterpretable results warrant a repeated testing. Two positive BLPTs, either from the two independent labs or from repeat testing at the same lab are sufficient to consider a person beryllium sensitized. Analysis included examination of inter- and intra-laboratory variability by calculating a kappa statistic, and assessment of positive predictive value of BLPT with respect to forecasting chronic beryllium disease (CBD). The level of agreement between the first and the second test for all three labs (intra-lab variability) was fair to moderate with kappa ranging between 0.3 and 0.5. The inter-lab agreement depended on the survey site ranging from poor (kappa=0.1) in Elmore to good (kappa=0.8) in Tucson. An abnormal BLPT test was approximately 50% predictive of CBD as detected on subsequent bronchoscopy. The BLPT has a high degree of variability, its positive predictive value is acceptable, but the sensitivity and specificity are still unknown. BLPT may serve as a useful medical surveillance tool, however it does not meet the criteria for a screening test.

1454 BIOMONITORING FOR BERYLLIUM: EXPERIENCE WITH A U.S. WORK FORCE.

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Experiments in animals indicate that beryllium (Be) has a very low bioavailability, a moderate to long half-life, and that urine is the primary route of excretion for absorbed Be. The feces is the primary route of excretion of unabsorbed Be following oral or inhalation exposure (via mucocilliary escalator). Studies conducted in the 1960s indicated that Be workers had elevated levels of urinary Be but a correlation between levels of Be in urine or blood and airborne concentrations of Be in work areas could not be established. There have been suggestions that workers may be absorbing Be by routes other than inhalation. Therefore, the purpose of this study was to determine whether a biomarker approach (using urine or blood) could be used to evaluate the extent and route of exposure using more modern analytical techniques. If so, the method would be useful in assessing whether workplace behaviors or exposure interventions are effective in limiting human exposures to beryllium in the workplace. The population evaluated consisted of individuals working with beryllium metal, beryllium oxide, and alloys. A control population of administrative personnel from buildings that are separate from the manufacturing facility were included in the survey. Of the193 urine samples collected, only 18 had detectable levels of Be (detection limit of 0.2 μg/L). Based on a comparison to nationwide data collected as part of the National Health and Nutrition Examination Survey (NHANES III), the data indicated that workers exposed to different forms of beryllium in the workplace had urinary levels that were indistinguishable from background levels in the United States. These preliminary results suggest that a more sensitive method for detecting beryllium in biological media is needed. Therefore, studying blood or urine to identify Be exposure will only be appropriate when the quantitative technique is sensitive enough to distinguish Be concentrations in exposed and unexposed populations.

1455 DEVELOPMENT OF METHODS FOR ANALYSIS OF BIOMARKERS OF BENZENE EXPOSURE USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) AND MATRIX ASSISTED LASER DESORPTION IONIZATION (MALDI).

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Because benzene is used extensively in industry and is a widespread industrial pollutant, it is important that biological monitoring accurately reflect the history of exposure. To provide more information on previous exposures than a single biomarker can provide, it is desirable that a battery of biomarkers be used. This work describes the development of assays for longer and shorter-term biomarkers of benzene exposure. Recent biomonitoring studies have focused on assessment of urinary levels of S-phenylmercapturic acid (S-PMA) and trans,trans-muconic acid (t,tMA) as they are specific to benzene and are biomarkers of recent exposure. Methods to assay a specific longer lived biomarker, S-phenylcysteine adducts, in albumin and hemoglobin have also been published, but require extensive preparatory work. Previous studies of the urinary metabolites have largely extracted and analyzed S-PMA and t,tMA separately, by splitting the samples prior to analysis. In this study, methods were developed for the combined extraction, derivatization and analysis of S-PMA and t,tMA from urine via liquid extraction, followed by derivatization using hydrochloric acid/methanol and GC/MS analysis. Recoveries were 90% for S-PMA and 78% for t,tMA from spiked water samples. The limit of detection for t,tMA was 10 ηg/mL and 20 ηg/mL for S-PMA. The best sensitivity for the combined analytes was obtained using positive ionization, although negative ionization was more sensitive for t,tMA alone. MALDI analysis of S-phenylcysteine adducts in albumin was accomplished using tryptic digests and provided sensitivity comparable to current literature methods. (Supported by U.S. EPA Assistance Number R826249-01-0.)

1456 ANALYSIS OF URINARY METABOLITES OF BUTADIENE.

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The toxicity of 1,3-butadiene (BD) varies greatly in rodent species and raises the question of whether the risk of BD exposure in humans is more like that of the sensitive species, the mouse, or more like that of the resistant species, the rat. Numerous studies have indicated that one reason for the species differences in response to BD is that the blood and tissues of BD-exposed mice contain high levels of both the mono- and the diepoxide metabolite of BD, while the tissues and blood of exposed rats contain very little of the diepoxide. The diepoxide is far more mutagenic than the monoepoxide, and it is reasonable that the diepoxide plays a major role in tumor induction in the mouse. A major question is the extent to which humans metabolize BD to the diepoxide, but evidence for diepoxide formation in humans is difficult to obtain. The diepoxide would be expected to clear rapidly from the blood, as is seen in BD-exposed mice. Currently we are testing the hypothesis that the diepoxide along with MI and MII, which are water soluble, would be excreted in the urine and could be used as markers of exposure. To extend this research to humans, diepoxide, MI and MII were assayed for in urine. Diepoxide was studied by monitoring diepoxide protein-binding in urine using a matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method. Briefly, the method involves a protein affinity capture step followed by mass spectrometric detection. The protein that we chose to assay for was β_2-microglobulin (B2M) which is a urinary protein common to primates and rodents and contains many possible adduct sites for diepoxide. Agarose beads are crosslinked with an antibody towards the B2M and this then becomes our affinity column. Results from rat and mouse exposures indicate the presence of diepoxide in the urine of mice but not rats. Urines from workers exposed to BD are being analyzed by electrospray ionization mass spectrometry (LC/MS/MS) for MI and MII. The urine was first cleaned-up by a solid phase extraction method, then simultaneously analyzed for the two metabolites by LC/MS/MS. (Research supported by HEI 97-1, NIH Grants ES-06015 and ES-09401, and CMA Research Agreement OLF-16.0-RES-LRRI.)

1457 AN EMPIRICAL MODEL OF BENZENE EXPOSURE BASED ON MULTIPLE BIOMARKERS.

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To improve human health risk assessment for benzene exposure, an empirically based model using a battery of biomarkers from blood, exhaled air, and urine samples is being developed. For development, testing and validation, the model is being formulated based on experimental measurements from controlled exposure conditions in mice. The critical factors in the development of the model are the use of biomarkers that: 1) can be measured non-invasively, 2) have different clearance patterns, and 3) are relatively specific to benzene. Benzene in exhaled breath, benzene in blood, and phenylcysteine adducts on albumin and on hemoglobin will be used in the model. Although

not necessarily specific to benzene exposure, chromosomal aberrations will also be incorporated as a long-term marker of damage. The logic of the model is as follows: Consider two biomarkers, each with a simple negative exponential clearance as a function of time. Generally, such curves are fit with the fraction or percent of the biomarker as the dependent variable so that all curves are normalized to describe clearance relative to deposition. Because the model should predict exposure, not deposition, this formulation will not provide enough information. The total deposition must be described by some other equation that is a function of the exposure concentration. With two biomarkers, then, there are two equations which can be solved to predict the two unknowns-exposure concentration and time since exposure. The model is being developed for benzene, but if it can be applied successfully to quantifying human doses, the conceptual framework may readily be extended to other compounds. (Supported by U.S. EPA Assistance Number R826249-01-0.)

1458 3-NO_2-ACETAMINOPHEN FORMATION BY REACTIVE NITROGEN SPECIES.

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Reactive nitrogen species (RNS) play a role in a number of physiological and pathological processes. Identifying specific RNS involved in this process is difficult to assess. The reaction of some RNS with acetaminophen (APAP) was evaluated. ^{14}C-APAP (0.06 mM) was incubated with 1 mM spermine-NONO; 1μg/ml myeloperoxidase, 0.05 mM H_2O_2, and 0.1 mM $NaNO_2$; 0.1 mM $NaNO_2$ and 0.1 mM HOCl; 0.1 mM $ONOO^-$; 2 mM Sin-1. Incubations contained 0.1 mM DETAPAC, and were at pH 7.4, 37°C, and for 30 min. Metabolism was assessed by HPLC. Incubations with myeloperoxidase, $ONOO^-$, and Sin-1 yielded a new product representing 7, 3.3, and 1% of the total radioactivity recovered by HPLC. The final pH of these incubations did not change by more than ±0.1 pH unit. The myeloperoxidase reaction was completely prevented with 1 mM NaCN, while the $ONOO^-$ reaction was prevented by preincubation of $ONOO^-$ for 10 min. The new product was identified by ^{1}H NMR and EI/ESI mass spectrometry as 3-NO_2-acetaminophen. Human polymorphonuclear neutrophils incubated with APAP and phorbol ester also synthesized 3-NO_2-acetaminophen. These results suggest that 3-NO_2-acetaminophen may be useful as a marker for certain RNS.

1459 APPLICATION OF SOLID PHASE MICROEXTRACTION TO THE MEASUREMENT OF URINARY BENZENE IN WORKERS EXPOSED TO BENZENE.

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The application of solid-phase microextraction to the determination of urinary benzene (UB) levels and the utility of UB as a short-term biomarker of exposure to benzene was investigated in urine samples collected from 42-exposed workers and 41 controls from Shanghai, China as a part of a cross-sectional study of benzene biomarkers. The mean UB levels in exposed workers (28.3 μg/L) were statistically higher than the controls (0.145 μg/L) ($p=0.0001$). When the log-transformed UB levels were regressed on log-transformed individual benzene exposure (8-h TWA) and other urinary metabolites, significant correlations were observed (UB vs. exposure: $r = 0.80$: UB vs. phenol: $r = 0.83$; UB vs. catechol: $r = 0.85$; UB vs. hydroquinone; $r = 0.87$; UB vs. muconic acid: $r = 0.87$) with $p<0.001$. The use of UB at low levels of benzene exposure will be investigated. (Supported in part by NIH grant P42ES05948.)

1460 SPECTROPHOTOMETRIC ANALYSIS OF SOLUBILIZED RAT HAIR PROTEINS FOLLOWING INTRAPERITONEAL INJECTION OF 2,5-HEXANEDIONE.

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Neurotoxic industrial solvents n-hexane and methyl n-butyl ketone are toxic by virtue of their common metabolite 2,5-hexanedione (2,5-HD). Our previous work (Johnson, et al., J. Toxicol. Env. Health, 1995) has shown that pyrrole-like substances in solubilized rat hair proteins from rats injected ip) daily with 2,5-HD were maximally absorbed in the 530 nm spectral region following reaction with the Ehrlichs reagent (p-dimethylaminobenzaldehyde). Modification of current analytical methods to achieve high specificity and lower detection limits with small sample quantities could have important implications for monitoring human populations. Adult male Sprague-Dawley rats weighing approximately 350 g were housed in individual metabolic cages with food and water provided ad libitum. Individual rats were injected (ip) daily with either 50 mg/kg 2,5-HD or physiologic buffered saline (PBS). Plucked hair samples (dorsal, right flank, and left flank) were obtained from each rat before and at seven-day intervals post 2,5-HD or PBS exposure for 28 days. Hair protein solubilization and extraction procedures were adapted from Marshall and Gillespie (J. of Forensic Sci. Soc.1982). One ml of dialyzed hair protein solution (2,5-HD or PBS control) was read against a reference cuvette containing water or reagent blanks. Analyses were done utilizing Shimadzu UV 160 V recording spectrophotometer at an absorbance spectral range of 450-600 nm. In all spectral tracings absorbance (530 nm) of pyrrole-like substances were only detected in samples from 2,5-HD treated rats. Absorbance at 530 nm was detected starting from 7-14 days post exposure. Further studies utilizing solubilized human hair proteins devoid of pigmentation, with the goal of developing a technique to detect exposure to neurotoxic solvents in human populations occupationally at risk are being carried out.

1461 METALLOTHIONEIN ISOFORM 3 AS A POTENTIAL BIOMARKER FOR HUMAN BLADDER CANCER.

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Bladder cancer is the fifth most common cancer in the United States. Currently, there are no widely accepted tumor markers that allow screening for the early presence of bladder cancer or for the detection and monitoring of advanced metastatic disease. The goal of the present study was to determine if the expression of MT-3 may serve as a biomarker for human bladder cancer. Immunohistochemistry, immuno-blot and RT-PCR analysis were used to define the localization and expression of MT-3 protein and mRNA in fresh and archival biopsy specimens. Immunohistochemical analysis disclosed no immunoreactivity for MT-3 in cells comprising normal bladder. MT-3 mRNA and protein could not be detected in normal bladder using RT-PCR or immuno-blot respectively. Immunohistochemical analysis demonstrated that MT-3 was expressed in *carcinoma in situ* (CIS), high grade bladder cancer, low grade bladder cancer and dysplastic lesions. MT-3 immunostaining was intense in both CIS and high grade bladder cancer, and low to moderate in low grade and dysplastic lesions. The cDNA from the RT-PCR primed for MT-3 was demonstrated to contain a *Fok I* restriction site, a site unique to MT-3. This study demonstrates that MT-3 is up-regulated in human bladder cancer and that this up-regulation increases with increasing tumor grade. The finding that MT-3 expression is minimal in normal bladder suggests that MT-3 could be developed into an effective biomarker for bladder cancer.

1462 METALLOTHIONEIN ISOFORM 1 AND 2 GENE EXPRESSION IN THE HUMAN BLADDER: EVIDENCE FOR UPREGULATION OF MT-1X AND DOWN REGULATION OF MT-1E mRNA IN BLADDER CANCER.

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Metallothioneins (MT) are a family of low molecular weight, cysteine-rich intracellular proteins that bind transition metals. There is a family of genes that underlies the expression of these proteins. Studies have shown an association of metallothionein (MT) over-expression with tumor type and grade. The goal of this study was to define the expression of MT-1 and MT-2 genes

and protein in normal bladder and to demonstrate that the expression of MT-1 and 2 isoforms are altered in bladder cancer. Immunohistochemistry was used to localize MT-1/2 protein and RT-PCR to determine expression of the MT-1 and MT-2 isoform-specific mRNAs. Normal bladder was not immunoreactive for the MT-1/2 protein but did express mRNA for the MT-2A, MT-1E and MT-1X genes. In contrast, bladder cancers were immunoreactive for the MT-1/2 protein with reactivity localized to the cytoplasm in all MT positive tumors and to the nucleus in a small sub-set of these tumors. In general, the intensity of MT immunoreactivity increased with tumor grade, with *carcinoma in situ* having pronounced reactivity. RT-PCR analysis of tumor specimens demonstrated a constant expression of MT-2A mRNA, down regulation of MT-1E mRNA and an upregulation of MT-1X mRNA in bladder cancers. This study demonstrates that there is a variation in MT expression in bladder cancer.

1463 CLONING OF ZONA RADIATA PROTEINS FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*): A MARKER FOR INVESTIGATING ESTROGENIC ENDOCRINE MODULATORS IN FISH.

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We have investigated induction of vitellogenin and zr-proteins in rainbow trout (*Oncorhynchus mykiss*) liver and plasma, after *in vivo* treatment with estradiol-17β (E2) and alpha-zearalenol (α-ZEA). A method for quantification of zr-proteins and vitellogenin PCR products has been developed using flourescent labeled probes (TaqMan assay). A partial sequence of rainbow trout zr-protein has been cloned (GenBank accession number AF185274) by alignment of available zr-protein sequences from four different fish species. The cloning was performed using reverse transcriptase polymerase chain reaction (RT-PCR) with an oligo dT primer followed by rapid amplification of cDNA ends (RACE) PCR and two degenerate zr-protein primers designed from a conserved region of the aligned sequences. This strategy resulted in a 700 bp long fragment that showed high amino acid sequence identity to that of salmon (77%), winter flounder (64%), carp (63%) and medaka (61%) zr-proteins. For the *in vivo* experiment juvenile rainbow trout (50-100 g) were kept 10 fish per 370 liter tank at 15°C (± 1) and acclimatized for 7 days prior to treatment, following a single intraperitoneal injection with E2 (0.01, 0.1, 1.0 or 10 mg/kg fish) or α-ZEA (0.1, 1.0 or 10 mg/kg fish). Enzyme-linked immunosorbent assay (ELISA) showed induction for both proteins in blood plasma from fish treated with E2 or α-ZEA. The potency of α-ZEA was approximately 10-fold less when compared to E2. Furthermore, preliminary data using the TaqMan assay indicate a dose-dependent induction of vitellogenin and zr-proteins after treatment with E2 and α-ZEA.

1464 INDUCTION OF CYTOCHROME B_5 BY CCl_4 IN RAINBOW TROUT LIVER.

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Several reports suggest that fish are a valuable model for testing environmental and occupational xenobiotics and also serve as nonmammalian vertebrate surrogates for understanding drug metabolism in humans. The present study was undertaken to investigate what effect if any CCl_4 will have in rainbow trout (RT) liver on cytochrome b_5. For comparative purposes cytochrome P-450 was also determined. RTs were given ip corn oil and CCl_4 (0.3 and 0.5 ml/100 g body weight, in 1:1 corn oil). The livers were excised after 24 hrs. Microsomes were prepared for determination of cytochrome b_5 and cytochrome P-450. Administration of CCl_4 showed a significant decrease in cytochrome P-450 content compared to the control. There was no difference between the two CCl_4 treated groups. However CCl_4 treatment showed a gradual but significant increase in the 0.3 ml group whereas the 0.5 ml CCl_4 treated showed a four fold increase compared to the control. These results suggest that cytochrome b_5 and cytochrome P-450 are differently regulated by CCl_4 in RT liver. Cytochrome b_5 is upregulated and cytochrome P-450 is down regulated. Exactly how CCl_4 is modifying the expression of cytochrome b_5 remains to be elucidated. The increase in cytochrome b_5 could be used as a useful marker for monitoring various environmental chemicals. (Supported by the Linus Pauling Institute.)

1465 DETERMINATION OF TOPICALLY APPLIED ANTIBIOTICS AND OTHER ENVIRONMENTAL CHEMICALS BY USING A TRANSDERMAL SWEAT PATCH AND GC/MS ANALYSIS.

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Sweat analysis is being evaluated as a minimally invasive means of testing patients for therapeutic drugs and drugs of abuse. Sweat can be collected in a transdermal patch for periods of up to seven days and subsequently analyzed by a variety of methods after the components of the sweat is extracted from the patch. In the present study, gas chromatography-mass spectrometry (GC/MS) was used to evaluate the possibility that airborne or topically-applied chemicals or their metabolites could be collected in a transdermal patch. Volatile laboratory solvents and topical creams containing bacterial or fungal antibiotics or insect repellents were chosen for the study. Subjects exposed to these materials because of occupational or medical reasons wore patches for one to seven days. Blood specimens were drawn at the end of this period to allow data for the patch to be compared to the more traditional sampling methodology. The results indicate that antibiotics and insecticides can be detected after dermal application, and the lower volatility organic compounds were detected presumably after inhalation. The utility of transdermal patches for detecting environmental and consumer chemicals will be demonstrated. These results suggest applications in occupational settings whereby environmental exposures for individuals can be monitored.

1466 SENSORY NERVE-MEDIATED NASAL VASODILATORY RESPONSE TO ETHYL ACRYLATE.

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Ethyl acrylate is a sensory irritant with an RD50 of roughly 1000 ppm in the rat. Our previous studies have characterized the immediate nasal vascular response to acrolein and acetaldehyde. The current study was aimed at characterizing this response to the ethyl acrylate. Nasal vascular responses were assessed by continuously monitoring the nasal uptake of an inert vapor (acetone) in an anesthetized F344 rat model. Ethyl acrylate exposure produced an immediate concentration dependent vasodilatory response. The response occurred in the absence of change in nasal air flow resistance. The LOEL for this response was 100 ppm; the NOEL 25 ppm. Pretreatment of rats with capsaicin (50 mg/kg, 1 week prior) to defunctionalize sensory nerves abolished the response to 200 ppm ethyl acrylate providing strong evidence that it was sensory nerve-mediated. The response was significantly diminished by treatment with the CGRP antagonist hCGRP 8-37 suggesting an important role for the sensory nerve-derived neuropeptide CGRP. Pretreatment with the nNOS selective inhibitor 7-nitroindazole also significantly diminished the response. Thus, at concentrations of 100 ppm or above ethyl acrylate induces an immediate sensory neuronal nasal vasodilatory response that appears to be mediat ed via release of CGRP and activation of nNOS and release of NO.(This work supported by NIEHS R01-08765.)

1467 CORRELATION OF REGIONAL HYDROGEN SULFIDE FLUX PREDICTION WITH THE DISTRIBUTION OF NASAL OLFACTORY LESIONS IN SUBCHRONICALLY EXPOSED RATS.

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Hydrogen sulfide (H_2S) is a toxic gas generated by natural and industrial sources. At high concentrations (>1000 ppm), it induces rapid respiratory paralysis and sudden death but at lower concentrations usually encountered in the environment H_2S appears to target primarily the olfactory system. Rats acutely exposed to H_2S exhibit dose-dependent necrosis of the olfactory mucosa. To test the hypothesis that the flux of inspired H_2S to the airway walls determines the distribution of tissue damage within the olfactory region of the rat nose, the nasal lesions of adult, male CD rats exposed by inhalation to 30 or 80 ppm H_2S (6 hrs/day for 70 consecutive days) were mapped. Neuronal loss and basal cell hyperplasia evaluated by the change in thickness and cellular composition of the olfactory neuronal cell layer were used as markers of damage to the olfactory epithelium. The incidence of the lesions was determined regionally at two cross-section levels of the nose corresponding to the mid-point of the nasal cavity (section 1) and the middle portion of the ethmoid recess (section 2). A three-dimensional, anatomically accurate, computational fluid dynamic model of the rat nasal passages was used to predict regional

H_2S flux. Four regions on the first section and 39 regions on the second section were ranked separately by lesion incidence and predicted H_2S flux, and the rankings were compared. Statistical analysis showed that H_2S flux and lesion incidence were correlated ($p<0.005$) within the olfactory epithelium for both 30 and 80 ppm exposure. These results indicate that airflow-driven patterns of H_2S uptake play an important role in the distribution of olfactory lesions and suggest that species-specific regional airflow in the nasal cavity must be taken into consideration when extrapolating rat lesions to human risk estimates.

1468 POTENTIATION OF OZONE-INDUCED MUCOUS CELL METAPLASIA BY ENDOTOXIN IS NEUTROPHIL-DEPENDENT.

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Exposure of laboratory animals to ozone, the primary oxidant gas in photochemical smog, causes neutrophilic inflammation and mucous cell metaplasia (MCM) in the nasal transitional epithelium (NTE) of rats and monkeys. Bacterial endotoxin is a common airborne biogenic agent that induces acute neutrophilic inflammation, but not MCM, in NTE. It does, however, potentiate ozone-induced MCM in rat nasal airways (Fanucchi et. al., *Toxicol. Appl. Pharmacol.* 152:1-9, 1998). In the present study, F344 rats exposed to 0 or 0.5 ppm ozone (8 h/d x 3 d) were intranasally instilled with 0 or 100 μg endotoxin 24 h and 48 h after the third ozone exposure. To determine the role of neutrophilic inflammation in endotoxin-induced potentiation of the MCM caused by ozone, half of the rats were depleted of circulating neutrophils prior to saline or endotoxin instillations. Rats were killed 6 h or 3 d after the last intranasal instillation and nasal tissues were processed for 1) light microscopy and morphometric analysis to determine the number of infiltrating neutrophils and the volume density (amount) of stored mucosubstances in the NTE, and 2) for quantitative RT-PCR analysis of steady-state mucin gene (rMuc-5AC) mRNA levels in the NTE. Endotoxin induced a transient influx of neutrophils in both air and ozone exposed rats that was completely blocked by neutrophil depletion. Endotoxin increased rMuc-5AC mRNA levels in the NTE of ozone-exposed rats, however, neutrophil depletion had no effect on endotoxin-induced upregulation of mucin gene mRNA levels. Endotoxin potentiated (4x) the ozone-induced increase in stored mucosubstances, but only in neutrophil sufficient rats with endotoxin-induced inflammation. These data indicate that endotoxin's potentiation of ozone-induced upregulation of mucin gene (rMuc-5AC) mRNA levels is neutrophil-independent while it's effects on intraepithelial production and storage of mucous glycoproteins is dependent on the presence of neutrophils. (Research funded by NIH/NHLBI grant HL59391-01.)

1469 NEWBORN MICE DIFFER FROM ADULT MICE IN CHEMOKINE AND CYTOKINE EXPRESSION AFTER OZONE EXPOSURE BUT NOT ENDOTOXIN.

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Neonatal animals of many mammalian species are more tolerant to pulmonary oxidative stress-inducing toxicants than are adults. Previous studies using exposure to acute hyperoxia demonstrated a rapid increase in the abundance of inflammatory chemokine and cytokine mRNA species early in the injury process, while the adult response was not seen until lethal exposure levels were achieved. Our current hypothesis is that such differential response is due to differences in specific cellular injury and not a consequence of altered regulation of inflammatory responses. To test this hypothesis we utilized two distinct models of pulmonary injury and inflammation: ozone (O_3) which causes epithelial injury and bacterial endotoxin which causes inflammation independent of direct epithelial injury. Mice (C57BL/6J) either 36 hours or 8 weeks of age were exposed to varying concentrations of ozone for 4 through 24 hours or to an LPS aerosol (total estimated deposited dose 10 ng) and examined at 2, 6, or 24 hours post inhalation. Adult mice displayed enhanced sensitivity to ozone as demonstrated by increased abundance of mRNA encoding eotaxin, macrophage inflammatory protein (MIP)-1α, MIP2, interleukin (IL)-6 and metallothionein (Mt). In newborn mice only Mt was increased. In contrast, 2 hours after exposure to endotoxin, mRNA for tumor necrosis factor (TNF) α, eotaxin, MIP-1α/β, MIP-2, interferon γ inducible protein (IP)-10 and monocyte chemoattractant protein (MCP)-1 were all increased in both adults and newborns. In this model, IL-6 and interferon-γ mRNA were increased acutely only in adults. The ability of neonatal animals to increase abundance of multiple inflammatory mediator mRNAs following endotoxin suggests that the lack of response of these genes following ozone is not due to the inability to mount an inflammatory response but is secondary to enhanced epithelial injury in the adult lung and a role for the epithelium in regulating the expression of certain inflammatory mediators. (Supported by grants from the CIAR, EPA STAR grant R826784, EPA PM Center R827354, and ES 01247.)

1470 COMBINED EFFECTS OF CIGARETTE SMOKE AND OZONE IN A MOUSE MODEL OF SMOKE-INDUCED EMPHYSEMA.

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Both cigarette smoke (CS) and ozone (O_3) cause pulmonary inflammation in humans and experimental animals, and cigarette smoking is the main risk factor for emphysema in humans. In this study we are examining the effects of combined exposures to CS and O_3 on antioxidant status, inflammatory responses, proteases, and development of emphysema in a mouse model of CS-induced emphysema. Female $B6C3F_1$ mice are exposed in whole-body chambers to 250 mg CS particulate matter/m³ or filtered air, 6 hours/day, 5 days to week and to O_3 at either 0, 0.08 (low O_3), or 0.3 (high O_3) ppm 8 hours/night, 5 nights/week. Mice were sacrificed at scheduled intervals to collect bronchoalveolar lavage (BAL) supernatant and cells and lung tissue for antioxidant status, histopathology, and stereology. O_3 caused an exposure-related increase in BAL supernatant total glutathione that was comparable to, and additive with, the effects of CS. The effect at 4 weeks was considerably greater than at 1 week. O_3 alone had little effect on BAL inflammatory cells at 1 week, but caused a dose-related increase in neutrophils and macrophages at 4 weeks. In combination with CS, which caused a marked, progressive increase in BAL neutrophils and macrophages, low O_3 did not affect BAL inflammatory cell numbers, but high O_3 suppressed the CS-induced increase in neutrophils at 1 week, and the CS-induced increase in both neutrophils and macrophages at 4 weeks. BAL protein was increased by both CS and O_3 at 1 and 4 weeks. Gelatinolytic activity in BAL was increased slightly by high O_3 and markedly by CS at both 1 and 4 weeks. However, at 1 week, the combination of CS and high O_3 induced markedly less activity than CS alone, whereas at 4 weeks, the combination induced activity comparable to that of CS alone. These results indicate complex interactions between the effects of CS and O_3 that may be additive or inhibitory depending on the endpoint and exposure concentration. (This research was conducted by the National Environmental Respiratory Center with support from the U.S. Environmental Protection Agency under Cooperative Agreement CR826442-01-1 with the Lovelace Respiratory Research Institute. This report has not been subjected to the Agency's peer or policy reviews and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.)

1471 LONG-TERM OZONE EXPOSURE INCREASES CYP2B ACTIVITY AND WORSENS INJURY TO THE CENTRAL ACINUS BY 1-NITRONAPHTHALENE.

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It is well recognized that repeated exposure to ozone at concentrations resulting in acute injury and inflammation render the centriacinar epithelium resistant to further oxidant injury. However, human populations exposed to oxidant gases like ozone are also exposed to a variety of environmental contaminants that require bioactivation for their toxicity. Whether changes that render the lung resistant to injury by an oxidant gas alter the metabolic activity required for bioactivation of other toxicants is not known. We tested the hypothesis that the tolerance produced by long-term exposure to ozone (O3) does not apply to bioactivated lung toxicants. Rats were exposed to 0.8 or 1 ppm O3 or filtered air (FA) for 90 days. On the morning following the 90th exposure, rats were administered the bioactivated lung toxicant 1-nitronaphthalene (1-NN) at 0, 50, or 100 mg/kg or were euthanized immediately for P450 measurements. We compared the level of toxicity in centriacinar areas with activity of CYP1A1, CYP2B and NADPH cytochrome P450 reductase in airway compartments isolated by microdissection. Based on high resolution histopathology and morphometry, there was significantly greater cell necrosis and epithelial exfoliation in O3-exposed rats administered 50 or 100 mg/kg 1-NN, compared to FA-exposed rats administered the same dose. In O3-exposed rats, CYP2B activity was elevated 3-fold ($p<0.02$) in samples containing the centriacinar region. Immunohistochemically detectable CYP2B protein was elevated in nonciliated terminal bronchiolar epithelium. In centriacinar epithelium, long-term exposure to ozone elevates: 1) CYP2B activity; 2) immunoreactive CYP2B protein; 3) 1-NN toxicity. We conclude that repeated, long-term exposure of human populations to trophospheric ozone elevates

the risk of pulmonary injury by contaminants activated by the cytochrome P450 monooxygenase system. (This work was supported by NIEHS ES 00628, ES 09681, ES 04311, and T32 ES 07059.)

1472 MECHANISMS OF AGE-RELATED SENSITIVITY TO OZONE IN WISTAR RATS.

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Ozone (O3) induces lung inflammation in humans and rodents. Evidence suggests age-related sensitivities to O3-induced lung responses in humans and rodents as well. These studies examined age-related O3-sensitivity in 14, 21, 28 and 60 day old Wistar rats exposed to 0.0, 1.0 or 2.0 ppm O3 for 2 hours. Polymorphonucleocytes (PMNs) in bronchoalveolar lavage fluid (BALF) increased in all age-groups after 1.0 and 2.0 ppm O3. Viable cells decreased after 2.00 ppm O3, most severely (87% to 48%) in 14 day olds, and epithelial cells increased 7.6X in 14 and 3X in 21 day olds. Total Protein (TP) increased 312% in adults at 2.0 ppm O3 but showed non-concentration related changes in 14 and 21 day olds with baselines elevated compared to adults. PGE2 recovered from 14 day olds increased 5 fold after 1.0 and 2.0 ppm O3 whereas 21 and 28 day olds increased only at 2.0 ppm O3. Mechanisms of altered arachidonic acid (AA) metabolism after O3 exposure *in vivo* were examined in 14 and 60 day old rats. Compared to age-matched controls, secretory PLA2 in lavage fluid showed no change in activity. Cyclooxygenases I & II showed no change in protein or mRNA in macrophages or tracheal epithelium. AA re-esterification was examined in tracheas and macrophages by incubation with 3H-AA alone, 3H-AA with 10uM non-radioactive AA or 3H-AA with Triacsin C pretreatment. In all studies, O3 decreased incorporation of 3H into phospholipids, with only increased recovery of unesterified AA in 14 day old tracheas. Adult tracheas and macrophages from both age groups showed increased incorporation of 3H into triglycerides. Epithelia of 14 and 60 day olds may have different capacities for redirecting storage of AA after alteration of esterification pathways. These biochemical alterations may contribute to increased PGE2 in BALF from 14 day olds. These findings support earlier studies which suggest younger Wistar rats are more sensitive to O3 than adults. (Supported by CT902908. This abstract does not represent EPA policy.)

1473 PRODUCTION OF EXHALED BREATH CARBONYLS IN HUMAN SUBJECTS EXPOSED TO OZONE.

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Responses to ozone (O_3) exposure such as lung inflammation are postulated to be mediated through secondary reaction products of O_3 with lung biomolecules. Carbonyls and peroxides are believed to be the main reaction products. Additionally, carbonyls may also be derived from lipid peroxidation and be indicative of the level of oxidative stress in a tissue. We have previously shown hydrogen peroxide to be increased in the exhaled breath of O_3-exposed human subjects. In this study we examined the production of exhaled breath carbonyls (as a measurement of lung carbonyl content) induced by O_3 exposure, and the effect of dietary anti-oxidant supplementation on carbonyl formation. Subjects were exposed to air or 0.4 ppm O_3 for 2 hr with intermittent exercise. Subjects had their diet supplemented with anti-oxidants (vitamins C and E, and V-8 juice) or placebo (orange soda and sugar pills) for 2 wk prior to the O_3 exposure . Breath was collected just prior to exposure and immediately after the last exercise period into inert Tedlar bags, passed through cartridges coated with the carbonyl derivatizing agent 2,4-dinitrophenylhydrazine (DNPH); the derivatives eluted with acetonitrile, and quantitated by HPLC-UV. The data suggested: 1) in placebo-treated subjects, total carbonyls (i.e., defined as the total amount of 3 to 11 carbon straight chain carbonyls) significantly increased after O_3 exposure, but not air exposure (relative to pre-exposure values) ($p<0.05$; $n=5$); 2) subjects pretreated with anti-oxidants did not have an increase in total carbonyls after air or O_3 exposures. Therefore lung carbonyls were increased in response to O_3 exposure, and may be markers of an exposure to this pollutant as well as mediators of lung responses. Anti-oxidant pretreatment attenuated the O_3-induced increase in carbonyls. (This is an abstract of a proposed presentation, and does not necessarily reflect US EPA policy.)

1474 INHALED NAPHTHALENE (NA) CAUSES DOSE DEPENDENT CLARA CELL CYTOTOXICITY IN MICE.

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Current OSHA standards for human NA exposure are set at 10ppm (TWA) with a STEC of 15ppm. While several studies have addressed the dose dependence of NA injury by intraperitoneal (i.p.) administration, the pattern and severity of injury by inhalation exposure is unknown. To determine the regio-specific and dose dependent differences with inhalation exposure, mice were exposed (4 hrs) to a range of NA concentrations (0-100ppm). Mice were killed and lungs were fixed via tracheal infusion for high-resolution histopathology 24 hours after the completion of NA exposure. NA (≤200mg/kg) administered i.p. causes Clara cell cytotoxicity which is limited to distal airways. Higher doses (>300mg/kg) extend the injury pattern toward the lobar bronchus. In contrast, NA inhalation exposure causes injury to lobar bronchus epithelium at 2ppm; injury increases in severity and in a dose dependent fashion up to 75ppm. Terminal airways of exposed mice exhibited little or no injury at low doses (2-10 ppm); doses exceeding 10ppm were required to produce injury to Clara cells in the terminal airways. We conclude: 1) Clara cells of mouse airways are exquisitely sensitive to inhaled NA at concentrations well below the current OSHA standard; 2) by inhalation exposure the pattern of NA injury moves proximal to distal with increasing severity in a dose dependent fashion and; 3) susceptibility to NA injury in naive animals is not related to airway level but to dose distribution. (Research funded by ES04311, ES00628, ES06700, ES05707, ES04699, ES09681.)

1475 LUNG TUMORIGENICITY FROM EXPOSURES OF F-344 RATS TO COMBINATIONS OF BERYLLIUM AND PLUTONIUM.

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Beryllium is classified as either a demonstrated or probable carcinogen in humans, whereas radiation is a known carcinogen. We have shown that inhaled beryllium metal caused a high incidence of rat lung tumors. This study was conducted to examine (1) dose-response relationships between beryllium exposure and lung tumor induction in rats at lower doses than those previously studied, and (2) potential interactions between beryllium and plutonium in producing lung tumors. Equal numbers of male and female F344 rats received single, nose-only inhalation exposures to either filtered air, beryllium metal (target initial lung burdens [ILBs] of either 0.3, 1, 3, 10, or 50 μg), or 239PuO2 (target ILBs of either 230 or 450 Bq). The 230 Bq ILB of plutonium was combined with either the 1 or 10 μg ILB of beryllium. Animals were held for life-span observation. Neither exposure-related mortality nor life-span shortening was observed. Because histopathological evaluations are not complete, we estimated lung tumor incidence from necropsy findings of lung masses or nodules in apparently non-leukemic rats on study for at least 1 yr. Estimated incidences of 4, 4, 12, 50, 61, and 91% were observed for the 0, 0.3, 1, 3, 10, and 50 μg ILBs of beryllium, respectively, and 28 and 46% for the 230 and 450 Bq ILBs of plutonium, respectively. Incidences of 62 and 89% were seen in the groups receiving ILBs of 230 Bq plutonium and either 1 or 10 μg of beryllium, respectively. These data demonstrate a no-effect level and dose-response relationship for the induction of beryllium-induced lung tumors in rats; and suggest an additive to super-additive effect of combined exposure. However, definitive conclusions must await completion of the histopathology and statistical evaluations. (Research supported by the US Department of Energy under Cooperative Agreement DE-FC04-96AL76406.)

1476 PULMONARY RESPONSES OF RATS, MICE, AND HAMSTERS TO INHALED TITANIUM DIOXIDE (TiO_2).

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Rats are unique in that the inhalation of high concentrations of poorly soluble, relatively noncytotoxic particles, can result in an "overload" of clearance mechanisms and ultimately can lead to pulmonary tumors. The objective of the present study was to examine the pulmonary responses of rodents to inhaled pigmentary TiO_2 to elucidate the basis for the species-differences in the long-term outcome of chronic exposure. Female mice, rats, and hamsters were exposed to 10 (L), 50 (M), or 250 (H) mg/m3 TiO_2 for 6 h/d, 5 d/wk, for 13 wk with recovery groups held for an additional 4, 13, 26 or 52 wk period.

Following exposure and at each recovery time the TiO_2 burden in the lung was determined. The inflammatory response of the lung (LDH, total protein, and cytologic parameters in bronchoalveolar lavage fluid) was assessed at each time point. Burdens (mg/g) of TiO_2 in the lung following exposure to L, M, or H TiO_2 were; (mouse) 5.2, 53.5, 170.2; (rat) 7.1, 45.1, 120.4; and (hamster) 2.6, 14.9, 120.3. Lung burdens decreased with time after exposure; the decline was more rapid in hamsters than in mice or rats. Increases in macrophage and neutrophil numbers and in soluble indices of inflammation were observed in all three species following exposure to M and H TiO_2 (rats > mice, hamsters) and remained elevated, relative to controls, at 26 weeks post exposure. Histopathologic examination of lungs showed a concentration dependent distribution pattern of particles which tended to be species specific at the L and M exposure concentrations. Hypertrophy and hyperplasia of alveolar epithelium and fibrotic changes were observed in rats exposed to H TiO_2. These data suggest that rats exposed to concentrations likely to induce overload differ from mice and hamsters in their cellular responses and in the way they clear and sequester particles. (Supported in part by Chemical Manufactures Association and European Chemical Industry Council.)

1477 SEVERITY OF PULMONARY EPITHELIAL AND INFLAMMATORY RESPONSES IN CARBON BLACK-EXPOSED RATS IS DEPENDENT ON PARTICLE SURFACE AREA.

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Rats chronically exposed to high levels of carbon black particles (Cb) develop lung tumors in association with chronic active inflammation and epithelial cell proliferation. The purpose of the present study was to determine the relationship of the particle surface area with the severity of the induced epithelial proliferation and inflammation in the lungs of rats exposed to Cb. F344 rats were exposed to three concentrations (1, 7, 50 mg/m3) of high surface area Cb particles (HSCb) and one concentration (50 mg/m3) of low surface area Cb particles (LSCb) for 6 h/day, 5 days/wk for 13 wks. The retained particle burden of LSCb was the same as that of the mid dose HSCb in terms of surface area, but was 1.6 times greater than the high dose HSCb in terms of particle mass. Rats were sacrificed one day after the end of exposure. The left lung lobe of each rat was intratracheally fixed at a constant pressure and further processed for light microscopy and morphometric analysis. Numeric densities of alveolar macrophages, neutrophils, and type 2 cells in the alveolar parenchyma, and epithelial cells in terminal bronchioles were morphometrically determined. Compared to controls, rats exposed to HSCb had a dose-dependent increase in the numeric densities of all cell types. For example, there was a 40, 75 and 128%, increase in the numeric density of type 2 cells in rats exposed to 1, 7, and 50 mg/m3 of HSCb, respectively. Numeric cell densities in the lungs of rats exposed to 50 mg/m3 LSCb were consistently lower than those in rats exposed to 50 mg/m3 HSCb. In addition, more necrosis of alveolar macrophages was observed in rats exposed to HSCb as compared to rats exposed to LSCb. These results suggest that the magnitude of the pulmonary proliferative and inflammatory lesions induced by Cb exposure is dependent, in part, on particle surface area. (Research funded by the International Carbon Black Association.)

1478 CYTOKINE, OXIDANT, AND MUTATIONAL RESPONSES AFTER LUNG OVERLOAD TO INHALED CARBON BLACK.

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Complex interactions between humoral and cellular mediators orchestrate the pathogenic effects of inhaled toxins leading to pulmonary inflammation, fibrosis and tumorigenicity. Chronic inhalation of poorly soluble particles at levels that cause persistent, marked inflammation has been associated with increased levels of adenomas and carcinomas in the rat lung. This response seems to be unique to the rat and has not been observed in mice, or hamsters. At present, the mechanisms underlying the development of particle-induced rat lung tumors are not completely understood, but increasing evidence indicates the rat lung tumor response is due to a secondary, non-genotoxic mechanism such as inflammation. Hence, the recruitment of neutrophils during the inflammatory response and subsequent release of oxidants from these cells may play an important role in the pathogenesis of lung tumors. To investigate this hypothesis rats were exposed to air, 1, 7, 50 mg/m3 of a high surface area carbon black (HSCB) and 50 mg/m3 of a low surface area carbon black (LSCB) for 13 weeks. Immediately following inhalation exposure and 3 months post exposure animals were sacrificed, bronchoalveolar lavage (BAL) was performed and characterized for cell number, type, oxidant and antioxidant levels. Ex vivo mutational analysis of inflammatory cells was evaluated by co-incubating with a rat lung epithlial cell line (RLE). Additionally, lung tissue was evaluated for pro- and anti-inflammatory cytokines. There was a dose and time related effects with all the parameters evaluated as well as a particle size effect. LSCB at both time points was similar to mid dose HSCB. Particle surface area appears to be an important determinant for particle overload-induced oxidant generation and associated mutational events in the lung. (Sponsored by the ICBA.)

1479 PARTICLE SURFACE AREA-ASSOCIATED PULMONARY EFFECTS FOLLOWING OVERLOADING WITH CARBON BLACK.

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Rats, but not mice or hamsters, exposed to high levels of carbon black develop lung tumors in association with lung overload. This and the lack of direct genotoxicity suggest a mechanism of tumor formation based on inflammatory and proliferative processes. To evaluate mechanistic aspects of this response, female F-344 rats (8 wks) were exposed to three concentrations (1, 7, 50 mg/m^3) of high surface area carbon black (HSCb) and one concentration (50 mg/m^3) of low surface area carbon (LSCb) for 13 weeks; serial sacrifices were performed during and post-exposure. There was a dose-dependent increase in the lung burden of HSCb. The LSCb mass lung burden was about 1.6 times higher than the high dose of HSCb at the end of exposure. In terms of retained particle surface area, however, the LSCb was the same as the mid-dose HSCb. Cellular and biochemical parameters and the concentration of cytokines/chemokines increased in bronchoalveolar lavage (BAL) fluid with the dose of HSCb. For the LSCb, these parameters were either the same as mid-dose HSCb or in between the mid- and high-dose HSCb. These elevations persisted in the mid- and high-dose HSCb groups and for LSCb; however, there was some regression of response for the mid-dose HSCb. Strand break analyses revealed DNA damage in BAL cells from the HSCb groups, whereas this response appeared to be lower in the LSCb group; DNA damage was less in lung tissue cells of all groups. These results indicate that retained particle surface area plays an important role in particle-induced inflammatory processes. (This research was funded by the International Carbon Black Association.)

1480 TUMOR NECROSIS FACTOR-α (TNF-α) ADMINISTRATION MIMICS ALLERGIC ADJUVANT EFFECT OF RESIDUAL OIL FLY ASH (ROFA) PARTICLES.

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We have previously demonstrated that pulmonary exposure to ROFA results in enhanced sensitization to house dust mite (HDM) and promotes allergic lung disease after allergen challenge. This effect was associated with increased TNF-α and Th2 cytokines in the lung. TNF-α is a macrophage- and bronchial epithelial cell-derived cytokine which has been shown to upregulate the expression of adhesion molecules on endothlial cells, promoting granulocyte migration to the lung, and to influence B cell isotype switching to IgE antibody formation. The present study examined whether enhanced sensitization to HDM could be achieved by instillation of TNF-α. One day prior to pulmonary sensitization with 10μg HDM, BN rats (56 days old, female) were instilled via the trachea with either 2.0μg recombinant rat TNF-α, 2.0μg bovine serum albumin (BSA), or 1000μg ROFA, and were challenged with 10μg HDM 14 days later. Antigen-induced immediate bronchoconstriction responses were significantly elevated in the TNF and ROFA groups compared with BSA control. Antigen-specific IgE titers, lymphocyte proliferation to HDM, as well as bronchoalveolar lavage fluid biochemicals, cytokines (TNF-α and IL-6), and eosinophil numbers were also elevated in rats treated with ROFA or TNF compared with BSA-treated control rats after HDM challenge. This study demonstrates that TNF-α has the same activity as ROFA in the enhancement of allergic sensitization. (This abstract does not reflect US EPA policy.)

1481 *IN VIVO* AND *IN VITRO* CORRELATION OF PULMONARY MAP KINASE ACTIVATION FOLLOWING METALLIC EXPOSURE.

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Residual oil fly ash (ROFA) is a particulate pollutant produced in the combustion of fuel oil. Exposure to ROFA is associated with adverse respiratory effects in humans, induces lung inflammation in animals, and induces inflammatory mediator expression in cultured human airway epithelial cells (HAEC). ROFA has a high content of transition metals, including vanadium, a potent tyrosine phosphatase inhibitor which we have previously shown to disregulate phosphotyrosine metabolism and activate mitogen-activated protein kinase (MAPK) signaling cascades in HAEC. In order to study MAPK activation in response to *in vivo* metal exposure, we used immunohistochemical methods to detect levels of phosphorylated protein tyrosines (P-Tyr) and the MAPKs ERK1/2, JNK, and P38, in lung sections from rats intratracheally exposed to ROFA. After a 1 h exposure to 500μg ROFA, rat lungs showed no histological changes and no significant increases in immunostaining for either P-Tyr or phospho (P)-MAPKs compared to saline-instilled controls. At 4 hs of exposure, there was mild and variable inflammation in the lung which was accompanied by an increase in specific immunostaining for P-Tyr and P-MAPKs in airway and alveolar epithelial cells and resident macrophages. By 24 hs of exposure, there was a pronounced inflammatory response to ROFA instillation and a marked increase in levels of P-Tyr and P-MAPKs present within the alveolar epithelium and in the inflammatory cells, while the airway epithelium showed a continued increase in the expression of P-ERK1/2. By comparison, HAEC cultures exposed to 100μg/ml ROFA for 20 min resulted in marked increases in P-Tyr and P-MAPKs, which persisted after 24 hs of exposure. P-Tyr levels continued to accumulate for up to 24 h in HAEC exposed to ROFA. These results demonstrate *in vivo* activation in cell signaling pathways in response to pulmonary exposure to particulate matter, and support the relevance of *in vitro* studies in the identification of mechanisms of lung injury induced by pollutant inhalation. This abstract of a proposed presentation does not necessarily reflect EPA policy.

1482 RESIDUAL OIL FLY ASH SLOWS THE CLEARANCE OF *LISTERIA MONOCYTOGENES* FROM RAT LUNGS.

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Inhalation of residual oil fly ash (ROFA) has been shown to increase pulmonary morbidity and impair lung defense mechanisms. The objective of this study was to evaluate the effect of ROFA exposure on the clearance of a bacterial pathogen from the lungs. Elemental analysis revealed the ROFA particles to be comprised of Al, Si, S, Ca, V, Fe, and Ni. Young adult male Sprague-Dawley rats were dosed intratracheally with saline (control) or ROFA at a dose of 1 mg/100 g b wt. Three days later, ~500,000 *Listeria monocytogenes* were intratracheally instilled into the ROFA-treated and saline control rats. Bronchoalveolar lavage was performed on the right lungs at 3, 5, and 7 days after exposure to *L. monocytogenes*. The cells recovered were differentiated, and chemiluminescence, an index of macrophage activation, was measured. At the same time points, the left lung and spleen were removed, homogenized, and cultured on brain heart infusion agar at 37°C. Colony forming units were counted after an overnight incubation. Pre-exposure to ROFA significantly ($p<0.05$) delayed the pulmonary clearance of *L. monocytogenes* as compared to the saline control rats. ROFA had no effect on the influx of neutrophils into the lungs but caused a significant ($p<0.05$) decrease in macrophage chemiluminescence as compared to saline control rats. We have demonstrated that acute exposure to ROFA slowed the pulmonary clearance of *L. monocytogenes*. This is most likely due to a ROFA-induced suppression of macrophage activation, perhaps related to the elemental composition of the particles. Therefore, in an occupational setting, inhalation of ROFA may lead to increased susceptibility to pulmonary infection.

1483 METAL-CONTAINING PARTICULATE MATTER-INDUCED AUTOXIDATION OF SYNTHETIC LUNG EPITHELIAL LINING FLUID.

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Oxidative stress has been implicated in the toxicity of metal-rich particulate matter (PM). Lung epithelial lining fluid (ELF) contains several antioxidants and serves as the first line of defense against inhaled toxins and infective agents. We used synthetic ELF (sELF) as a model system to study the role of residual oil fly ash (ROFA, an ambient PM containing soluble transition metals) in autoxidation of ELF constituents and the effects of antioxidants on this process. sELF was formulated based on literature data and our own measured values of human bronchoalveolar lavage. Oxygen-18 labeling was used to trace the extent of oxidation by measuring ^{18}O incorporated into sELF components after exposure of sELF (200 μL, pH 7.4) containing ROFA (0-200 μg/mL) to 20% $^{18}O_2$ (24°C, 4 h). Ascorbate, urate, total glutathione, and non-protein sulfhydryls were also measured. ROFA induced autoxidation of sELF significantly in the presence of ascorbate and this effect was slightly enhanced by glutathione. Ascorbate became inhibitory at high concentrations. Catalase, superoxide dismutase, and glutathione peroxidase had no significant effect on the autoxidation at normal and 2X reported physiological concentrations. The consumption of ascorbate was ROFA-dose-dependent, while urate, total glutathione, and non-protein sulfhydryls were not depleted under any condition studied. Compared with maximum possible binding of ROFA metals to sELF components, oxygen incorporation was 2-9X higher (on a nmol/mL basis). These results suggest that ROFA-induced oxidative stress might be a significant contributor to toxicity and that ascorbate and glutathione act as pro-oxidants in this process. (Supported by EPA/UNC CT #902908. This abstract does not represent EPA policy.)

1484 SURFACE CHARGE AND SIZE AS CONTRIBUTING FACTORS TO PARTICULATE MATTER (PM) TOXICITY.

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The heterogeneous composition of PMs has complicated identifying which physicochemical component(s) contribute to their inflammatory effects. In vitro exposure of airway cell targets to various urban and industrial PM pollutants results in differential inflammatory changes (e.g., cytokine release, oxidative burst). In this study, nine PMs from various urban, natural and industrial sources (e.g., urban air particles, diesel, woodstove, oil fly ash) were described in terms of their surface charge (i.e., zeta potential) and particle size. The different PMs were examined in field suspension (fine and course particles) and after 0.22 um filtration (ultrafine particles). The zeta potentials of suspended field PMs (1-30 um in diameter) did not differ significantly across sources, but were dependent on the vehicle milieu. For example, zeta potential values of PM suspended in 1 mM KCl (pH 7.4) ranged from averaged values of -27 to -42 mV, whereas, when suspended in serum-supplemented culture media, their zeta potentials ranged from -6 to -12 mV. In contrast, the zeta potentials of the ultrafine particles (i.e., after 0.22 um filtration) were highly variable, ranging from averaged values of +13 mV to -109 mV in 1 mM KCl (pH 7.4). Exposure of mouse sensory neurons and human bronchial epithelial cells (i.e., BEAS-2B) to the individual suspended field PM or to their filtrates produced a differential release of the proinflammatory cytokine IL-6. Collectively, these data suggest that both surface charge and particle size are contributing factors to the inflammatory effects of PMs seen in cultured airway target cells. Although charge appears not to be the discriminative factor for the differential inflammatory effects of PM >1 um, it may be relevant for the ultrafine PM fraction.(This abstract has been reviewed by NHEERL and does not necessarily reflect EPA policy).

1485 CHANGES IN HEART RATE VARIABILITY IN YOUNG AND ELDERLY HUMANS EXPOSED TO CONCENTRATED AMBIENT AIR PARTICLES.

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Epidemiology studies have shown an association between ambient air particles (PM) and mortality in the elderly related to cardiovascular causes. In addition, a recent study has shown an association between PM and decreased heart rate variability in elderly residents of a retirement village. 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. Fourteen healthy young volunteers (aged 18-35) and 14 healthy elderly volunteers (aged 65-80) were exposed to concentrated Chapel Hill ambient air particles (CAPS) for two hours. Heart rate variability was determined before, immediately after, and 24 hrs after exposure. Changes in both time (SSDN, PNN50) and frequency (LF, HF, LF/HF) domain were measured. There were no statistically significant changes in heart rate variability in young subjects. In contrast, elderly subjects developed significant decrements in both time and frequency domain immediately after exposure. Changes in some of these parameters persisted for at least 24 hrs. These data demonstrate that PM can induce decreased heart rate variability in the elderly, which can persist for many hours, suggesting a possible mechanism that may contribute to the epidemiology findings. (This abstract of a proposed presentation does not necessarily reflect EPA policy.)

1486 LUNG INJURY FROM EXPOSURE TO RESIDUAL OIL FLY ASH (ROFA), OTTAWA DUST (OTT), OR MT. ST. HELENS ASH (MSH) IN A RAT MODEL OF HYPOXIA-INDUCED PULMONARY HYPERTENSION (PHT).

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Previous studies to assess PHT as a susceptibility factor for particulate matter (PM) have used monocrotaline (MCT) to induce the impairment. While causing damage to pulmonary blood vessels, MCT also gives rise to substantial coexistent lung inflammation. The hypoxia model in the rat has virtually no inflammatory component while exhibiting considerable PHT and cardiomegaly. We hypothesized that PHT alone without coexistent inflammation would result in increased lung injury induced by PM exposure. Male, 60-day old SD rats (260-290 g) were subjected to 12 days of either normobaric normoxia or hypoxia (10% O2). Subgroups of normoxic and hypoxic rats were then intratracheally (IT) instilled with saline suspension of 3.3 mg/kg MSH (inert PM), OTT (ambient PM), or ROFA(oil combustion PM). At 24 hrs post IT, bronchoalveolar lavage fluid (BALF), blood and heart weights were analyzed. Hypoxic animals exhibited PHT (RV/LV+S: normoxia = 0.35±0.04, hypoxia 0.60±0.07; hematocrit: normoxia = 46.4±2.0, hypoxia = 56.0±2.9). PM exposure caused increases in BALF protein, albumin and LDH (ROFA>OTT>MSH) in both normoxic and hypoxic animals. BALF total cells and neutrophils also increased following exposure (ROFA>OTT>MSH). While hypoxia was associated with exacerbated ROFA and OTT-induced increases in BALF protein and total cells, neutrophilic inflammation remained similar in both normoxic and hypoxic rats. Exposure to ROFA and OTT caused plasma fibrinogen levels to increase slightly in normoxic but significantly in hypoxic rats. This study shows that preexistent PHT without pulmonary inflammation is associated with some degree of exacerbation of PM-induced pulmonary injury and systemic changes. (This abstract does reflect EPA policy; Supported in part by NCCU #CT827262.)

1487 1, N2 PROPANODEOXYGUANOSINE-DNA ADDUCTS AND ARTERIOSCLEROTIC PLAQUES IN COCKERELS EXPOSED TO ACROLEIN.

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Inhalation of 1.3 butadiene, a vapor phase component of second-hand tobacco smoke (SS) accelerates development of arteriosclerotic plaques. We asked whether inhaled acrolein (ACR), a reactive aldehyde that is also a prominent vapor phase component of SS, damages artery wall DNA and also accelerates plaque development. Cockerels inhaled 0, 1, or 10 ppm ACR mixed with HEPA-filtered air for 6 hr. Half were sacrificed immediately for detection of the stable, premutagenic 1, N2 propandeoxyguanosine ACR-adduct in aortic DNA via a 32P-postlabeling/HPLC method and half were sacrificed after 10 days for assessment of adduct repair. After 1 day, ACR-DNA adducts were 5x higher in the 1 & 10 ppm groups than in HEPA-filtered air controls. However, after 10 days, adduct levels in the 1 & 10 ppm ACR groups were reduced to the control adduct level. For the plaque studies, cockerels inhaled 1 ppm ACR (6 hr/day, 8 wk) mixed with the same HEPA-filtered air inhaled by controls. Plaque development was measured blind by computerized morphometry. Unlike the case with butadiene, ACR inhalation did not accelerate plaque development. Thus, even though repeated exposure to ACR alone has no effect on plaque size under the exposure conditions used here, a single, brief inhalation exposure to ACR elicits repairable DNA damage to the artery wall. These results suggest that frequent exposure to SS may lead to persistent artery wall DNA damage and thus provide sites on which other SS plaque accelerants can act. (Supported by NIEHS # 08624.)

1488 THE RESPONSE OF HUMAN AIRWAY EPITHELIAL CELLS TREATED WITH DIESEL PARTICULATES IN VITRO WILL VARY ACCORDING TO THE COMPOSITION OF THE PARTICULATES.

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Strong epidemiologic evidence suggests that increased morbidity and mortality are associated with pollution episodes. These studies further suggest that particulate matter (PM), a major constituent of ambient pollution, is an important player in the adverse pulmonary effects observed. Many sources contribute to the particulate fraction of ambient pollution, including diesel exhaust particulates (DEP). The composition of diesel exhaust particulates varies greatly dependent upon multiple factors, including the engine driving cycle conditions. We have examined the effect of DEP components derived from different driving cycles on the responses of normal human bronchial epithelial cells (NHBE) *in vitro*. Specifically, we have looked at NIST standard references materials DEP 1650 and 2975 (parent), an idling workload at KW~0 (extract 2A) and a high workload at KW~85 (extract 5A). NHBE cells were treated for 2 hrs at 37C, 5% CO2 with 0 to 500µg/0.5ml of each DEP in HBSS + 0.005% BSA. Cell culture medium was then added to the cells. Cell supernatants were collected for measurement of cytotoxicity and inflammatory mediators at 24 hrs. Preliminary findings show that all 3 DEPs have a slightly cytotoxic effect on NHBE cells at 500µg compared to untreated controls as measured by LDH release. The release of the inflammatory cytokine IL-8 was decreased 2.3-fold and 4.7-fold in cells treated with 500µg parent and extract 2A, respectively, whereas cells treated with 500µg extract 5A showed a 2.4-fold increase in IL-8 release. Basal release of PGE2, another marker of inflammatory responses, did not appear to differ from controls in cells treated with extract 2A but was increased as much as 2.5-fold in cells treated with parent and extract 5A. Melittin stimulation of the cells showed that parent and extract 2A had an inhibitory effect on PGE2 release at 500µg, but all 3 compounds showed increased melittin stimulated PGE2 production at 50 and 100µg. The data suggests that the response of NHBE cells to treatment with diesel exhaust will vary dependent upon the engine load which affects the exhaust components. (This is an abstract of a proposed presentation, and does not necessarily reflect US EPA policy.)

1489 EFFECTS OF LUNAR AND MARTIAN SOIL SIMULANTS ON HUMAN ALVEOLAR MACROPHAGES (HAM).

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In this study the potential toxicity of JSC-Mars 1, a Martian regolith simulant, and JSC-1, a lunar mare soil simulant, were considered in preparation for long-term space missions. HAM were treated with the Martian or lunar dust simulant *in vitro* for 24 h. Cells were examined for viability by Trypan blue exclusion and for apoptosis by morphological and cell death ELISA assays. Treatment with either particulate resulted in significant HAM apoptosis. To elucidate a possible mechanism, by which these particles induce apoptosis, we investigated the involvement of scavenger receptors (SR) in this process. Cells were pretreated with polyinosinic acid (poly I; 0 or 250 µg/ml) 15 min prior to treatment with one of these particulates; poly I competitively inhibits binding to SR. Pretreatment with poly I significantly inhibited apoptosis. Therefore, HAM apoptosis induced by these particulates may be due to the interaction with SR resulting in initiation of one or more signaling pathways leading to apoptosis. Furthermore, treatment of HAMs with either particulate resulted in a shift in the RFD1+:RFD1+7+ HAM subpopulation ratio toward RFD1+. This shift suggests particle-induced activation of the immune system.

1490 LUNG INFLAMMATION AND DAMAGE AFTER SILICA INHALATION IN RATS: IS THERE RECOVERY?

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Crystalline silica (quartz) is a well established lung inflammatory and fibrogenic occupational dust. Although the pulmonary effects of silica exposure in animal models has been well studied, the relationships between silica exposure and clearance (recovery) on lung inflammation and damage has not been investigated. To investigate these relationships, rats were exposed to filtered air (control) or 15 mg/m^3 silica aerosol (6 hours/day,5 days/week). Rats were exposed for 20, 40 or 60 days and each exposure group had a corresponding exposure plus 36 day recovery group. Rat lungs were lavaged to isolate bronchoalveolar lavage (BAL) cells and acellular BAL fluid, while samples of whole blood were collected to monitor peripheral leukocytes. Pulmonary inflammation was monitored by measuring BAL polymorphonuclear leukocytes (PMN). BAL PMN cell counts were elevated in silica-exposed versus control rats, and PMN counts increased further during recovery. Blood leukocyte counts displayed a pattern similar to the BAL PMN counts. Silica cytotoxicity was measured by analyses of BAL fluid lactate dehydrogenase (LDH) activity and albumin (ALB) concentration. BAL fluid LDH activities and ALB concentrations were higher in silica-exposed versus control rats, and these parameters continued to increase during recovery. These data indicate that progressively severe silica-induced lung inflammation and damage occurs in response to the duration of silica inhalation and these processes continue after silica exposure has ended.

1491 EXPOSURE TO SILICA ACTIVATES MACROPHAGES AND INCREASES THE PULMONARY CLEARANCE OF *LISTERIA MONOCYTOGENES* IN RATS.

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Alveolar macrophages (AM) play a crucial role in protecting the lungs from infectious agents. In this study, we evaluated the effect of silica on macrophage function using a rat *Listeria monocytogenes* (LM) infection model. Male Sprague-Dawley rats were instilled intratracheally with saline or silica (20 mg/rat). Thirty-five days later, the rats were then inoculated intratracheally with either ~5,000 or 500,000 LM. At 3, 5 and 7 days post-infection, the left lung was removed, homogenized and cultured on brain heart infusion agar at 37°C. The numbers of viable LM were counted after an overnight incubation. Bronchoalveolar lavage (BAL) was performed on the right lungs and BAL cell differentials, acellular LDH activity and acellular albumin content were determined. Macrophage chemiluminescence (CL) and nitric oxide (NO) production were assessed as a measure of macrophage function. Pre-exposure to silica significantly increased the pulmonary clearance of LM as compared to saline controls. Exposure to silica caused significant increases in BAL neutrophils, LDH and albumin in non-infected rats. The generation of CL by AM was also enhanced in silica-treated rats. In summary, the results demonstrated that subchronic exposure of rats to silica increased neutrophil infiltration and macrophage activation. These enhanced activities may be responsible for increasing the pulmonary clearance of LM.

1492 LUNG FLUID ACTIVATION OF INHALED P-ARAMID RFP IS A PREREQUISITE FOR BIODEGRADABILITY: DEVELOPMENT OF *IN VITRO* CELLULAR AND NONCELLULAR MECHANISTIC STUDIES.

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Numerous inhalation toxicity studies have demonstrated that inhaled p-aramid RFP are biodegradable, i.e., break down transversely into smaller fragments in the lungs of exposed rats and hamster. The finding of p-aramid RFP shortening in the lungs of two rodent species and the lack of lung tumors in rats have been considered by IARC in 1997 as evidence supporting a Category 3 classification (Inadequate evidence for carcinogenicity). In the current study, we have investigated the mechanisms of p-aramid RFP biodegradation in the lungs of animals. We have hypothesized that lung fluids interact with and activate the inhaled p-aramid RFP following deposition in the lung; subsequently, the RFP become vulnerable to enzymatic attack in the lungs. To test the hypothesis, p-aramid RFP and a biopersistent control organic fiber-type, cellulose RFP were instilled into the lungs of rats and the lungs digested 24 hrs post-instillation, using two different digestion techniques, 1) a conventional ethanolic KOH method, and 2) an enzyme method (designed to simulate pulmonary enzymes). Following tissue processing, the RFP lengths were assessed by scanning electron microscopy. The results demonstrated that the enzyme method but not the KOH method artificially cleaved the p-aramid RFP recovered from rat lungs. In contrast, neither the enzyme or KOH method affected the length dimensions of cellulose RFP. Additional *in vitro* studies were conducted wherein p-aramid or cellulose RFP were incubated with lavaged fluids from a normal rat and then underwent simulated digestion procedures with either KOH or enzyme methods. The lengths of p-aramid RFP were shortened when incubated with BAL fluids and then "digested" with the enzyme method but not with KOH, thus confirming the *in vivo* results. In contrast, no change in fiber lengths were measured in the cellulose RFP following incubation with BAL fluids and subsequent "digestion" with either KOH or enzyme method. Additional studies are ongoing, wherein saline and BAL-treated p-aramid and cellulose RFP have been incubated with alveolar macrophages and with macrophage/epithelial cell co-cultures for one or two weeks. Preliminary data generated from these experiments demonstrate that macrophages phagocytize the BAL-treated p-aramid RFP, and this could account, in part, for the biodegradability of inhaled p-aramid measured in the lungs of exposed rats. To summarize our findings, these data suggest that components of lung fluids coat and catalyze the p-aramid, rendering the RFP vulnerable to enzymatic attack by macrophages or other pulmonary cell-types. This could represent a mechanism through which inhaled p-aramid RFP are shortened in the lungs of exposed rats and hamsters. Cellular studies are ongoing to assess the contribution of pulmonary macrophages to the clearance and shortening of p-aramid RFP.

1493 BIOSOLUBILITY AND SHORT-TERM LUNG INFLAMMATION CAN BE USED TO ASSESS THE TOXIC POTENTIAL OF SYNTHETIC VITREOUS FIBERS.

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A new bio-soluble fiber glass (JM 902) was developed for specialty insulation and filtration. JM 902 was tested for *in vitro* dissolution, biopersistence in rats, and early effects on bronchoalveolar (BAL) and pleural (PL) lavage fluid in rats. In vitro dissolution of JM 902 (kdis at pH 7.4=150 ng/cm2/hr) was faster than that of another fiber glass (kdis=100) that was innocuous in a rat chronic inhalation study. JM 902 also cleared rapidly from the lungs of rats: following 5 d fiber inhalation, lung clearance weighted half-time for fibers >20 mm in length was 6.8 days (satisfies the EU criterion for Category 0, non-carcinogen); following intratracheal instillation, JM 902 demonstrated a clearance half-time for fibers >5 mm in length of 20 days (satisfies the German criterion for a non-hazardous fiber). BAL and PL inflammatory indicators were measured in rats 1, 14, and 30 days after 5 d of fiber inhalation. Results for JM 902 were compared with those for 2 other fiber glasses (JM 901 standard insulation and JM 475 special application fiber) and amosite asbestos. (In previous rodent chronic inhalation studies, JM 901 was non-pathogenic, while JM 475 and amosite were pathogenic.) All 4 fibers induced elevated cellular and biochemical markers for inflammation in BAL at d 1 and in PL at d 14. However, only JM 475 and amosite asbestos continued to induce BAL/PL elevations through d 30, indicating sustained inflammation. In rats exposed to JM 901 and 902, BAL/PL parameters returned to normal levels by d 30. Thus, bio-solubility as well as short-term measures of sustained inflammation can be used to assess the potential toxicity of SVFs.

1494 THE INHALATION BIOPERSISTENCE AND MORPHOLOGIC LUNG DISPOSITION OF PURE CHRYSOTILE SERPENTINE ASBESTOS IN COMPARISON TO TREMOLITE AMPHIBOLE ASBESTOS IN RATS.

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Chrysotile is a serpentine composed of many fine fibrils which have an exposed layer of magnesium. Amphiboles (e.g tremolite) are rod-like fibers in which magnesium is sandwiched within the silicon matrix. McDonald (1997) has postulated that the presence of tremolite which occurs in many chrysotile mines, may account for much of the excess mortality found in chrysotile workers. **Design:** Pure chrysotile from the Cana-Brava Mine was investigated to determine the kinetics without amphiboles/tremolite. The study determined lung clearance (biopersistence), translocation and distribution within the lung. Chrysotile exposure aerosol had 465 fibers/cm^3 longer than 20μm. **Clearance:** Through 12-months post inhalation exposure (5 day, 6 hrs/day), lungs were analysed by TEM for total fiber number and size. This procedure digests the entire lung with no possibility of localising fibers in the lung.

Distribution: Confocal microscopy was used to determine the anatomic fate, orientation and distribution of fibers in airways, alveoli and lymphatics. **Results:** Pure chrysotile is rapidly removed from the lung. Fibers L>20 μm are cleared with a $T_{1/2}$=1.3 days. Fibers 5-20μm had a $T_{1/2}$=2.4 days. Fibers were never found clumped together but appeared as fibrils in corners of alveolar septa and within macrophages. In lymphatics, they appeared free or within lymphocytes. **Conclusion:** Pure chrysotile has been found to clear rapidly from the lung. The remaining short fibers were found in alveoli and lymphatics. These results support the evidence that the carcinogenicity of commercial chrysotile largely depends on its tremolite content.

1495 CRITICAL ROLE OF FIBER LENGTH IN THE BIOACTIVITY AND CYTOTOXICITY OF GLASS FIBERS.

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A critical question in fiber research is the relative contribution of chemical properties vs physical dimensions to the potential pathogenicity of an inhaled fibrous particle. To address this question, it is essential to obtain fiber samples of discrete lengths for investigation. Recently our laboratory has developed a method, which utilized a dielectrophoretic classifier, to separate fiber fractions of narrowly defined lengths. The objective of the present study was to analyze the effects of fiber length on the ability of macrophages to phagocytize these fibers and to determine the potency of fibers of various lengths to activate nuclear transcription and cytokine production and to elicit cytotoxicity. Glass fibers (JM-100) were separated into five discrete size fractions (lengths of 3, 4, 7, 17, and 33 μm). Fibers ≤ 7 μm long were phagocytized by macrophages *in vitro*, while fibers ≥ 17 μm in length were too long to be completely engulfed, resulting in frustrated phagocytosis. There was a clear distinction in the bioactivity and cytotoxicity of fibers too long to be completely engulfed compared to shorter fibers. Glass fiber fractions having 17 μm or 33 μm lengths exhibited similar cytotoxicity on macrophages *in vitro*, measured as lactate dehydrogenase release or inhibition of zymosan-stimulated chemiluminescence. However, these long fibers had a toxic potency nearly two orders of magnitude greater than fiber fractions of 3, 4, and 7 μm lengths. Bioactivity was measured as the ability of glass fiber fractions to activate the DNA binding of the transcription factor, nuclear factor kappa B (NFkB), to activate the gene promoter for tumor necrosis factor alpha (TNFα), and to increase TNFα production by macrophages *in vitro*. Long fibers (17 μm) were significantly more potent bioactivators than short fibers (7 μm). This bioactivation was inhibited by N-acetyl-L-cysteine, an antioxidant, indicating that the generation of oxidants contributed to this induction. These results suggest that length plays an important role in the potential pathogenicity of fibrous particles with effects being magnified when fibers are too long to be phagocytized completely.

1496 DIETARY VITAMIN E SUPPLEMENTATION DOES NOT PREVENT LUNG MITOCHONDRIAL DYSFUNCTION INDUCED BY *IN VITRO* AMIODARONE AND N-DESETHYLAMIODARONE.

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We have previously shown that mitochondrial dysfunction may be an initiating mechanism of amiodarone (AM)-induced pulmonary toxicity, and that dietary vitamin E supplementation decreases pulmonary injury resulting from intratracheal administration of AM to the hamster. In whole lung mitochondria isolated from hamsters maintained on a control diet, complex I-supported state 4 (resting) respiration was inhibited to a similar extent (by 72-89%) by equimolar concentrations (100, 200 or 400 μM) of AM or *N*-desethylamiodarone (DEA). In mitochondria from vitamin E-supplemented hamsters (500 IU d,l-α-tocopherol acetate / kg chow for 6 weeks), 400 μM DEA inhibited state 4 respiration more than 400 μM AM did (95% vs. 72%) ($p<0.05$), whereas 100 or 200 μM AM and DEA produced inhibition of the same magnitude (by 63-91%). For complex II, all concentrations of both drugs (50-400 μM) inhibited state 4 respiration in mitochondria from both diet groups, but 100, 200 and 400 μM DEA (by 90-100%) had greater effects than equimolar concentrations of AM (by 60-78%) ($p<0.05$). In addition, inhibition by DEA at both complexes occurred more rapidly than did inhibition by AM. For both diet groups, mitochondrial membrane potential (measured by safranine dye fluorescence) was decreased to the same degree by equimolar concentrations of both drugs, but at 200 and 400 μM, the rate of decrease was greater (by 229-600%) for DEA than for AM ($p<0.05$). Lipid peroxidation in mitochondria from the control diet group, measured as thiobarbituric acid-reactive substance (TBARS) production, was not increased by either drug (50-400 μM) in incubations lasting up to one hour. While differential effects of AM and DEA with respect to respiration and membrane potential were observed, the minimal effect of dietary vitamin E supplementation in this study suggests that the protective mechanism(s) of vitamin E against AM-induced pulmonary toxicity is not exerted at the level of the mitochondrion. (Supported by Medical Research Council of Canada grant No. MT-13257.)

1497 AMIODARONE (AM) DISRUPTS MITOCHONDRIAL MEMBRANE POTENTIAL IN ISOLATED LUNG CELLS: POTENTIAL MECHANISM OF AN INDUCED PULMONARY TOXICITY.

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Amiodarone (AM), a commonly prescribed antidysrhythmic agent, causes potentially fatal pulmonary fibrosis. In the present study, we investigated perturbation of mitochondrial function as a possible initiating mechanism of AM induced cytotoxicity in hamster lung cells. Preparations enriched with alveolar macrophages (95=98%), alveolar type II cells (75-85%) and non-ciliated bronchiolar epithelial (Clara) cells (35-50%), were isolated from male golden Syrian hamsters by protease digestion followed by centrifugal elutriation and density gradient centrifugation. In intact cells, mitochondrial membrane potential was measured cytofluorometrically with the 5,5',6,6'-tetrachloro 1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1)probe (argon laser excitation 488nm). Following incubation for 2 h, the ratio of mean red (high membrane potential) to mean green (low membrane potential) fluorescence (i.e. mean 575nm fluorescence intensity/mean 525nm fluorescence intensity SD) of vehicle treated (0.1% ethanol) alveolar macrophages (1.19 0.3) alveolar type II cells (1.00 0.4) and Clara cells (1.14 0.3) was significantly higher ($p<0.01$, n=5) than in the 100 M AM treated cells (0.69 0.2), (0.49 0.2), and (0.52 0.2) respectively. Following preloading with [3 H] adenosine for 1 h, and subsequent incubation with 100 & M AM or vehicle for 2 h, [3 H] ATP, [3 H] ADP and [3 H] AMP were separated by reverse phase HPLC and quantitated by scintillation counting. In contrast to the membrane potential results, no significant differences in the amounts of [3 H] ATP, [3 H] ADP or [3 H] AMP were observed between control and AM treated cells. Although we have previously demonstrated significant AM-induced cytotoxicity within these enriched fractions at 24h, cell viability (measured by 0.5% trypan blue exclusion) was not altered by 100 M AM at 2h. This demonstrates that AM disrupts mitochondrial membrane potential early during AM exposure, at a time when effects on ATP content and cell viability are not apparent. Hence, the initiating mechanism of AM-induced cytotoxicity may be perturbation of mitochondrial function. (Supported by MRC of Canada Grant# MT-13257.)

1498 ASSESSMENT OF ACUTE LUNG INJURY IN RATS EXPOSED TO DIPHENYLMETHANE-4,4'- DIISOCYANATE (MDI): ANALYSIS OF BREATHING PATTERNS AND LUNG LAVAGE.

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The early acute pulmonary response of Wistar rats exposed nose-only to diphenyl-methane-4,4'-diisocyanate (MDI) aerosol was examined to investigate both the relationship between acute pulmonary irritation and ensuing disturbances of the air-blood barrier. The first was addressed by analysis of changes in breathing patterns, the second was addressed in rats exposed for 1 x 6-hr to 0.6 to 20 mg MDI/m³, including analysis of the time-response relationship of MDI-induced acute lung injury during a postexposure period of 1-week. Bronchoalveolar lavage fluid and cells were analyzed for markers indicative of injury and changes in surfactant homeostasis. The analysis of breathing patterns demonstrated that respirable MDI aerosol caused a concentration-dependent apneic pause with minor changes on respiratory rate which is suggestive of stimulation of J-receptors. Serial sacrifices of rats exposed for 6-hr to 0.6-20 mg MDI/m³ revealed that most endpoints were maximal on postexposure day 1 and changes returned to the level of the control group on day 7. The analysis did not reveal a time-dependent exacerbation of effects. The relative comparison of effects suggests that a dysfunction of the air-blood barrier was characterized the increase in BAL-ACE (ACE: angiotensin-converting enzyme) activity and marked increase of intracellular phospha-tidyl-choline. In summary, this study suggests that respirable MDI aerosol appears to stimulate extravasation of plasma proteins via stimulation of J-receptors rather than cytotoxicity. It is believed that the increase of intracellular phospholipids indicates the involvement of pulmonary surfactant.

Thus, it appears that changes in pulmonary surfactant function is important for the understanding of the pathomechanism of respirable MDI aerosol.

1499 FATE OF DIESEL SOOT-ADSORBED BENZO[A]PYRENE FOLLOWING DEPOSITION AND RETENTION IN THE LUNGS OF DOGS.

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The rate and extent to which carcinogenic hydrocarbons on diesel soot particles are released in the lungs may contribute to the suspected activity of diesel emissions as lung carcinogens in humans. The alveolar absorption of tritiated benzo(a)pyrene (BaP) adsorbed on the carbonaceous core of diesel soot particles was measured in the Beagle dog. The concentration of BaP was 0.015 μg/μg soot, which corresponds to 25% of a monomolecular coating of BaP on the particle surface. The dogs were exposed to a bolus of resuspended diesel soot in a single deep breath, and the subsequent appearance of BaP in the systemic circulation was monitored by repeatedly sampling blood from the ascending aorta and the posterior vena cava. At 5.6 months after the exposure, diesel soot was recovered from peripheral lung tissue and the tracheobronchial lymph nodes through ultracentrifugation. The release rate of BaP from the soot was highest at the moment of deposition, but decreased rapidly with time. Soot recovered from lung and lymph nodes 5.6 months after the exposure had 16% and 10%, respectively, of a monomolecular layer of BaP-equivalent activity remaining. The bioavailability of BaP from soot particles was, thus, significantly higher in lymph nodes than in lung parenchyma. Of the radioactivity extracted into toluene from the recovered soot particles, ~30% was BaP parent compound, and the rest was metabolites/decay products of BaP. After toluene extraction, 7% of a BaP monolayer remained on the soot. A substantial fraction of soot-adsorbed BaP was extracted in the lung, but still was less than that extractable into organic solvents. (Research sponsored by the Health Effects Institute and the Swedish Council for Working Life Research, under Cooperative Agreement DE-FC04-96AL76406 with the US DOE.)

1500 GENETIC MODELING OF TOLERANCE TO ZINC OXIDE INHALATION IN INBRED MICE.

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In a variety of occupational settings, workers are exposed to inhaled toxicants such as zinc oxide (ZnO) fumes generated from welding processes. Inhalation exposure to ZnO can induce a variety of rapidly onsetting adverse pulmonary effects including inflammatory cellular influx, and can also give rise to systemic effects such as "inhalation fever." As a result of frequent and repeated exposures to various inhaled toxicants, many workers develop tolerance (or tachyphylaxis) to the exposures and exhibit reduced symptoms. The implications of tolerance to health risks in humans are numerous. We hypothesize that inter-individual variability in the development of tolerance to inhaled toxicants is controlled by host genetic factors. Studies were conducted using inbred strains of mice to begin identifying genes controlling the development of tolerance. Ten inbred mouse strains were exposed to 1.0 mg/m^3 for 3 h a single time (1X) or on 5 successive days (5X). Pulmonary inflammation was assessed after 1X and 5X exposures by inflammatory cell counts and total protein content in bronchoalveolar (BAL) lavage. Results demonstrated marked inter-strain variation exists in the ability to develop tolerance to ZnO-induced pulmonary inflammation, as well as in the initial pulmonary response to inhaled ZnO. In general, only a tolerant and a non-tolerant phenotype was displayed among the inbred strains which suggests that a single gene may be responsible for a significant amount of variance in the tolerance phenotype. BALB/cBy and DBA/2 mice were identified as tolerant and non-tolerant strains, respectively. Inflammatory cell and protein concentrations in BAL fluid from F1 offspring of these two strains exposed to ZnO were like that of the tolerant BALB/cBy progenitor. This is indicative that the ZnO-induced pulmonary tolerance phenotype is an inheritable dominant trait. This genetic model provides a unique opportunity to investigate mechanisms and regulation of pulmonary tolerance. The observations from this research will allow for the identification of genes that control the development of tolerance through the use of linkage analysis and oligonucleotide microarray techniques in future studies.

1501 DNA SEQUENCE POLYMORPHISMS OF INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR-α IN SILICOSIS.

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Silicosis is an inflammatory /fibrotic lung disease and represents a major health problem worldwide. Many gene products which include inflammatory and fibrotic mediators are involved in the progression of this disease. Since IL-1 and TNF-α promote inflammation and fibrosis and play a central role in the pathogenesis of many chronic inflammatory diseases, it is now believed that the polymorphisms of these cytokines play an important role in silicosis. To investigate the relationship between severity of silicosis and the genetic variations associated with interleukin-1(IL-1) and tumor necrosis factor-α (TNF-α) production, we examined DNA collected from lung autopsy tissues from 150 miners diagnosed with silicosis as well as in 150 control miners with no disease. We analysed functional SNPs at the TNF- α (-308 and -238), IL-1β (+3953), IL-1α (+4845) and IL-1 receptor antagonist (RA) (+2016) loci by RT-PCR to determine each genotype. Our preliminary results suggest that IL-1RA (+2016) and TNF-α (-238) may have a role in the pathogenesis of silicosis. These findings provide new insight into the possible role of IL-1 and TNF-α polymorphisms in the severity of this disease.

1502 EFFECTS OF SULFUR DIOXIDE EXPOSURE ON NEUTROPHIL RESPIRATORY RESPONSE IN CATTLE.

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Sulfur dioxide (SO_2) is one of the pollutants that is suspected in affecting the health and productivity of cattle raised on pastures located in close proximity to sour gas and petroleum processing operations in Alberta, Canada. The objective of this investigation was to assess the effects of SO_2 on cell-specific respiratory responses in peripheral neutrophils of cattle. For inhalation exposures to SO_2, two steers were sequentially exposed to atmospheres containing 0, 5, and 20 ppm of SO_2 for 6 hr/day for a period of 6-7 days per exposure in a controlled environmental system. Blood from each animal was collected before, during and after exposure to SO_2. The respiratory rates of neutrophils were measured in non-stimulated cells (basal rate) and in phorbol myristate acetate (PMA)-stimulated cells (respiratory burst or stimulated rate). A flow cytometric procedure involving the oxidation of dihydrorhodamine to rhodamine was used to measure the respiratory responses in neutrophils. Exposure to SO_2 did not cause any significant change in the basal rates; however, the respiratory burst response was lowered by 25-30% after exposure to 5 ppm, and by 60% after exposure to 20 ppm SO_2 concentrations. In experiments conducted *in vitro*, the direct treatment of isolated neutrophils with sulfite ($SO_3^=$), a primary hydrated form of SO_2 at physiological pH, caused inhibition of cellular respiration and superoxide anion production in PMA-stimulated neutrophils. The sensitivity of these cell-specific responses was dependent on $SO_3^=$ concentration and duration of treatment. The stimulus-dependent respiratory responses were severely inhibited (> 80%) after a 24 hr treatment of cells with fairly low concentrations of $SO_3^=$ (e.g., 1 mM). This research will ascertain the exposure sensitivity, its nature, and associated implications for cattle health.

1503 CHANGES IN GENE EXPRESSION IN NHBE CELLS EXPOSED TO TRANSITION METALS.

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Metals (Cu, Fe, Zn, As, V) present in particulate pollution have been shown to cause lung injury and inflammation in animals and in cultured human lung epithelial cells. Earlier work demonstrated that individual metals did not all activate the same signal transduction pathways or transcription factors, suggesting that metals may exert their effects by different mechanisms. In order to better understand differences in mode of action of individual metals, we have employed gene array technology to compare patterns of gene expression after exposer of human lung primary epithelial cells to Ni(II), Fe(II), V(IV), Zn(II), or As(III). Cells were exposed to these metals for 4 hours and mRNA levels of 588 genes were quantified using the Atlas Human cDNA Expression Array (Clontech). The expression of mRNAs coding for nearly 100 different genes was significantly altered by these metals; both increased and decreased

amounts of mRNA were observed. Preliminary analysis indicates that Fe and Zn followed similar gene expression patterns as did V and Ni while As-induced gene expression was most closely related to that of V and Ni. Specifically, V, Ni, and As upregulated genes such as cyclic-AMP-dependent transcription factor ATF-4, RANTES, and calgranulin A and B generally associated with increases in inflammation and down-regulated genes such as IL-13 associated with decreases in inflammation whereas Fe and Zn upregulated more genes associated with apoptosis such as UV excision repair protein RAD23 and c-myc transcription factor (puf) as well as certain glutathione transferases. In order to facilitate analysis of the large data set, we chose to focus on the changes in expression in inflammatory genes. Metal-induces changes in expression of the subset of inflammatory-associate genes were confirmed by RT-PCR analysis of mRNAs present after 4, 8, and 24 hour exposures. These data should provide new insights into transition metal toxicity as well as illustrating the utility of gene array technology in identifying patterns of gene expression. (This abstract of a proposed presentation does not necessarily reflect EPA policy.)

1504 EFFECT OF CRF RECEPTOR-1 INHIBITION ON LEYDIG CELL TESTOSTERONE PRODUCTION.

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A role for the corticotrophin releasing factor (CRF) in androgen biosynthesis by testicular Leydig cells has been proposed. We evaluated the potential effects of a series of selective CRF receptor-1 (R1) antagonists (SK696, SP904, and SP397) on testosterone production in primary cultures of rat, dog and monkey Leydig cells. Cells were isolated by differential density gradient separation of enzymatically digested testicle. Enrichment for Leydig cells was confirmed by 3-b hydroxysteroid dehydrogenase staining and cytology. Cells were cultured for 1 to 3 hours in the presence or absence of 2 IU/mL (rat, monkey) or 5 IU/mL (dog) of human chorionic gonadotrophin (hCG) after which time testosterone was assayed in supernatant by radioimmunoassay. Ketoconazole, used as a positive control, produced an expected dose-dependent decrease in basal and hCG-stimulated testosterone production in all species. CRF itself did not alter baseline testosterone production and R1 antagonists did not modify basal or hCG-stimulated testosterone production in any species. A role for CRF R1 in Leydig cell testosterone biosynthesis was not demonstrated. Therefore, pharmacologic inhibition of CRF R1 is not expected to alter Leydig cell function in humans.

1505 INTERACTION OF METHOXYCHLOR AND RELATED COMPOUNDS WITH ESTROGEN RECEPTOR ALPHA AND BETA, AND ANDROGEN RECEPTOR: STRUCTURE-ACTIVITY STUDIES.

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We have previously demonstrated the differential activity of the methoxychlor metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) with estrogen receptor alpha (ERα), beta (ERβ), and androgen receptor (AR). In this study we characterize the ERα, ERβ, and AR activity of structurally related Methoxychlor metabolites. Human hepatoma cells (HepG2) transfected with human ERα, ERβ, or AR expression plasmids plus and a steroid-responsive reporter gene, were dosed with various concentrations of HPTE or structurally related compounds in the presence (for detecting antagonism) or absence (for detecting agonism) of 17β-estradiol or dihydrotestosterone. The monohydroxy analog of methoxychlor as well as mono- and dihydroxy analogs of 2,2-bis(p-hydroxyphenyl)-1,1-dichloroethylene had ERα agonist activity and ERβ and AR antagonist activities similar to that observed for HPTE. The trihydroxy analog of methoxychlor displayed only weak ERα agonist activity and did not alter ERβ or AR activity. Replacement of the trichloro- or dichloroethane with a methyl group resulted in a compound with agonist ERα and ERβ activity while retaining AR antagonist activity. This study identifies some of the structural requirements for differential ERα, ERβ, and AR activity and demonstrates the complexity involved in determining the mechanism of action of endocrine active chemicals that may act as agonists or antagonists through one or more hormone receptors. (ES09106 and ES04917)

1506 ANDROGEN RECEPTOR AGONIST DIHYDROTESTOSTERONE REDUCES ESTROGENIC RESPONSES IN RAINBOW TROUT.

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We investigated the capacity of dihydrotestosterone (DHT), a potent, non-aromatizable androgen, to act as an endocrine disruptor in rainbow trout, *Oncorhynchus mykiss*, by altering vitellogenin (Vg), 17β-estradiol (E_2) and total cytochrome P450 levels. DHT (0, 50 or 100 mg/kg/day) and flutamide (0 - 1250 mg/kg/day), an androgen receptor antagonist, were fed to juvenile rainbow trout for 2 weeks. No toxicity was observed between groups as observed grossly and measured by body weight and liver somatic index. Vg and E_2 levels were suppressed by over 80% and 50%, respectively, in trout fed DHT. In trout fed DHT and flutamide, Vg levels were similar to groups fed DHT alone, suggesting that the DHT effects on Vg were not mediated through direct androgen receptor binding. Flutamide alone, however, was as effective as DHT in decreasing E_2 in both sexes, but decreased Vg significantly in males only. This observed difference in Vg production in response to E_2 decreases may represent sex differences in crosstalk between the androgen and estrogen receptor pathways regarding Vg production. Cytochrome P450 repression was quantified to explore possible indirect effects of DHT. DHT reduced total P450 content to less than 20% of controls in both sexes. Co-treatment with flutamide somewhat inhibited these decreases in males, suggesting that DHT induced P450 decreases may be partially mediated through androgen receptor binding. In females fed DHT, flutamide did not increase P450 levels suggesting a sex difference in the sensitivities to androgen receptor mediated effects by DHT. The antiestrogenic and P450 effects of DHT may or may not be related. These studies indicate that androgen receptor agonists/ antagonists can elicit significant antiestrogenic effects that may not necessarily be mediated through receptor binding. (Supported by ES03850 and ES07060.)

1507 THE CONSTITUTIVE ANDROSTANE RECEPTOR BETA MEDIATES THE TRANSCRIPTIONAL REGULATION OF CYP2B GENES BY PHENOBARBITONE.

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The rodent hepatocarcinogen phenobarbitone (PB) induces expression of the mouse cyp2b10 gene and rat CYP2B1/2 genes at the transcriptional level. Within the promoter of each gene, an essentially identical regulatory element has been defined which mediates PB transcriptional induction, termed the PB-Responsive Enhancer Module (PBREM) [Honkakoski, P. & Negishi, M. (1997) J. Biol. Chem. 272, 14943-14949]. The constitutive androstane receptor-beta (CAR-beta) nuclear receptor exhibits intrinsically high transcriptional activity which is abolished by the mammalian pheromone 5-alpha-androst-16-en-ol (androstenol) [Forman, B.M., Tzameli I., Choi H.S., Chen J., Simha D., Seol W., Evans R.M. and Moore D.D. (1998) Nature 395, 612-5], and binds to a direct repeat (DR4) element within the cyp2b10 PBREM [Honkakoski, P., Zelko I., Sueyoshi T. and Negishi M. (1998) Mol. Cell. Biol. 18, 5652-8]. In HepG2 cells, PB activates CAR-beta at a substantially high concentration than that observed for induction of the cyp2b10 in mouse hepatocytes [Sueyoshi, T., Kawamoto, T., Zelko, I., Honkakoski, P., and Negishi, M. (1999). J. Biol. Chem. 274, 6043-6]. We have constructed a luciferase reporter gene containing the cyp2b10 PBREM DR4 element and carried out co-transfection assays in CV-1 cells with mouse CAR-beta. CAR-beta caused a 4-fold induction of reporter gene activity, which was ablated by administration of 10microM androstenol for 20 hours Addition of 500microM PB for the same period did not alter reporter gene activity in the presence of androstenol. However, 300nM of the potent PB-like inducer 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) restored reporter gene activity to control levels when incubated with 10microM androstenol. In contrast, when CAR-beta was co-transfected in rat hepatocytes with the same reporter gene, both 300microM PB and 300nM TCPOBOP induced reporter gene activity (3 and 5 fold respectively) and this was suppressed by 50microM androstenol. Our results imply that CAR-beta mediates the transcriptional effects of PB, although clearly the cellular context is important for this event.

1508 TRANSCRIPTIONAL ACTIVATION OF DNA POLYMERASE α BY ESTROGEN IN MCF-7 CELLS REQUIRES INTERACTION OF ESTROGEN RECEPTOR α/Sp1 WITH A GC-RICH ELEMENT.

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17β-Estradiol (E2) is mitogenic in human breast cancer cells and stimulates DNA synthesis, cell cycle progression, and genes associated with cell proliferation. Treatment of MCF-7 breast cancer cells with 10nM E2 results in increased DNA polymerase α mRNA levels, and in MCF-7 cells cotransfected with estrogen receptor α (ERα) plus a construct containing the -1515 to +45 region of the DNA polymerase α gene promoter linked to a luciferase reporter gene (pDNAP1), E2 induced a 2.8-fold increase of luciferase activity. Deletion analysis of the DNA polymerase α gene promoter identified a region from -116 to -65 required for ERα-mediated transactivation, and this sequence contained a GC-rich site and a c-AMP response element. Subsequent mutational analysis of this region of the promoter showed that only the GC-rich site was required for transcriptional activation by E2. Gel electromobility shift assays and antibody supershifts showed that nuclear extracts from MCF-7 cells bound this region of the promoter and formed retarded bands with both Sp1 and Sp3 proteins. ERα did not directly bind to the promoter but enhanced Sp1-DNA complex formation. *In vitro* footprinting experiment demonstrated that recombinant human Sp1, but not ERα, protein bound the GC-rich sequence (-106 to -100); however, coincubation with both Sp1 plus ERα proteins demonstrated that ERα significantly enhanced the Sp1 footprint. These results demonstrate that transcriptional activation of DNA polymerase α by E2 requires ERα/Sp1 interactions with a proximal GC-rich element in the gene promoter. (CA76636 and ES09106)

1509 ENHANCED DNA-INDEPENDENT TRANSCRIPTIONAL ACTIVITY OF ZINC FINGER DOMAIN - DELETED MOUSE ESTROGEN RECEPTOR THROUGH THE ESTROGEN RECEPTOR/SP1 PROTEIN INTERACTION.

K. H. Kim and S. Safe. *Texas A&M University, Department of Veterinary Physiology & Pharmacology, College Station, TX.*

Several estrogen-inducible genes are transactivated through estrogen receptor α (ER_α)-Sp1 protein interactions that do not require direct binding of ER_α to estrogen response elements (EREs). To further characterize these interactions, we generated a mouse estrogen receptor (MOR) mutant using PCR-based mutagenesis. Twenty-one amino acids from one of the zinc finger domains of wild-type MOR (aa 189 to 209) were deleted. The mutant (MORΔZF1) did not bind [^{32}P]-ERE in a gel mobility shift assay, whereas both wild-type ER_α and ER_β bound as homo or heterodimers to give specifically-bound retarded bands. In MDA-MB-231 cells transiently transfected with a construct containing a triple ERE promoter insert ($pERE_3$) and wild-type MOR expression plasmid, treatment with 10 nM E2 resulted in 3.1-fold induction of reporter gene (luciferase) activity. In contrast, replacement of MOR with MORΔZF1 expression plasmid resulted in loss of E2-induced activity, and this is consistent with the failure of the mutant to bind DNA. In MCF-7 and MDA-MB-231 cells cotransfected with a construct containing a consensus triple GC-rich Sp1 binding motif ($pSp1_3$) and MOR expression plasmid, treatment with E2 resulted in a 2- and 2.3-fold induction of luciferase activity, respectively. However, replacement of MOR with MORΔZF1 expression plasmid resulted in a significantly increased 77- and 10.6-fold induction of reporter gene activity in these cell lines respectively, suggesting that unknown inhibitory protein(s) may be interacting with aa 189 to 209 of the DNA binding domain of wild-type MOR. Current studies are investigating transactivation potential of this mutant compared to MOR mutants of the second zinc finger motif and human analogs and their functional differences in ER/Sp1-mediated transactivation in various cell lines.

1510 IDENTIFICATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA-DEPENDENT GENES RESPONSIVE TO A HEPATOCARCINOGENIC PEROXISOME PROLIFERATOR CHEMICAL.

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Peroxisome proliferators (PPs) are a diverse group of rodent non-genotoxic carcinogens that include hypolipidemic drugs and industrial chemicals. Short-term treatment of rats and mice with PP results in an increase in liver peroxisome number, hepatomegaly and target genes transcription. Chronic treatment of rodents with PP results in hepatocarcinogenesis. It is important to determine the risks to humans of environmental and therapeutic exposure to these compounds by understanding the mechanisms of non-genotoxic heptocellular carcinoma formation in rodent. Earlier studies suggested that a member of steroid hormone receptor called peroxisome proliferator-activated receptor (PPAR) mediates the action of PP (e.g. Wy-14,643). There are three subtypes of PPAR within vertebrates termed alpha, beta and gamma and PPAR alpha appears to be the one that is most strongly activated by PP. Gene-knockout mice lacking PPAR alpha definitely confirmed that PPAR alpha mediates PP-induced peroxisome proliferation, hepatomegaly and hpatocarcinogenesis. However, the underlying mechanisms by which PPAR alpha mediates these pleiotropic responses are still not clear. It is suggested that activation of PPAR alpha by PPs will lead to altered transcription of some gene(s) involved in hepatocyte growth regulation. Identification of such PP responsive genes that are PPAR alpha dependent might gain an insight into the molecular mechanisms involved in the PPAR alpha-dependent pathway in PP-induced pleiotropic effects. The aim of the present study is to search genes that are differentially expressed in wild-type but not in PPAR alpha knockout mice after treatment with Wy-14,643 for two weeks by using fluorescent differential display RT-PCR technique. Up to now, twenty differentially expressed cDNAs were obtained and two of them were identified as Cyp4a10 and Cyp4a14. Further characterization of other cDNAs is underway.

1511 SPECIES DIFFERENCES IN RESPONSE TO PEROXISOME PROLIFERATORS: INCREASING HUMAN PEROXISOME PROLIFERATOR-ACTIVATED-RECEPTORα (PPARα) EXPRESSION LEVELS IN HUMAN CELLS IS NOT SUFFICIENT FOR FATTY ACYL-COA OXIDASE RESPONSIVENESS.

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We compared the ability of rat and human hepatocytes to respond to fenofibric acid and a novel phenylacetic acid PPARα agonist (compound 1). Fatty acyl-CoA oxidase (FACO) activity and mRNA were increased after treatment with either fenofibrate or compound 1 in rat hepatocytes. In addition, apoCIII mRNA was decreased by both fenofibric acid and compound 1 in rat hepatocytes. Both agonists decreased apoCIII mRNA in human hepatocytes; however, very little change in FACO activity or mRNA was observed. Moreover, other genes that are regulated in human hepatocytes, such as mitochondrial HMG-CoA synthase and carnitine palmitoyl transferase-1 (CPT-1), were also regulated in HepG2 cells in culture by PPARα agonists. Several stabily transfected HepG2 cell lines were established that overexpressed human PPARα (between 2 and 26-fold over normal human hepatocytes). These PPARα overexpressing cells had higher basal mRNA levels of mitochondrial HMG-CoA synthase and CPT-1; however, basal FACO mRNA levels were not affected. In addition, FACO mRNA levels were not increased by PPARα agonist treatment in the PPARα overexpressing cells, although mitochondrial HMG-CoA synthase and CPT-1 were both induced. These results suggest that other factors besides PPARα levels determine the species specific response of human and rat hepatocytes to the induction of peroxisome proliferation.

1512 EVIDENCE FOR DIVERGENT SIGNALING PATHWAYS IN REGULATION OF RECEPTOR MEDIATED EFFECTS OF PEROXISOME PROLIFERATORS.

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Peroxisome proliferators are a diverse group of environmentally, pharmacologically and economically important chemicals associated with widespread human exposure. In rodents, chronic exposure to these agents causes hepatocellular proliferation and hepatic tumors. It is established that many of the hepatic effects of peroxisome proliferators are mediated through the nuclear receptor, PPARα. Using primary mouse hepatocytes, we previously found that peroxisome proliferators also cause a rapid phosphorylation and activation of the extracellular signal-regulated kinase (ERK). Activation of ERK by Wy-14,643 is associated with an increase in replicative DNA synthesis, which could be blocked by inhibitors of both ERK kinase (PD098059) and phosphatidylinositol 3-kinase (LY294002). To further investigate the role of these pathways in mediating response to peroxisome proliferators,we are currently determining whether the ERK and PI3K pathways directly modulate or regulate PPARα transcriptional activity. In transfection experiments with luciferase reporter vectors to measure PPARalpa activation, the transactivation activity of PPARα was inhibited by low concentrations of LY294002, but

unaffected by concentrations of PD098059 which block ERK activation. In addition, although epidermal growth factor strongly activated ERK in a PI3K-independent manner, it did not specifically modulate PPARα transactivation. Therefore, we hypothesize that PI3K is an upstream effector of ERK activation by peroxisome proliferators and participates in the mitogenic response to these chemicals. In contrast, our results suggest PI3K and ERK play separate roles in modulating PPARα transactivation. (Supported in part by US DOE contract DE ACO6 76RLO 1830.)

1513 INHIBITION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA BY MK886.

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MK886 was originally identified as a potent inhibitor of leukotriene biosynthesis. Its mechanism is related to its ability to inhibit 5-lipoxygenase activating protein (FLAP), and this has also been presumed to underlie the apoptosis inducing activity of MK886. It is now evident that MK886-induced apoptosis is unrelated to lipoxygenase (*Biochem. J.* **340**: 371 [1999]). FLAP is a fatty acid binding protein, and MK886 may bind to other such proteins. Peroxisome proliferator activated receptors (PPARs) have been labeled as 'fatty acid receptors' because they are activated by endogenous fatty acids as well as exogenous peroxisome proliferators. The ability of MK886 to inhibit PPAR activity was assessed using both transient and stable transfection reporter assays in CV-1, keratinocyte 308, and CHO cells. In all systems examined, 1-10 μM MK886 inhibited PPARα ~80%. Higher doses appeared to be less effective, perhaps due to a partial agonist effect. Effects on PPARδ were substantial (~50%) using the stable transfection assay in CV-1 cells, but were not evident using the transient transfection reporter assay or in keratinocytes, perhaps because of low basal activity. Inhibition of PPARγ was substantially lower than the other receptor subtypes. Although there are numerous PPAR activators, only one xenobiotic was recently shown to antagonize PPARγ, and MK886 is the first xenobiotic shown to inhibit PPARα and PPARδ. (Supported by HL51005 and Center Grant ES07784.)

1514 THE MAPK SIGNAL TRANSDUCTION PATHWAY REGULATES PPARα THROUGH DIRECT PHOSPHORYLATION OF THE RECEPTOR.

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Peroxisome proliferators (PPs) induce the expression of target genes by activating a member of the steroid receptor superfamily, Peroxisome Proliferator-Activated Receptor alpha (PPARα). To further elucidate the PP signaling pathway, the ability of the mitogen-activated protein kinase (MAPK) pathway to regulate PPARα activation was examined. The specific MAPK/Erk kinase (MEK) inhibitor, PD098059 inhibited the transcriptional activity of ectopically expressed PPARα. The effect of PD098059 on PPARα was further demonstrated to be due to MEK inhibition since co-transfection of a dominant negative MEK gave similar results. Further, when a rat hepatoma cells were pretreated with PD098059, the Wy-14,643-mediated induction of the PP-responsive gene fatty acyl-CoA oxidase (ACO) was completely abolished. PD098059 pretreatment inhibited MAPK phosphorylation in these cells without affecting the expression of PPARα. In addition, MAPK was able to phosphorylate PPARα *in vitro*. Taken together, these results suggest that growth factor signal transduction pathways positively regulate PPARα, and this effect is through direct phosphorylation of the receptor by MAPK. (Supported by NIH ES07799 and DK49009.)

1515 ARSENITE ALTERS SIGNAL TRANSDUCTION BY INDUCING THE PRB-E2F PATHWAY AND NOT *VIA* EFFECTS ON P53.

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Human exposure to inorganic arsenic in drinking water is associated with cancers at a number of sites. Yet little is known of the molecular biological changes of such long-term low dose exposure. While not a mutagen itself at low concentrations, arsenite (AsO_2-) enhances the mutagenicity of other genotoxicants. Previous studies have shown that nontoxic levels of arsenite interfere with DNA repair mechanisms although not through direct inhibition of DNA repair enzymes. We investigated whether chronic, low-dose arsenite interferes with DNA damage responses involving the p53 checkpoint. WI38 normal human diploid fibroblasts were grown under standard conditions in media containing 0.1 μM sodium arsenite for at least 5 days. DNA damage was induced by X-rays (XIR, 6 Gy) and after 4 hr, p53 protein was measured in whole cell lysates by immunoprecipitation and Western Blotting. XIR induced p53 protein and its downstream targets p21 and gadd 45. However, arsenite did not prevent WI38 fibroblasts from mounting these responses. Short-term (18 hr) effects of higher concentrations of arsenite were also examined. P53 protein elevation occurred only at a toxic dose of arsenite (50 μM). The response to 6 Gy XIR was not inhibited at any dose. However, proteins in the pRb-E2F pathway did exhibit quantitative changes after long-term (30 days, 0.1μM) and short-term (18 hr, 0.5 μM) exposure to arsenite. Immunohistochemical staining of whole cells revealed increased amounts of E2F1 and cyclin D1, proteins controlling progression of the cell cycle into S phase. These results indicate that environmentally relevant levels of arsenite may alter signal transduction in the G_1 to S phase of the cell cycle, encouraging cell cycling at the expense of DNA repair and providing an explanation for arsenite's observed effects on comutagenesis and gene amplification.

1516 INHIBITION OF GAP JUNCTION COMMUNICATION, ACTIVATION OF MAPK, AND THE RELEASE OF ARACHIDONIC ACID BY SPECIFIC ISOMERS OF METHYLATED ANTHRACENES.

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The mitogenic activity of tumor promoters requires the removal of an initiated cell from growth suppression by inhibiting gap junctional intercellular communication (GJIC) and the activation of intracellular mitogenic pathways. We tested the structure activity relationship of two isomers of 1- and 2-methylanthracene (1-MeA and 2-MeA) on the temporal activation of mitogen activated protein kinase (MAPK), the release of arachidonic acid (AA) from the plasma membrane, and the inhibition of GJIC in F344 rat liver epithelial cells. Noncytotoxic doses of 1-MeA, which contains a bay-like region, reversibly inhibited GJIC, induced the release of AA, and strongly activated ERK 1 & 2 and P38 but only weakly activated SAPK/JNK. In contrast, the linear-planar isomer 2-MeA had no effect on GJIC, AA release, and MAPK activation. MAPK was activated 10-20 min after the down-regulation of GJIC and release of AA, which indicates that MAPK is not involved in the initial regulation of GJIC and that AA may play an upstream role in activating all MAPKs. Our results are consistent with the hypothesis that mitogenesis requires the removal of growth suppression by inhibition of GJIC and then the activation of mitogenic pathways. (This research was supported by the NIEHS Superfund grant #P42 ES04911-07.)

1517 MAP KINASE ACTIVATION IN HUMAN AIRWAY EPITHELIAL CELLS EXPOSED TO AMBIENT AIR PARTICULATE MATTER.

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Exposure to ambient particulate matter (PM) in the Utah Valley (UV) has previously been associated with a variety of adverse health effects. In the present study we investigated intracellular signaling mechanisms that may contribute to pulmonary responses to PM inhalation in human airway epithelial cells exposed to PM collected from the UV. Aqueous extract of PM sampled in the year before (Y1), during (Y2), and after (Y3) the closure of a local steel mill located in the UV were used to expose primary human airway epithelial cell cultures (HAEC). UV PM induced IL-8 expression in a dose- and time-dependent manner in HAEC with a potency rank of Y1>Y3>Y2. Transfection with dominant negative constructs of ERK1 or ERK2 effectively blocked UV PM-induced IL-8 promoter reporter activity in HAEC. In contrast, overexpression of dominant negative P38 or JNKK1 had no effect. Similarly, the P38 inhibitor SB203580 failed to block UV PM-induced IL-8 release. The ERK MAP kinase kinase (MEK1/2) inhibitor PD98059 significantly diminished IL-8 released in response to UV PM, as did the EGF receptor kinase inhibitor tyrphostin AG 1478. Western blotting analyses showed UV PM-induced phosphorylation of EGF receptor tyrosine, MEK1/2 and ERK1/2, which could be ablated with AG1478 or PD98059 in HAEC. For all findings the potency of UV PM collected during Y2 was found to be lower relative to that of Y1 and

Y3. These data demonstrate that UV PM can induce IL-8 expression through the ERK signaling pathway by activating ERK upstream kinases, including the EGF receptor kinase. Moreover, these results show that UV PM collected during the closure of the steel mill is less active in inducing IL-8 expression through ERK signaling activation in HAEC. This study suggests that activation of the ERK MAP kinase pathway is a mechanism that can mediate pulmonary responses to ambient PM inhalation. (This abstract of a proposed presentation does not necessarily reflect EPA policy.)

1518 SARIN AND STRESS MODULATION OF NICOTINIC AND MUSCARINIC ACETYLCHOLINE RECEPTORS IN SARIN EXPOSED RATS.

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Stress is known to affect central nervous system (CNS) functions. The role of stress in CNS toxicity of organophosphate esters such as sarin (isopropyl methyl phosphonofluoridate) remains to be established. In the present study, we evaluated the role of stress in the manifestation of low dose sarin toxicity in the CNS of rats. Male Sprague-Dawley rats were exposed to stress 5 min./day, by placing the rats individually into a Plexiglas® restraint tube (60 days) followed by a single subclinical dose (0.1 X LD_{50}) of sarin (10ug/kg, i.m.). Twenty-four hours after the sarin treatment, nicotinic acetylcholine receptor (nAChR) and muscarinic receptor was determined in different brain regions using receptor-specific ligands. Sarin exposure caused a significant decrease in nAChR binding in cortex, brainstem and midbrain, while there was no change in the cerebellum. Stress exposure in animals caused further decrease in acetylcholine receptor nAChR binding. In contrast, sarin exposure caused upregulation in m2 muscarinic receptor binding in midbrain, cortex and brainstem, while there was no change in cerebellum. Stress exposure did not exhibit any modulating response on m2 muscarinic receptor binding. Sarin exposure did not cause any change in brain choline acetyl transferase and acetylcholinesterase activity whereas plasma acetylcholinesterase and butrylcholinesterase activities were slightly inhibited. No effect of stress, however, was observed in plasma cholinesterase activities. These data suggest that sarin at low subclinical doses may cause CNS-related abnormalities through differential regulation of nicotinic and muscarinic ACh receptors, with stress playing a critical role in manifestations of CNS toxicity by sarin. (Supported in part by a DOD grant DAMD 17-98-C-8027.)

1519 A NOVEL MECHANISM FOR ELECTRICAL SIGNALING BY INTERLEUKIN-1.

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Sponsor: L. G. Sultatos.

Interleukin-1 (IL-1) is a proinflammatory cytokine implicated in a wide variety of diseases, but little is known about its central actions. It has been associated with Alzheimer's, Down's, and AIDS-related dementia. In the hippocampus, IL-1 has been reported to affect changes in synaptic plasticity by enhancing synaptic inhibition and mitigating long-term potentiation. In the present study, it was postulated that IL-1 receptor (IL-1R) might behave as or be homologous with an ion channel(s)in the brain. GCG programming was employed to search Genebank using IL-1R as a template. This search revealed that IL-1R has a high degree of homology and identity with a specific voltage-gated, chloride channel (CLC-3), localized in the brain. A chloride-flux assay was employed using CHO cells stably transfected with IL-1R and IL-1R accessory protein, whose presence is required for biological activity. These assays were performed in microtiter plates using radiolabeled chloride. Termination was effected by vacuum suctioning assay buffer and quantitation of chloride uptake was performed by scintillation counting. A time course of specific uptake, relative to naïve cells, demonstrated a two-fold increase in chloride uptake in the presence of 0.1uM IL-1.This uptake was significantly blocked (~75%) with a 10-fold excess of IL-1R antagonist. In conclusion, functional genomic searching of Genebank has revealed an association between IL-1R and CLC-3.Ion flux assays demonstrated that IL-1R might have channel activity itself. The inhibitory influence of such channel activity by IL-1R, or the possible interaction of the IL-1 ligand with CLC-3 in the brain, would constitute a mechanism by which IL-1 could affect dementia.

1520 STIMULUS-RESPONSE RELATIONSHIPS DEMONSTRATE THE CONTRIBUTIONS OF AFFINITY AND INTRINSIC EFFICACY TO ARYL HYDROCARBON RECEPTOR LIGAND POTENCY.

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Models of receptor action describe properties of the ligand-receptor interaction and are necessary for risk assessment of receptor-mediated toxic effects. In order to build such a model for the aryl hydrocarbon receptor (AHR), binding affinities and CYP1A induction potencies were measured in PLHC-1 cells for ten AHR ligands: TCDD, TCDF, and PCB congeners 77, 81, 105, 118, 126, 128, 156 and 169. From these data, an operational model of AHR action was used to: 1) determine intrinsic efficacies of these ligands, 2) predict their properties in mixtures, and 3) determine the relationship between receptor occupancy and response. TCDD, TCDF, and non-ortho-substituted PCBs 77, 81, 126 and 169 behaved as full agonists and displayed high intrinsic efficacy. In contrast, the ortho-substituted PCBs bound to the AHR but displayed decreased or no intrinsic efficacy. PCB 156 was a full agonist, but with lower intrinsic efficacy. PCB 118 was a very weak partial agonist. PCBs 105 and 128 were competitive antagonists. When the properties of PCBs 126, 128 and 156 were measured in mixtures with TCDD, PCBs 156 and 128 inhibited the response to TCDD. Thus, the relative insensitivity of fish to ortho-substituted PCBs is a result of both lower receptor affinity and decreased intrinsic efficacy. The stimulus-response model predicts that only 1-2% of the receptors in the cell are occupied for 50% CYP1A induction by one of the high intrinsic efficacy agonists, demonstrating the existence of "spare" receptors in this system. Separation of AHR ligand action into the properties of affinity and intrinsic efficacy allows for comparison across species and toxic endpoints. (This work was supported by the EPA (R823889), NIH (ES06272), and WHOI Sea Grant.)

1521 DIFFERENTIAL EXPRESSION OF ARYL HYDROCARBON RECEPTOR ISOFORMS IN A FISH MODEL OF DIOXIN RESISTANCE.

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Fundulus heteroclitus, an estuarine teleost, is a well-characterized fish model of aryl hydrocarbon toxicity noted for the expression of two distinct genes for the aryl hydrocarbon receptor (AHR), AHR1 and AHR2. The *F. heteroclitus* population in New Bedford Harbor, MA (NBH), a Superfund site, exhibits heritable resistance to the toxic effects of halogenated aromatic hydrocarbons, including 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). To investigate the role of AHR signaling in TCDD resistance, we used quantitative RT-PCR to measure relative levels of mRNA for AHR1 and AHR2 in whole embryos at different developmental stages, comparing expression of these genes in NBH fish with fish from a reference site (Scorton Creek, MA; SC). We also compared the tissue distribution of these mRNAs in adult fish from both populations. AHR1 and AHR2 transcripts were present in eggs, decreasing over the forty-eight hours after fertilization, but subsequently increasing to steady state levels by the hatch (day 10). Embryonic expression of AHR1 mRNA was delayed relative to AHR2. At all stages examined, embryos of TCDD-resistant fish exhibited no measurable differences in expression of either AHR isoform compared to SC fish. In adult fish from the reference site, AHR2 mRNA was detected in all organs examined, while AHR1 transcripts exhibited a restricted distribution, found primarily in the heart, brain, gonad, and spleen. In contrast, AHR1 mRNA was widely expressed in NBH fish, appearing with unusual abundance in gill, gut, kidney, and liver. This striking discrepancy suggests that AHR1 expression may play a role in the TCDD-resistant phenotype of the NBH *Fundulus* population. Ongoing studies are examining the heritability and functional importance of ubiquitous AHR1 expression in this fish model of TCDD resistance. (Support: NIH ES07381 (MEH) and ES05800 (WHP), WHOI Sea Grant, the Donaldson Charitable Trust, and the WHOI Postdoctoral Scholar Program.)

1522 ANALYSIS OF THE NUCLEAR EXPORT SIGNAL OF THE MURINE ARYL HYDROCARBON RECEPTOR.

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The aryl hydrocarbon receptor (AHR) is a ligand activated basic helix-loop-helix protein that forms a heterodimer with the ARNT protein to mediate the effects of aromatic hydrocarbons. Previous studies from this lab (*J. Biol.*

Chem. 274:28708) have shown that the AHR protein is degraded in the cytoplasm and that the process is inhibited when nuclear export is blocked by treatment of cells with leptomycin B (LMB). Thus, it is likely that nuclear export and degradation are important steps in regulation of gene expression and necessary for termination of the ligand induced signal. The current study was designed to examine the effects of blocking degradation of AHR by mutating the Nuclear Export Signal (NES). The study of AHR mutated for nuclear export will permit analysis of AHR-mediated gene induction without concern for effects that may be caused by blocking global nuclear export with LMB. A first step in this process is the generation and functional analysis of AHR NES mutants. The NES for AHR protein spans residues 64-72 (LDKLSVLRL) in the helix 2 region that is also involved in protein dimerization with ARNT. In vitro mutagenesis was used to generate expression constructs with single residue mutations: L67A and L70A; or double mutations: L70A:L72A and L67A:L70M. The results show that the L70A:L72A does not form a functional AHR-ARNT heterodimer when analyzed by EMSA, and does not induce XRE driven reporter plasmids when transfected into cells even though it is functional in ligand binding and nuclear import. In contrast, L67A and L67A:L70M show DNA binding and gene induction to approximately 50% of wild type levels, while L70A appears to function to near wild type levels in both DNA binding and reporter gene assays. The reduced function of the L70A:L72A mutant shows the importance of these residues in dimerization and the difficulty of analyzing a sequence with dual functionality. The continued analysis of L67A, L70A and L67A:L70M will be important in determining the relationship between degradation of AHR and regulation of gene expression. (Supported by ES08980.)

1523 ANALYSIS OF RAINBOW TROUT AH RECEPTOR PROTEINS *IN VITRO*.

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Two cDNAs for the Ah receptor (AHR) have recently been isolated from rainbow trout (Oncorhychus mykiss). The cDNAs are highly homologous showing >97% sequence identity within the amino acid and nucleotide sequences including regions of the 3' and 5' untranslated regions. Both of the encoded proteins show sequence homology to the AHR 2 isoform initially isolated from fundulus. Studies were initiated to develop techniques to study aquatic receptors *in vitro* and compare the function of these two proteins to mammalian AHR *in vivo*. To facilitate study of the rtAHR proteins, they were expressed with FLAG peptide at the NH-terminus. Both rtAHRa and rtAHRb were expressed in an *in vitro* transcription/translation system and could be detected with anti-FLAG antibodies. To assess these protein further, equal amounts were mixed with either mARNT or $rtARNT_b$ and activated at 20-30°C. The activated complexes were tested for their ability to associate with mammalian xenobiotic response elements (XRE) or aquatic XREs. In this assay, mFLAGAHR/mARNT and mFLAGAHR/$rtARNT_b$ showed strong TCDD-dependent binding to both XRE sequences, however, neither rtAHR isoform showed significant binding activity compared to the mammalian sequence. In vitro activation of cytosol derived from trout gonad cell line, RTG-2, also showed a low level of activity compared to cytosol derived from mouse Hepa-1 cells whether activated at 4, 20 or 30°C for 2-5 hours. These results suggest trout AHR protein is extremely sensitive to *in vitro* analysis likely due to instability of the protein at the temperatures used for the assay. Thus, it appears that the analysis of these protein requires cell culture models that will support the integrity of the rAHR complex. (Supported by ES08980.)

1524 SCREENING FOR GENETIC VARIABILITY OF THE HUMAN *ARNT* GENE AND ANALYSIS OF THE 5'- FLANKING REGION.

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The human ARNT (aryl hydrocarbon receptor nuclear translocator) is widely expressed in human tissues. As a member of the basic-helix-loop-helix (bHLH) superfamily of proteins it acts as a heterodimerizartion partner of various structurally related proteins to form active transcription complexes influencing diverse cellular processes. The most prominent heterodimerization partner is the dioxin receptor/aryl hydrocarbon receptor (AhR). Ligand activation of AhR leads to induction of responsive genes via AhR/ARNT, some of them encoding for enzymes such as CYP1A2 that can metabolize xenobiotics to carcinogenic intermediates. Several studies have reported interindividual differences in the susceptibility towards effects of AhR agonists and in the activities of AhR/ARNT-regulated enzymes. In this study we investigate if there are any genetic variations in the *ARNT* gene that may contribute to these differences or that affect other cellular processes in which ARNT is involved. The *ARNT* gene including part of the 5'-flanking region has been isolated and characterized from a human BAC genomic library. DNA was isolated from peripheral blood samples in a cohort of 94 healthy Caucasian volunteers. Subsequently, PCR-SSCP (single strand conformation polymorphism) analysis was carried out. Fragments that showed a variant pattern were cloned and sequenced. The fragments investigated so far did not show genetic variants of possible functional significance. Completion of the analysis will give further insights. The promoter region of the human *ARNT* gene was found to contain a number of putative regulatory elements such as a TATA-box, a cAMP-responsive element, an E-box and a CAAT-box.

1525 SUBCELLULAR LOCALIZATION OF THE ARNT PROTEIN IN RAINBOW TROUT, MOUSE, AND CHICKEN OVER DEVELOPMENTAL TIME.

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The aryl hydrocarbon receptor nuclear translocator (ARNT) is a basic-helix-loop-helix/Per, ARNT, Sim (bHLH/PAS) transcription factor that is involved in multiple signaling pathways. The gene targeted deletion of ARNT in a mouse model resulted in a lethal phenotype on embryonic day 10.5, emphasizing ARNT is critical for development. It is important to comprehend the subcellular localization of ARNT in tissues to obtain a complete understanding of its function. However, studies investigating ARNT in tissues over developmental time are limited and concentrate on mammalian models. Previous immunocytochemical studies on a range of cell lines indicate that the ARNT protein is found mainly in the nucleus, which is logical considering vertebrate ARNT has a nuclear localization signal. In contrast, one study on mammalian ARNT localization in tissues found cytoplasmic staining, as well as, nuclear staining. Thus, the precise localization of ARNT protein in tissue remains controversial. This study focuses on the presence and localization of ARNT in a range of tissues over developmental time in the rainbow trout, mouse, and chicken. Three different species were chosen to determine the consistency of the ARNT staining pattern. Immunohistochemical techniques were used to stain sections of embryos over at least three developmental time points for each species. Mice were stained on embryonic day 10.5, 12.5, and 15, and the tissues evaluated showed predominant nuclear staining. Developing chicken were stained on embryonic day 2, 4, and 10, and the tissues evaluated also showed predominant nuclear staining. Studies using developing rainbow trout show predominantly nuclear ARNT staining. Therefore, in all tissues examined, regardless of the species, ARNT was found to be expressed and was predominately nuclear over all the time periods investigated. At no time point has ARNT reactivity been detected in the cytoplasm to a greater level than in the nucleus. These data overwhelmingly support the localization of ARNT in tissues to be nuclear and are consistent with and the location of ARNT in cell culture lines the presence of a nuclear localization signal in the ARNT protein. (Supported by ES08980.)

1526 THYROID DISRUPTING CAPACITY OF SELECTED PESTICIDES IN *XENOPUS*: POTENCIES AND MODES OF ACTION.

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The thyroid disrupting capacity of eight pesticides or herbicides, including p,p-DDE, o,p-DDE, dieldrin, methoxychlor, methoprene, pyriproxyfen, sulfometuron methyl, and nicosulfuron, were evaluated using the 14-d *Xenopus* metamorphosis assay. In this assay, tail resorption during metamorphic climax (stage 60 to stage 66) was used as a morphological marker of thyroid disruption. Methoxychlor was most potent in its capacity to inhibit tail resorption, yielding no adverse effect concentrations (NOAEC) of 0.01μg/L and lowest adverse effect concentrations (LOAEC) of 0.1μg/L. o,p-DDE, dieldrin and p,p-DDE were found to be somewhat less inhibitory with NOAEC and LOAEC values of 0.1 and 1.0μg/L, 1.0 and 10.0μg/L, and 10.0 and 100.0μg/L, respectively. Methoprene, pyriproxyfen, nicosulfuron and sulfometuron methyl were the least inhibitory, with NOAEC and LOAEC values of 1,000 and 10,000μg/L, respectively. Results from these studies indicate that methoxychlor, dieldrin and o,p-DDE possessed the greatest capacity to slow the rate of tail resorption. The remaining test materials possessed a lesser capacity to inhibit the rate of tail resorption. The *Xenopus* tail resorption assay appears to be capable of evaluating the effect of selected environmental compounds on amphibian metamorphosis. More work will be required to

fully elucidate whether this assay is specific for determining effects on the thyroid axis or a more generalized ecotoxicology assay for amphibians.

1527 ECDYSTEROIDS ELICIT LATE LIFE-CYCLE TOXICITY WITHOUT REDUCING FECUNDITY IN *DAPHNIA MAGNA*.

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Daphnia magna were exposed for 21 days to the ecdysteroids, 20-hydroxyecdysone (20-HE), the accepted molting hormone, and Ponasterone A, an exogenous ecdysteroid found in some crustaceans and many plants. Daphnids were monitored for alterations in molting, fecundity, and death. 20-HE elicted no effects on reproduction in healthy animals and had no effects on molting until the death of adult daphnids. Typically, death came to daphnids that were presumably healthy and usually greater than 14 days old. At that time nearly 100% of the daphnids died because of incomplete ecdysis, indicating that the exogenous ecdysone elicited a molting effect without effects on reproduction although these two processes are closely co-regulated in daphnids. Ponasterone A, which has been reported to have approximately 10X higher affinity for the ecdysone receptor than 20-HE and is probably a constituent of pulp and paper mill effluents, was examined next. We hypothesized that Ponasterone A may be comparatively more potent *in vivo* because it may not be as easily metabolized as 20-HE. However, Ponasterone A elicited late-stage molting effects at approximately 10X lower concentrations than 20-HE. Ponasterone A also did not elicit a reproductive effect, suggesting that in Daphnia magna, Ponasterone A is an ecdysteroid that is easily metabolized and eliminated. Thus, both 20-HE and Ponasterone A show similar effects and the overall difference in toxicity is mostly likely due to recepor affinity. Furthermore, the overall chronic toxicity of these steroid hormones was less than anticipated.

1528 REPRODUCTIVE CHANGES IN THE ESTUARINE FISH CUNNER (*TAUTOGOLABRUS ADSPERSUS*) EXPOSED TO 17β-ESTRADIOL AND ETHINYL ESTRADIOL IN THE LABORATORY.

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Both 17β-estradiol and ethinyl estradiol are environmental estrogens that have been shown to cause estrogenic effects in fish collected from rivers receiving sewage treatment effluent. Cunner (*Tautogolabrus adspersus*) are being studied in our laboratory to evaluate how 17β-estradiol and ethinyl estradiol affect aspects of their reproductive endocrinology and reproductive success. Cunner was selected because this species is common in estuarine areas, easily obtainable, amenable to laboratory holding and spawns daily. Fish of both sexes were exposed through subcutaneous implant of the test estrogen in a controlled release matrix (ethylcellulose and ethoxylated castor oil). In two eight-week experiments, male and female laboratory-held cunner were taken from winter conditions, injected with the appropriate treatment, and gradually acclimated to spring spawning conditions. Treatments tested in these experiments were uninjected control, matrix-injected control, and 0.05, 0.5 or 2.5 mg/kg concentrations of either 17β-estradiol or ethinyl estradiol. Plasma was taken from a subsample of treated fish over the course of these experiments and analyzed to determine circulating levels of the steroid hormones 17β-estradiol and testosterone, as well as the estrogen-induced yolk protein vitellogenin. Egg production and egg viability were recorded daily over the eight-week exposure period. Over the course of the entire experiment, fish treated with the lowest concentration of 17β-estradiol produced more eggs per female than any other treatment. Cunner given the highest concentration of 17β-estradiol produced fewer viable eggs. In the fish treated with ethinyl estradiol, the viability of eggs from fish receiving the lowest concentration was half that of the controls. Gonadosomatic index (GSI) was determined at the end of each experiment for all surviving fish. Males treated with 17β-estradiol showed a concentration-related increase in GSI, while those treated with ethinyl estradiol exhibited a concentration-related decrease. Overall, results of these experiments suggest that exposure to estrogens can impact the reproductive success of cunner.

1529 VITELLOGENIN TRANSCRIPTIONAL ACTIVATION IN SHEEPSHEAD MINNOW FOLLOWING ACUTE ESTROGEN EXPOSURE *IN VIVO*.

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Many environmentally persistant xenobiotics appear to disrupt the endocrine system by acting as ligands for endogenous steroid receptors, such as the estrogen receptor. Activation of these receptor systems in wildlife using *in vivo* model systems is not well characterized. By characterizing transcriptional and translational activation of genes following exposures to 17β-estradiol (E_2), baseline physiological responses in gene regulation can be established. We have cloned partial cDNA fragments of vitellogenin (VTG) from sheepshead minnow (*Cyprinodon variegatus*). Two fragments (α and β) with 70% nucleotide sequence homology were found, each corresponding to a homolog in fundulus heteroclitus, suggesting that sheepshead minnow have two VTG genes. The fragments isolated can be used as sensitive genetic probes to study early steps in the response of exposed fish to estrogen or to estrogen mimics. We have quantified VTG gene induction in sheepshead minnow *in vivo* using two routes of exposure; IP-injection with E_2 and flow-through water exposures with E_2 and 17α-ethinylestradiol (EE_2). VTG messenger ribonucleic acid (mRNA) induction was characterized by Northern analysis and quantified using slot blots and *in vitro* synthesized RNA standards. Plasma VTG protein was identified by Western and quantified using a previously validated enzyme-linked immunosorbent assay specific for sheepshead minnow. VTG mRNA exhibited a maximum induction of 1300 pg/μg total RNAwith E_2-injection and 120 ng/μg total RNA with E_2 water exposure, under the conditions and doses tested. The data illustrates hormone induction profiles of VTG mRNA and protein to E_2 and EE_2.

1530 NON-MAMMALIAN ESTROGENICITY SCREEN: RAINBOW TROUT ESTROGEN RECEPTOR BINDING.

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The U.S. EPA has been mandated to screen industrial chemicals and pesticides for potential endocrine activity. Current assays for measuring endocrine activity are primarily mammalian-based. The appropriateness of extrapolating mammalian results to non-mammalian species is uncertain, primarily due to the lack of non-mammalian data from which to make such an assessment. The primary objective of the study was to establish the relative binding affinity of a number of xenobiotics in a widely used non-mammalian toxicity model, the rainbow trout. Binding affinity to the rainbow trout estrogen receptor (rtER) was measured by radioligand competition assay. Trout liver cytosol (300μl) was incubated (20 hr at 4°C) with a saturating concentration (5 nM) of ^{3}H-estradiol (^{3}H-E2) and a range of xenobiotic concentrations. Bound xenobiotic was determined as the amount of ^{3}H-E2 displaced from the rtER. The relative binding affinity (RBA) was calculated as the EC_{50} for displacement of xenobiotic relative to the EC_{50} for displacement of estradiol (E2). E2 bound to rtER with high affinity (K_d = 1.5 nM; B_{max} = 13.3 nM) as did known ER agonists, diethylstilbestrol (RBA ~ 400) and ethinyl estradiol (RBA ~ 400). A variety of other chemicals, including p,p'-methoxychlor (MXC), 4-tert-pentylphenol (PTPP), and 4,4'-diaminostilbene-2,2'-disulfonic acid (DAS) were tested and found to have orders of magnitude lower RBAs or did not bind to the rtER at solubility-limited concentrations (e.g., MXC). The absolute affinity and capacity of xenobiotic binding to the rtER are generally lower than in mammalian systems, however, the relative binding affinities of various chemicals are comparable. These data will be used to help establish the basis for extrapolating estrogenic potential of xenobiotics across species. (This abstract may not represent EPA policy.)

1531 SYNERGISTIC EFFECTS OF THE XENOESTROGEN 4-OCTYLPHENOL (4-OP) AND UV-B RADIATION ON SOMATIC DEVELOPMENT AND GENE EXPRESSION IN THE FOREBRAIN OF THE LEOPARD FROG (*RANA PIPIENS*).

D. Crump, V. L. Trudeau and D. R. S. Lean. *University of Ottawa, Ottawa, Ontario, Canada.* Sponsor: M. E. Hahn.

Newly-hatched *Rana pipiens* tadpoles were exposed to three concentrations of 4-OP (1nM, 1μM, 10μM) and two levels of UV-B radiation (25 μW/cm^2, 6

μW/cm²) in a ten-day static renewal experiment. An RNA-arbitrarily primed PCR (RAP-PCR) strategy was used to identify candidate genes differentially expressed in the forebrain (including pre-hypothalamic tissue) of the exposed tadpoles. Twenty tadpoles from each treatment group were raised to metamorphosis in order to assess any possible effects on later development. A cDNA fragment (774 base pairs) approximately 66% identical to the 3'-end of human and rat plectin at the predicted amino acid level was up-regulated by 1 nM 4-OP as defined by the differential display technique. Plectin is a cytoskeletal intermediate filament binding protein which provides mechanical strength to cells and tissues. Another transcript (762 base pairs) up-regulated by 1 nM 4-OP was 89% identical to human Nck, Ash and phospholipase C gamma-binding protein (NAP4). NAP4 is implicated in coordinating various signaling pathways including growth factor and cell adhesion receptors. One transcript (517 base pairs) was down-regulated by both 1 nM and 1 μM 4-OP and was 59% identical to human brain-specific angiogenesis inhibitor 2 (BAI2). BAI2 is a candidate for involvement in the development of glioblastoma (primary brain tumor development). Finally, a transcript (403 base pairs) was down-regulated by the high UV-B treatment (25 μW/cm²) and was 56% identical to rat arcadlin. Arcadlin is a novel cadherin molecule expressed at the synapses that may play an important role in activity-induced synaptic reorganization underlying long term memory. Prior to hind-limb emergence (stage 30), tadpoles from the combination group of UV-B (6 μW/cm²) and 4-OP (1 μM) were significantly larger ($p<0.05$) while those in the UV-B treatment (6 μW/cm²) displayed a significant reduction in weight ($p<0.05$). These results indicate that levels of 4-OP commonly found in the environment and sub-ambient levels of UV-B radiation alter developmentally important genes and disrupt normal growth patterns. (Research supported by NSERC [DL] and CNTC [VLT].)

1532 INVESTIGATION OF THE POTENTIAL *IN VIVO* INTERACTION OF ESTRADIOL AND TCDD IN *FUNDULUS HETEROCLITUS*.

T. A. Young and K. R. Cooper. *Rutgers, The State University of New Jersey, UMDNJ, Piscataway, NJ.*

The estuarine fish *Fundulus heteroclitus* comes into contact with mixtures of contaminants that have the potential to interact the organism. 17β-estradiol (E2) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have previously been found to be antagonistic in *in vitro* and *in vivo* mammalian and some fresh water fish systems. In the present study plasma vitellogenin (as assessed by plasma alkaline phosphate (PALP)) and hepatic ethoxyresorufin deethylase (EROD) were used as markers of estrogenic and dioxin-like activity, respectively. Animals were treated for 6 days by intraperitoneal injection, on days 0 and 3, with E2 in olive oil. TCDD was given in a krill gelatin food mixture on day 3, or both days 0 and 3. No reduction of EROD activity was observed from 2.2ng TCDD/g in animals pretreated with 0.25-0.5mg E2 / g fish. No TCDD related reduction in PALP was observed in animals co-administered 0.6-4.9ng TCDD/g and 0.25mg E2 / g body weight. A dose responsive relationship was, however, found for each ligand and its respective marker. Based on these results there does not appear to be any interaction between E2 and TCDD using this protocol. These results may be altered by pretreatment for longer periods with either E2 or TCDD. The potential for interactions between toxic chemicals is of great importance when assessing complex mixtures in the field. The results presented here reinforce the importance of investigating different organisms, and multiple pathways of toxicity. (USEPA CR825437, NIEHS ES07148, ES005022)

1533 AN ADVANCED PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR *MYA ARENARIA* USING 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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A more robust physiologically-based pharmacokinetic model (PBPK) was developed for the soft-shelled clam (*Mya arenaria*). The version 2.0 model was optimized using the ^{14}C-inulin data and validated using 28-day 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) elimination data. The original (version 1.0) model was improved by the addition of the foot and siphon into the model, and the incorporation of binding parameters. At 24 hours post mantle injection (2.13 pg/g), the highest TCDD level was in the siphon (137 pg). The highest TCDD concentration at day 28 was in the gonad followed by the siphon, gill, mantle, digestive gland, kidney, foot, adductor muscle, heart, and hemolymph. TCDD was initially assumed to distribute within the clam according to lipid content based on passive diffusion with no metabolism. The model based solely on lipid content fit the TCDD data poorly by over estimating and underestimating concentrations in the various tissues. Examination of the literature suggested that the gonad might have a binding protein for 2,3,7,8-TCDD. Incorporating a binding parameter in the gonad compartment resulted in a better fit to the data. The PBPK model predicted the uptake and elimination concentrations for 2,3,7,8-TCDD within each of the clam tissue compartments based on the flow rates, tissue compartment sizes, lipid content of each organ, binding parameters, and blood circulation. The version 2.0 model can be further modified to incorporate metabolic processes for compounds that undergo metabolism. This PBPK model can be valuable for estimating tissue burdens following environmental exposures.

1534 MODELING BIOACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN AN AQUATIC ECOSYSTEM.

K. H. Watanabe and R. Luna. *Tulane University, New Orleans, LA.* Sponsor: M. B. Anderson.

In 1996 and 1997 the Tulane/Xavier Center for Bioenvironmental Research (CBR) supported ecological studies in the LaBranche Wetlands of Louisiana. The study area was selected because Bayou Trepagnier was the receiving stream for effluents from a petrochemical manufacturing complex for nearly 50 years ending in 1995. As a result of the industrial discharges to the system, the bayou is contaminated with heavy metals and polycyclic aromatic hydrocarbons (PAHs). Samples of sediment, water, and biota were collected from fixed stations along the bayou and analyzed for approximately 50 semi-volatile organic compounds. Spotted gar gut contents were examined to determine feeding preferences and establish the food web for the bioaccumulation model. We developed a spatially referenced food-web bioaccumulation model for spotted gar using established bioaccumulation modeling assumptions for hydrophobic organic chemicals and site specific data. In this study we focused on seven PAHs: naphthalene, anthracene, phenanthrene, benz(a)anthracene, chrysene, benzo(a)pyrene, and dibenz(a,h)anthracene. Model simulations show that bioaccumulation occurs through the benthic invertebrates consumed by the gar. For octanol water partition coefficients between $10^{3.3}$ and $10^{4.6}$ the highest concentrations in the food web are found in the benthic invertebrates. These concentrations range from 61 μg/kg for naphthalene to 150 μg/kg for anthracene. For PAHs with partition coefficients between $10^{5.6}$ and $10^{6.5}$, the predicted gar concentrations are highest among organisms in the food web ranging from 206 μg/kg for benz(a)anthracene to 547 μg/kg for benzo(a)pyrene.

1535 PCB TOXICITY IN FISH, SEAL AND POLAR BEAR.

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PCB have 209 congeners. Chlorinating atoms increase fat solubility, reduce metabolic degradation from P450 nonspecific oxidase, or biphenyls dioxygenase and prolonged the biological half-life. Bioaccumulation from fish to seal, or birds and to polar bear are summarized. There is an overall 3 or more order of magnitude increase in concentration of these chemicals from plankton to polar bear via fishes and birds up the food chain. Planktonivorous, herbivorous and carnivorous fishes accumulate different congeners from their diets. Different fishes in different tributaries have different patterns. Migratory salmon have less PCB than the bass, that stay in the contaminated locale. Fishes exposed to PCB have lost the P450 inducibility from xenobiotics. Gulls have a high load of PCBs because of its habitat. Seals consumed contaminated fishes developed endometrosis, fetal abortion and reproductive failures not to mentioned other stigmata from hyperaldosteronism. Disclaimer: The opinions and conclusions expressed in this paper are those of the authors and do not necessarily reflect those of affiliated institutions.

1536 AURAL ABSCESSES IN WILD-CAUGHT TURTLES: POSSIBLE INVOLVEMENT OF ORGANOCHLORINE-INDUCED HYPOVITAMINOSIS A.

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Wild-caught box turtles (Terapene carolina carolina) with aural abscesses were observed over a period of several years. Histopathologic evaluation of the lining of the middle ear and other epithelial tissues (conjunctiva, pharynx, trachea, auditory tube) revealed mucosal hyperplasia and squamous metaplasia, and accumulated keratin-like material in the middle ear cavity. Together these manifestations suggested the possibility of vitamin A deficiency in the wild turtles. Ten additional turtles were therefore collected (5 with an externally visible aural abscess and 5 without an externally visible aural abscess). Mean serum and hepatic vitamin A levels in turtles with abscesses were 71% and 49% of respective levels found in turtles without abscesses. Using gas chromatography, eight different organochlorine (OC) compounds were detected in livers of turtles. Three individual OC compounds (a-benzene-hexachloride, heptachlor epoxide, and oxychlordane) and total mg/g OCs were significantly higher in livers from turtles with abscesses compared to turtles without abscesses. No OC compounds were higher in turtles without abscesses compared to turtles with abscesses. These data suggest a possible effect of environmental chemicals on the metabolism or utilization of vitamin A in wild box turtles, resulting in a state of hypovitaminosis A.

1537 TOXICIT EVALUATION OF 1,3,5-TRINITROBENZENE (TNB) IN SHREWS (*Cryptotis parva*).

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TNB has been detected as an environmental contaminant of soil and water at certain Army installations and production waste disposal sites. At present, there are no toxicity data on TNB in small wild mammals that might be used for ecological risk assessment. Therefore, a 14-day toxicity study was conducted to determine the No Observed Adverse Effect Level (NOAEL) of TNB in the least shrew (*Cryptotis parva*). Male and female shrews (10/group/sex) were fed a mixture of wet-dry Purina Cat Chow containing 0, 5, 10, 20 and 40 ppm for 14 days. The calculated average consumed doses were 0, 10.68, 22.24, 37.79 and 98.27 mg/kg/day for males and 0, 10.75, 21.60, 45.26 and 98.72 mg/kg/day for females. There were no significant differences in food and water consumption among the groups. However, there was a decrease in body weight in males (10 to 40 ppm) and a significant increase in body weight in females in the 20 ppm dose group. The relative spleen weights were increased in females (10 to 40 ppm). There were no significant changes in hematology parameters in females while in males there was a significant decrease in RBC and an increase in WBC in the 40 ppm dose group. Histpathological change noted was erythroid cell hyperplasia in the spleen of males receiving 20 and 40 ppm TNB diet. The NOAEL for male and female shrews was determined as 10.75 mg/kg/day for the 14-day study. (Abstract does not reflect US EPA/ US Army policy.)

1538 STUDIES EVALUATING THE ENVIRONMENTAL TOXICITY OF ARSENIC PENTOXIDE, CUPRIC CHLORIDE, PENTACHLOROPHENOL, AND 1,2,3,6,7,8 HEXACHORODIBENZO-*P*-DIOXIN IN SINGLE AND BINARY MIXTURES.

S. H. Adair, B. Buckley and K. R. Cooper. *Rutgers, The State University of New Jersey, New Brunswick, NJ.*

Arsenic pentoxide, cupric chloride, pentachlorophenol, and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin are common contaminants found in combination in the environment. The first three compounds listed are currently used for wood treatment, while 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin is one of the most toxic contaminants found in technical grade pentachlorophenol. Dose-response curves were developed for these compounds based on the experimental results obtained from the Japanese Medaka (Orzias latipes) Embryo-Larval Assay (MELA). The LC50 values estimated from the single compound dose-response curves for arsenic pentoxide, cupric chloride, pentachlorophenol, and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin were 50 mg/l, 5 mg/l, 84 μg/l, and 2.3 μg/l, respectively. Tube heart and pericardial edema were frequently observed lesions at the highest concentrations tested during each of the single compound MELA studies. Additional compound specific lesions included vitelline vein dilation and hemorrhage for cupric chloride, hypopigmented blood and yolk-sac cloudiness for arsenic pentoxide, hypopigmented blood and swollen spleen for pentachlorophenol, and brain swelling, brain and caudal area hemorrhage, and hypopigmented blood for 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin. The LC25 values of 4.1 mg/l for cupric chloride and 48.5 mg/l for arsenic pentoxide were determined from their respective dose-response curves for the cupric chloride/arsenic pentoxide binary study. The binary mixture of cupric chloride and arsenic pentoxide appeared to have a greater than additive effect on lesions in the Japanese Medaka embryos. Complex mixtures at wood treating facilities may pose a greater risk than determined from single compound studies. (USEPACR825437, NIEHSES07148, ES005022)

1539 CHANGES IN ARSENIC SPECIATION DURING MICROBIAL GROWTH.

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Arsenic (As) is ubiquitous in the environment, both as a natural component of rocks and minerals and as a contaminant from human activities. Arsenic in the soil often leaches into water supplies. Sub-ppm levels of As in drinking water have been shown to lead to skin, bladder, and lung cancer. Different forms of As have different chemical and physical properties and therefore different cellular toxicities and soil binding properties. The As species present in the soil, and therefore the toxicity and risk from the As, is affected by biotic and abiotic influences. This study's aim was to determine the changes in As species when a soil microbial community is grown with the four common forms of As—- arsenite (AsIII), arsenate (AsV), monomethylarsonate (MMA), and dimethylarsenate (DMA). Minimal salts media (glucose as the carbon source and pyrophosphate as the phosphorous form, pH 6.00) containing 0, 30, or 300ppm As as AsIII, AsV, MMA, or DMA was inoculated with microbes from a non-contaminated soil and microbial growth measured as optical density at 660nm over time. During different points of the growth curve, As speciation was determined by HPLC-hydride generation-atomic-fluorescence-spectrometry and compared to non-inoculated controls. Results were similar with either 30ppm or 300ppmAs. While there were no changes in either the level or form of As when the initial As species was MMA or DMA, AsV was consistently reduced to AsIII during the exponential growth phase. When the initial As species was AsIII, oxidation to AsV was found in one of the three replicates, while the other two had similar AsIII levels as controls. This indicates that the ability to reduce AsV to AsIII may be more prevalent than As oxidation in the soil community. Reducing AsV to AsIII may increase the bioavailability and the toxicity of the As in the environment. (Work supported by NIH Grant ES06644, NIEHS Grant ES07091, Center Grant P30ES06694, and the Caldwell Health Sciences Research Fellowship.)

1540 HEAVY METALS, PESTICIDES AND ENVIRONMENTAL CONTAMINANT RESIDUES IN RESIDENT GIANT CANADA GEESE (*BRANTA CANADENSIS*) FROM NORTH EAST AND SOUTH WEST OHIO.

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In contrast to forty years ago when Canadian geese (*Branata canadensis*) approached extinction due to loss of wetland habitat, unrestricted egg taking, and over-hunting, they are now common residents of American golf courses, ponds and lakes. Because of protection, their numbers have increased from a few thousand to well over a million, and the Canadian goose population is now widely viewed as out of control. Many risks to animal, human and environmental health are associated with the geese, from transmitting diseases to other animals, eutrophication of ponds and lakes and decreasing water quality through extensive droppings, viciously attacking humans, and damaging landscapes. States are now beginning efforts to reduce their Canadian goose population. In the present investigation, FSIS cooperated with the state of Ohio to reduce the geese over-population while simultaneously trying to meet food requirements of needy families. To determine the suitability of these wild birds for human consumption, 18 geese were collected from northeastern (NE) and southwestern (SW) Ohio. Muscle, liver, kidneys and fat samples were analyzed for a number of metals (aluminum, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, manganese, mer-

cury, molybdenum, nickel, sodium, zinc), pesticide residues (dieldrin, DDE, HCB, chlorinated organophosphate, and PCBs). Fat contained traces of dielderin, heptachlor, PCBs and HCB. None of the detected levels exceeded the pesticide tolerances. However, livers from these geese (both locations) contained elevated levels of some of the examined metals. Livers from SW contained 2-fold increase of lead (1.06±0.21 vs. 0.57±0.17 ppm) and iron (1540±172 vs. 766±145 ppm), and a 1.7X increase of molybdenum (1.71±0.41 vs. 1.05±0.06 ppm) compared to those found in NE livers. Kidneys from NE geese lead concentrations that were 4X that found in livers from the same group. Surprisingly, this difference was not repeated in the SW geese. Data suggests that the muscle tissues of these animals are fit for human consumption. The migration path may affect the range and level of contaminants in the examined birds. Risk assessment and through analysis of these animals before using them in any food program is important procedure.

1541 PARASITE SUSCEPTIBILITY AND STRAIN-SPECIFIC RESPONSES TO LONG-TERM CADMIUM EXPOSURE IN THE FRESHWATER GASTROPOD, *BIOMPHALARIA GLABRATA*.

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Different strains of Biomphalatia glabrata can be resistant or susceptible to trematode parasites that cause schistosomiasis. Our interest is to ascertain whether such differences can be related to differences in resistance to Cd toxicity. Initial experiments showed that a parasite-resistant strain, BS90, is less tolerant of acute Cd exposure than a parasite-susceptible strain, NMRI. Strain-related responses were further examined in continuous exposures over three generations. NMRI was exposed to higher Cd concentrations to account for its greater Cd resistance. Low concentrations of 0.0125 μM for BS90 and 0.025 μM for NMRI had no effects on survival or reproduction. NMRI at the intermediate Cd concentration of 0.05 μM exhibited reduced growth and reproduction in the first generation and failed to produce viable offspring in the second. Strain-specific differences in Cd resistance were apparent at the 0.025 μM Cd exposure common to both strains: NMRI was more tolerant than BS90, reflecting the instrinsic difference in Cd resistance noted originally. At this concentration, the response of NMRI was similar to that of controls, while Cd-exposed BS90 exhibited decreased survival rates in each of the three generations. At 0.10 μM Cd, at which only NMRI was subjected, there was substantial mortality in the first generation, but not in subsequent generations, which were derived from the Cd-resistant survivors of the first. These results were consistent with selection for Cd resistance in NMRI. This rapid increase in Cd resistance (<3 generations) is indicative of only one or a few responsible genes. We determined the number of genes involved in Cd resistance in this species using a quantitative genetic approach and report a preliminary estimate of 1.35 genes. It appears, thus far, that selection for parasite resistance is associated with increased sensitivity to Cd toxicity and decreased ability to adapt to Cd exposure.

1542 GLYCEROL-MEDIATED PHOTOREDUCTION OF HEXAVALENT CHROMIUM.

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Chromium is an important industrial metal and an environmental pollutant. In the hexavalent [Cr(VI)] form, this metal is known to be a human carcinogen, while trivalent chromium [Cr(III)] is considered much less toxic. We have found that hexavalent chromium in the form of potassium dichromate can be rapidly photoreduced to trivalent chromium [Cr(III)] in aqueous solutions containing glycerol. This photoreduction occurred after irradiation with either UVA (320-400 nm) or a wide spectrum light source, but not a UVB (290-320 nm) light source. Photoreduction of hexavalent chromium was found to be pH-dependent and did not occur in dilute solutions of sodium hydroxide. In acidified solutions, the photoreduction occurred at elevated rates and at lower concentrations of glycerol. This reaction is apparently dependent on the unsubstituted alcohol groups of glycerol since alpha-phosphoglycerol or beta-phosphoglycerol did not support the photoreduction of hexavalent chromium. These findings may have significant applications in the remediation of hexavalent (carcinogenic) chromium at contaminated environmental sites. (Funded in part by the Consortium of Risk Evaluation with Shareholder Participation (CRESP), DOE# DE-FC01-95EW55084.)

1543 REPRODUCTIVE TOXICITY OF ERGOT ALKALOIDS IN MINK.

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Ergot alkaloids are synthesized by fungi of the Claviceps family that infect rye as well as other cereals and grains. Since a portion of the ranch mink diet is cereal, mink are at a risk of being exposed to ergot alkaloids. This study was performed to determine the reproductive toxicity of ergot alkaloids derived from ergot-contaminated wheat in mink. Four groups of 12 female mink each were fed diets containing 0, 3, 6, or 12 ppm ergot alkaloids from 2 weeks prior to the breeding season until the kits were approximately 33 days old (133 days). Females were mated with untreated males. Ergot alkaloids caused a transient decrease in feed consumption, but body weights were unaffected. The gestation period of the mink in the 12 ppm group was longer compared to controls. The number of mink whelping varied significantly with 9 mink whelping each in the control and 3 ppm group compared to 4 mink in the 6 ppm group and 1 in the 12 ppm group. Ergot alkaloids had a significant effect on kit survivability with no kits surviving in the 12 ppm group. Plasma prolactin was significantly depressed in the 3 ergot alkaloid groups compared to the controls. This study indicated that ingestion of ergot alkaloids at 3 ppm or higher resulted in reproductive toxicity in mink.

1544 MERCURY IN FISH FROM THE SHOALS AREA OF THE TENNESSEE RIVER.

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Kentucky, Pickwick and Wilson are the first three impoundment lakes from the mouth of the Tennessee River. Collectively, they extend from Kentucky Dam, TRM (Tennessee River Mile) 22, to Wheeler Dam in the Shoals area of northwest Alabama, TRM 275. Pickwick, the middle of these three contiguous lakes, goes from Pickwick Dam (TRM 206) to Wilson Dam (TRM 260). Pickwick Lake was the site of an historic discharge of mercury from a chlor-alkali facility. Fish tissue studies conducted by the Tennessee Valley Authority's Office of Water Management showed that average values for mercury in muscle tissue of largemouth bass from Pickwick Lake peaked in 1973 at 1.03 ppm. By 1979, this value had declined to 0.14 ppm. Similar data from Kentucky Lake (the next lake downstream) showed levels of 1.07 and 1.01 ppm for 1973 and 1974 respectively. Data from 1979 demonstrated a decrease of this average to 0.32 ppm. To determine if residual mercury in Pickwick Lake was currently being incorporated into the food chain, tissue samples from fish collected from the lake were analyzed for total mercury. Tissue was freeze-dried, weighed and solubilized with nitric acid and hydrogen peroxide at 45°C. Solvent controls were included with each assay. USEPA Method 245.1, Cold Vapor Atomic Absorption, was used for mercury analysis. NIST standard Reference Material 2976, mussel tissue, was used to validate the analytical procedures. Mercury was detected in all tissue samples from fish collected from Pickwick Lake. Tissue concentrations ranged from 0.02 ppm in muscle tissue from a white bass 25 cm long to 2.13 ppm in liver from a fresh water drum 43 cm in length. Fish collected from Kentucky Lake, down river from Pickwick, also contained mercury. These tissue concentrations ranged from 0.05 ppm for the liver of a 19 cm long drum to 0.18 ppm seen in a muscle sample from a 34.5 cm long drum. Unexpectedly, mercury was found in fish from Wilson Lake, up river from Pickwick. This lake is separated from Pickwick by a 30 m high dam. Mercury in Lake Wilson cannot be attributed to pollution from the chlor-alkali source that contaminated Pickwick. Highest tissue levels from Wilson were found in a liver sample from a blue catfish (3.28 ppm) and from the liver of a channel catfish (1.47 ppm). Possible sources of mercury pollution in this lake include down river wash from the estimated 75-150 tons of mercury released into East Poplar Creek by the Y-12 Plant at Oak Ridge National Laboratory. Another possible source is atmospheric deposition. Mercury contained in coal can be released into the atmosphere by stack gases from coal burning steam plants during the production of electricity. There is one such plant in the Shoals area.

1545 STRESS PROTEINS AND MDA.

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Relationship between oxidative stress and induction of stress proteins in copper exposed fathead minnows, Pimephales promelas.Induction of heat shock or stress proteins (hsp) in fish has been used as a biomarker of exposure to environmental contaminants. Although hsp are well studied with respect to exposure, little is known about the relationship of hsp to adverse biochemical or physiological effects. To investigate the relationship between heat shock proteins and cellular damage, 32 fathead minnows, Pimephales promelas were divided into 4 10-gallon tanks and exposed to 0, 10, 20, and 40 ppb copper. Fish were maintained under static-renewal conditions for 96 hours, at which time they were sacrificed with an overdose of MS222. Liver, gills, and muscle were dissected and frozen for later analysis. Heat shock proteins 60 and 70 were analyzed by Western blotting. Oxidative stress was assessed by HPLC analysis of malonaldehyde (MDA). Hsp60 in liver and muscle, but not gill, increased in a dose-dependent fashion, up to 200% above control. Hsp70 was induced 67% and 194% in the gills and muscle respectively, but not the liver. MDA levels were elevated up to 6-fold at 20 ppb copper in the liver only. No apparent relationship was observed between hsp70 and oxidative stress. The data suggests a relationship between hsp60 and oxidative stress may exist, but further analysis is required.

1546 MICROCYSTIN-LR OZONATION BY-PRODUCTS: CHEMICAL AND TOXICOLOGICAL CHARACTERIZATION.

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Cyanobacterial toxins pose a health risk for humans especially since these toxins, such as microcystins (MC), are suspected tumor promoters. It is therefore necessary to develop procedures to effectively destroy these toxins during water treatment. Ozonation of MC in raw water with 1.0 mg O3/l completely destroys the toxin after 9 min (Höger et al. Toxicol. Sci. 48 1-S, 152, 1999). During a bloom episode or when the organic load of the water is high, the oxidation potential might be insufficient to completely destroy the toxin. In such a situation, ozonation by-products are found. These by-products have not been adequately identified and the aim of this study was to characterize them according to their toxic effects and their chemistry. Products from treatment of MC with 0.5mg O3/l were fractionated by HPLC. Using an ELISA with Adda (the amino acid unique for MC/Nodularin) as the antigen, the products were tested for the presence of the Adda side chain. Their phosphatase inhibition and protein binding capacities were tested with an inhibition assay and by immunoblotting, respectively. The results show that one fraction, which had an HPLC retention only slightly shifted from that of MC-LR, still exhibited protein phosphatase inhibitory activity and was also detectable by ELISA and immunoblotting. These findings show that incomplete oxidation of cyanobacterial toxins may lead to products showing toxic effects and protein adducting activity.

1547 IMMUNOCHEMICAL DETECTION OF MICROCYSTIN-LR IN TISSUES OF RAINBOW TROUT AND CARP.

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Microcystins (MC) are cyclic heptapeptide toxins produced by cyanobacteria such as Microcystis aeruginosa and are known to possess hepatotoxic and tumor-promoting activity. Intoxication with MC has lead to death of humans and animals such as livestock or fish. In order to determine the localization of microcystin in various fish tissues, an anti-MC-antibody was employed. Microcystin was probed in tissue of carp and rainbow trout after *in vivo* exposure to the toxin. Both immunocytochemistry and immunoblotting were used to detect organ/cellular distribution and protein adducts respectively. In order to identify putative protein adducts, Western blots were also stained with anti-human protein phosphatase 2A (PP2A). Immunocytochemistry showed positive staining in the liver and kidney while immunoblotting detected three microcystin-protein adducts with approximate molecular weights ranging from 23 to 38 kD in the liver, kidney, intestine, skeletal muscle, and brain. One of these adducts had an approximate molecular weight of 36 kD and this band was identified as PP2A by using a specific PP2A antibody. Other microcystin-protein adducts were isolated for further identification.

1548 DETECTION OF CYANOBACTERIAL TOXINS IN WHITEFISH (*COREGONUS LAVARETUS* L.) FROM LAKE AMMERSEE.

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Lake Ammersee is one of the biggest lakes in southern Germany. Whitefish (*Coregonus lavaretus*) is the dominating fish species with great importance for the local fishery. In recent years whole age groups of the coregonid population in Lake Ammersee have been lost and reductions in body weight of fish were observed. The causes for this, however, remain unclear. The metalimnic cyanobacterium Planktothrix agardhii, known to produce the cyclic peptide toxin 3-desmethyl microcystin-RR (3dmRR), has become one of the dominating planktonic species causing regular blooms in summer. The aim of this study was to elucidate the effect these cyanobacterial blooms might have on coregonid population dynamics. During bloom episodes, gut contents of whitefish displayed a blue discoloration, possibly representing phycobiliproteins typical for cyanobacteria. Microcystins (MC) were determined in P. agardhii by protein-phosphatase assay and by HPLC. ELISA and immunoblotting techniques were used to detect microcystins in liver tissue samples and gut contents of whitefish. The cytotoxicity of the cyanobacterial bloom sample was tested with trout hepatocytes using the MTT assay. Toxin analysis of a massive P. agardhii bloom in July 1998, revealed an average of $1.6 \pm 0{,}1\mu g$ 3dmRR/mg dry weight (N=8). ELISA analyses of the gut content were positive for microcystin congeners. Similarly, immunoprobing with microcystin-antibodies demonstrated microcystin-protein adducts in liver homogenates of whitefish caught during P. agardhii blooms. In addition, extracts of the P. agardhii were cytotoxic when tested in the trout hepatocyte assay.

1549 AN ELISA WITH BROAD SPECIFICITY TO CYCLIC PEPTIDE CYANOBACTERIAL TOXINS.

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Cyanobacteria (blue–green algae) capable of producing hepatotoxins, e.g., *Microcystis spp.* can be present in fresh water worldwide. These toxins, inhibit ser/thr protein-phosphatases, and induce hepatocyte necrosis/apoptosis. They are toxic to man, other mammals, and to fish—including salmonid species. This group includes the microcystin (MC) heptapeptides (over 50 known variants) and nodularin, a pentapeptide. Antibodies raised against a novel cyanobacterial toxin analogue-cBSA conjugate were used, together with ovalbumin-toxin-conjugates as a plate coater to develop a competitive ELISA. The ELISA is designed to detect most cyanobacterial hepatotoxins with equal sensitivity. Although not fully optimised, it has a detection limit below 0.1 ng/ml, and a limit of quantitation lower than the WHO-proposed guideline (1 mg/l) for drinking water. The assay is more sensitive than other available ELISAs, thus water analyses can be performed without sample preconcentration. The assay shows good cross-reactivity with all microcystin analogues tested to date, and has ~100% cross-reactivity with nodularin (relative to MC-YR). The assay is robust and has been used successfully in the analysis of an array of aqueous matrices, including raw river and reservoir water, algal culture, and algal bloom samples. The broad specificity of the ELISA makes suitable for use as a quick screening procedure for the detection of cyanobacterial hepatotoxins in water and the aquatic food chain.

1550 LETHAL AND SUBLETHAL EFFECTS OF IVERMECTIN IN A FRESHWATER OLIGOCHAETE, *LUMBRICULUS VARIEGATUS.*

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Sponsor: S. Hendrich.

Ivermectin is a potent antiparasitic drug against nematode and arthropod parasites. Although lethal levels have been determined in some non-target species, the effects of sublethal doses have seldom been examined. In this study, we examined the lethal and sublethal effects of ivermectin in a freshwater oligochaete, *Lumbriculus variegatus* (order Lumbriculida). LC_{50} at 72 h after ivermectin treatment was 0.62 μM. The endpoints for sublethal effects of ivermectin focussed on several stimulus-evoked locomotor behaviors: (1) escape reflex controlled by giant interneuron pathways; (2) swimming and reversal in open water; and (3) crawling on wet surface or in confined spaces under water. Swimming, reversal and crawling are controlled by non-giant

interneuron pathways. Ivermectin inhibited swimming, reversal, crawling frequency, and crawling speed in a time- and concentration-dependent manner with an EC_{50} at 3 h of 1.1, 16, 91 and 51nM, respectively. Ivermectin at 0.3 nM, or higher, also significantly decreased the frequency of helical swimming waves after 3h treatment. Picrotoxin (Cl^- channel blocker) antagonized the ivermectin-induced decrease in swimming frequency, crawling frequency and crawling speed. The worms retained escape reflex function 3 h after treatment with 0.3μM ivermectin. Electrophysiological recordings showed that ivermectin had no effects on the conduction velocity of medial or lateral giant fiber systems (MGF or LGF), or on muscle potentials evoked by MGF action potentials. The results indicate that locomotor behaviors controlled by non-giant interneuron pathways are more sensitive to ivermectin than are those controlled by giant interneurons, and suggest that Cl^- channels are involved in mediating ivermectin's inhibitory effects.

1551 EFFECTS OF *CLAVICEPS PURPUREA* VAR *SPARTINAE* (*SPARTINA* ERGOT) FED TO ADULT JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Studies were carried out to examine the potential effects of *Spartina* ergot on fish that might consume the sclerotia. This variant is in the class with *Claviceps purpurea* but floats on the surface of water. The sclerotia might be consumed by fish inhabiting salt marshes contaminated by the *C.p. spartina*. Japanese medaka (*Oryzias latipes*) were fed a diet containing 10% sclerotia at an 8% body weight for 17 days. There was no difference between the treatment group and the control fish for any growth parameters. No visible lesions were observed in the control fish (N=10) while a number of lesions were observed in the exposed fish (N=9). The major lesions that were observed included hemorrhages in the small capillary beds (44-55%), brown appearing blood (55%), and hemorrhages along the trunk muscle (22.2%). Altered pigmentation was observed in treated fish. These studies demonstrate that the *Spartina* ergot if eaten by fish could result in severe lesions. The widespread distribution of *C.p. spartinae* in North America, South America and Europe could pose a potential risk to wildlife. (ES07148, ES005022.)

1552 BIOLOGICAL STUDIES CONDUCTED ON SOUTH MONMOUTH AND CAPE MAY SEWERAGE AUTHORITIES EFFLUENTS AND RECEIVING WATERS TO A MARINE FISH (*FUNDULUS HETEROCLITUS*), A FRESHWATER FISH (*ORYZIAS LATIPES*), AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*), AND A CRUSTACEAN (*MYSIDOPSIS BAHIA*).

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There are fourteen outfalls that discharge chlorinated, treated municipal wastewater into the coastal waters off New Jersey. Since none of these wastewater treatment plants have dechlorination systems, their effluent may cause toxic chlorine-produced oxidants (CPOs) to be released into the receiving waters as well as be in violation of the New Jersey Water Quality Criteria for these CPOs. Two of these plants were examined for toxicity on marine organisms, South Monmouth and Cape May Sewerage Authorities. Grab samples were taken from each plant periodically from September 1998 through August 1999. Some of the grab samples from each plant was sewage containing Rhodamine WT, a tracer dye, as well as sewage that was dechlorinated later in the lab. Acute and sub-chronic toxicity assay were conducted for a freshwater finfish (*Oryzias latipes*), and a crustacean (*Mysidopsis bahia*). These effluent collection assays showed significant differences ($P \leq 0.05$) for fecundity for *Mysidopsis bahia*, and embryo development (bubbling of chorion) for either one or both the South Monmouth and Cape May sewerage effluents when compared to controls. A transplant study was also conducted near the ocean outfall effluent pipe during July and August of 1999 for both sewage treatment plants. The animals that were used in the transplant were the American oyster (*Crassostrea virginica*), juvenile and embryo mummichog fish (*Fundulus heteroclitus*), and the shrimp *Mysidopsis bahia*. A significant increase in the underdevelopment, disintegration and death of mummichog fish (*Fundulus heteroclitus*)embryos was observed near the outfall pipe when compared to a farfield control site for both South Monmouth and Cape May sites. Also, juvenile oyster growth initially significantly ($P \leq 0.2$) increased for the nearfield outfall site when compared to the farfield for the Cape May site, but over 30 days the farfield oysters increase in growth was significantly ($P \leq 0.2$) larger than the nearfield oysters. This may suggest that the effluent may over time inhibit the growth of the oysters by contaminants bioaccumulating or other physical factors such as temperature or salinity changes from the effluent plume.

1553 *RANUNCULUS* SP: INVESTIGATION OF THE POTENTIAL FOR USE OF A TOXIC PLANT IN AMPHIBIAN HABITAT RESTORATION.

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Management strategies to control aquatic vegetation often conflict with the habitat and life cycle requirements of amphibians. The loss of aquatic plants leads to numerous direct and indirect effects upon amphibian populations. We report the results of a study to examine the persistence of two native aquatic plants, water buttercup (*Ranunculus* sp.) and creeping water primrose (*Jussiaea repens*), to grazing by grass carp (*Ctenopharyngodon idella*). These plants offer the potential for restoration of aquatic systems unbalanced by the introduction of grass carp. Water buttercups (buttercups) are a member of the Ranunculacae which produce ranunculin, a vesicant extremely irritating to mucous membranes. To test both the hypothesis that carp would avoid buttercup and creeping water primrose (primrose), we employed a two-part study. In part I, carp were allowed to graze freely on plants placed in the aquaria. In addition to primrose and buttercup, curly pondweed (*Potamogeton crispus*) and duckweed (*Lemna major*) were provided as control plants. Carp were placed in four treatment groups which consisted of the following: carp + buttercup; carp + all plant species except buttercup; carp + all plant species; and carp isolated from all plant species by nylon mesh and fed trout chow. Pondweed and duckweed were readily consumed, while both primrose and buttercup were avoided, even when buttercup was the sole source of food. In part II we delivered a filtrate of blended primrose, buttercup or pelleted food, by gavage, to the pharynx and esophagus. Carp were allowed to digest the material overnight, killed by immersion in MS222, and tissues were fixed in 10% NBF and routinely processed for histopathology. Eight of nine carp in the buttercup group manifested significant lesions characterized by effects on mucosal epithelial cells including granular clumping of cytoplasm, pyknosis, karyorrhexis and loss. These results are consistent with the proposal that buttercup and primrose can be used to enhance habitat complexity in the presence of grass carp.

1554 DETERMINATION OF CORRELATION COEFFICIENTS FOR AQUATIC TOXICITY DATABASE ENDPOINTS.

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An Ecotoxicity list in the LOLI(R) for Windows database has been developed to provide a standardized resource of aquatic toxicity data for use in ecological risk assessment, environmental health and safety, and hazardous chemical evaluation. This dynamic list is comprised of data derived from a large amount of acute toxicity testing conducted on freshwater aquatic species for over 600 commonly used industrial chemicals. An investigation was undertaken to determine if a correlation exists between the data obtained from toxicity testing of varying species of freshwater fish [fathead minnow (n=79), rainbow trout (n=54), and bluegill (n=51)], water flea (n=51), freshwater algae (n=15) and Photobacterium phosphoreum Microtox test outcomes. The correlation coefficients included in this abstract are calculated from the data obtained in the Ecotoxicity list comprised of approximately 600 chemicals. The r values for Photobacterium phosphoreum and the comparative species follow: fathead minnow (r=0.722), rainbow trout (r=0.643), bluegill (r=0.645), water flea (r=0.513), and algae (r=0.600). The results of our statistical analyses concluded that positive correlations do exist between Photobacterium phosphoreum and all of the species tested, with the strongest positive correlation between fathead minnow data and Photobacterium phosphoreum. This may be due to the differences in availability of data for the other test parameters (n values), the susceptibility of the other species to the chemicals, and/or the susceptibility of the other species to test conditions (duration of test, method of test, age of organism, pH, water hardness, and temperature).

1555 SYSTEMIC BIOCHEMICAL EFFECTS IN CATTLE EXPOSED TO REPEAT LOW DOSAGES OF A PETROLEUM CRUDE OIL AND DIESEL.

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In Alberta, Canada, the proximity of some oil-field operations to land used for cattle pasture has raised concerns that long-term exposures to operational contaminants are deleterious to cattle health. In this investigation, we have probed in cattle the systemic effects of an Alberta crude oil (ACO) and a commercial diesel fuel (CDF) on biochemical components responsible for xenobiotic metabolism. Cattle were dosed on day 0, 14, 28, and 42 with either water (control) at 10 mL/kg, or CDF at 5 mL/kg, or ACO at 2.5, 5 and 10 mL/kg. Animals were killed on day 56, and liver, kidneys and lungs were analyzed for biochemical activities. Cattle exposed to ACO and CDF showed a significant, dose-dependent increase as compared to the control animals in the activity of aryl hydrocarbon hydroxylase (AHH) in kidneys (2.5- to 8-fold) and lungs (2- to 10-fold). Among the other biochemical components, the activity of ethoxyresorufin-O-deethylase (EROD) was also markedly higher (2.5-fold) in lungs of CDF and ACO treated cattle than the control cattle. Although the frequency of higher activities than average values of the control group was found to be more in livers of CDF and ACO exposed groups for several biochemical components (e.g., cytochrome P-450, AHH, EROD), the treatment effects were masked due to biological variations between animals. Evaluation of hepatic AHH activity with inhibitory probes showed a different response pattern in ACO and CDF treated cattle as compared to control animals, suggesting alterations in the composition of cytochrome P-450 isoforms.

1556 ESTROGEN-INDEPEDENT EFFECTS OF ATRAZINE(ATR) ON MAMMARY GLAND(MG) DEVELOPMENT IN RATS.

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Because of its widespead use as a herbicide and its ability to induce MG tumors in rats, the effect of ATR on pubertal MG development was examined in Long-Evans hooded rats. On postnatal day (PND) 22, rats were either ovariectomized (OVX) (N=27) OR SHAM OPERATED (N=18). Beginning on PND26, ten OVX and 10 sham rats were gavaged with ATR (100 mg/kg/day). The remaining rats (9 vehicle, 10 ATR) were injected with estradiol (E, 10 µg/day, s.c.) and progesterone (P,10,g/day) for 7 days. All other animals received sesame oil only. During this 121 day treatement period, the animals were examined for vaginal opening (VO). Females were killed on PND 37, trunk blood collected, pituitaries and MG removed for histology. Sham rats dosed with ATR had delayed VO (con=PND33 vs. ATR=PND35). While, VO was absent in OVX rats, there was no difference in the age of VO in control or ATR OVX rats receiving E+P. ATR-treated rats had smaller pituitaries (both sham-ATR and OVX & E+P-ATR), decreased pituitary and serum prolatin, and decreased serum LH. The MGs of the ATR-sham rats had decreased lateral branching, puberty-induced lobul formation, and retained more terminal end structures than untreated controls. In the E+P-ATR OVX rats, decreased branching (less dense lobule formation) and more persistent terminal end structures were still evident. These data suggest that ATR decreases puberty-induced MG development by altering normal pituitary functions. (This abstract does not reflect EPA policy).

1557 ANTIOXIDANT PRETREATMENT PREVENTS SEIZURE-INDUCED LOSS OF CYTOCHROME C OXIDASE AND HIGH-ENERGY PHOSPHATES DEPLETION IN BRAIN REGIONS OF RAT.

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Cytochrome c oxidase (CO) of mitochondria is responsible for electron transport and oxidative phosphorylation yielding ATP. A reduced capacity of this enzyme can cause incomplete reduction of oxygen which leads to an increased production of reactive oxygen species (ROS). We have examined changes in CO activity and high-energy phosphates in brain regions of rats following kainic acid (KA, 15 mg/kg, sc)-induced status epilepticus (SE). With the onset of seizures, CO activity in amygdala (0.533 µmole/min/mg protein), hippocampus (0.514 µmole/min/mg protein) and frontal cortex (0.569 µmole/min/ mg protein) increased up to 140% of control during the first hour of SE, while ATP and posphocreatine (PCr) were significantly reduced to 34%-55% of control. Within 3 to 7 days after induced SE, both CO activity and high-energy phosphates values were significantly decreased. Pretreatment with the spin trapping agent N-*tert*-butyl-α-phenylnitrone (PBN, 200 mg/kg, ip) or the antioxidant vitamin-E (100 mg/kg, ip) prevented KA-induced decline in high-energy phosphates and significantly attenuated the reduction of CO activity in amygdala and hippocampus. These findings support the suggestion that SE-induced neuronal injury within these brain regions may be linked to excessive generation of ROS. (Supported by NIH Grant, ES04597.)

1558 PROTECTION BY ANTIOXIDANTS AGAINST DEPLETION OF HIGH-ENERGY PHOSPHATES IN RAT BRAIN REGIONS FOLLOWING CARBOFURAN-INDUCED SEIZURES.

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The effects of the carbamate carbofuran (CF, 1.25 mg/kg, sc) induced status epilepticus (SE) were studied on high-energy phosphates in cortex, amygdala and hippocampus of rats sacrificed by head-focused microwave. Control values of ATP, ADP, AMP, PCr and Cr were significantly higher in cortex (25-35%) than in amygdala or hippocampus. Within 1 hr following CF administration, at a time when AChE was maximally inhibited (75%), the induced brain hyperactivity caused a marked decline in these determinants (ATP, 33-49%; and PCr, 25-50%). Total adenine nucleotides (TAN) and total creatine compounds (TCC) were also reduced (TAN, 28-32%; and TCC, 26-29%). Adenylate energy charge, an indicator of high-energy phosphate bond availability, remained unchanged. Three days after the onset of SE, recovery was seen in the amygdala and hippocampus, but not in the cortex. Pretreatment of rats with an antioxidant, such as the spin trapping agent N-*tert*-butyl-α-phenylnitrone (PBN, 200 mg/kg, ip), or vitamin-E (100 mg/kg, ip), provided partial protection against CF-induced neurotoxicity and depletion of high-energy phosphates. These results suggest that CF-induced SE, associated with depletion of high-energy phosphates contributes to the generation of radical oxygen species. (Supported by NIH Grant, ES04597.)

1559 ANTIOXIDANTS PREVENT DEPLETION OF HIGH-ENERGY PHOSPHATES ASSOCIATED WITH *STATUS EPILEPTICUS* IN BRAIN REGIONS OF RAT.

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Systemic injection of the excitatory amino acid, kainic acid (KA, 15 mg/kg, sc) or the anticholinesterase diisopropylphosphorofluoridate (DFP, 1.3 mg/kg, sc) induced status epilepticus (SE) causing depletion of high-energy phosphates in brain regions of rats sacrificed by head-focused microwave. Control values of ATP and phosphocreatine (PCr) were significantly higher in cortex (2.50 and 11.02 µmol/g, respectively) than in amygdala (1.72 and 8.48 µmol/g) or hippocampus (1.77 and 7.46 µmol/g). A significant decline in ATP and PCr was seen as early as 1 hour following KA- or DFP-induced SE. Three days after KA- or DFP-induced SE, levels of ATP remained decreased in amygdala (45% or 84%) and cortex (56% or 49%) while hippocampus values returned to normal. Pretreatment with the spin trapping agent N-*tert*-butyl-α-phenylnitrone (PBN, 200 mg/kg, ip) prevented seizures and protected high-energy phosphates levels depleted by DFP but not by KA. Pretreatment with the antioxidant vitamin E (100 mg/kg, ip), afforded protection for ATP and PCr in amygdala and hippocampus of both KA- and DFP-induced seizures groups. These results suggest that SE-induced depletion of high-energy phosphates caused by KA are in part protected by antioxidants such as vitamin E and PBN. PBN prevented DFP-induced SE and associated ATP and PCr changes by protecting AChE from critical inhibition. (Supported by NIH Grant, ES04597.)

1560 THE CORNEAL EFFECTS OF 2-(2-NITRO-4-TRIFLUOROMETHYLBENZOYL)-CYCLOHEXANE 1,3-DIONE (NTBC) IN THE RAT.

M. Robinson and M. Provan. *AstraZeneca, Macclesfield, United Kingdom.* Sponsor: L. L. Smith.

NTBC is a triketone used for the treatment of tyrosinaemia type 1. Rats dosed with NTBC at 2-40mg/kg/day by oral gavage daily develop corneal lesions. By ophthalmoscopy the corneal changes ranged from hazy or complete areas of opacity 1mm diameter or affecting the whole corneal surface. Some eyes showed vascularisation of the cornea. The earliest histopathological lesion

was a focal disorganisation of the corneal epithelium. This was rapidly followed by inflammatory changes until a well developed keratitis was seen. After 14 weeks of dosing some rats were dosed vehicle only until week 21 to represent a recovery phase. At week 21 the eyes of these rats showed a marked reversal of the lesions. The ophthalmoscopic and histopathological features closely resemble those when rats are dosed orally with 5% L-tyrosine in a low protein diet. Previous work has shown that NTBC inhibits 4-hydroxyphenylpyruvate deoxygenase, the second enzyme in the catabolic pathway of tyrosine, and an increase in plasma tyrosine has been demonstrated after dosing rats with NTBC.It is concluded that the corneal lesions of NTBC in the rat are a result of increased plasma levels of tyrosine.

1561 THE EFFECT OF 2-(2-NITRO-4-TRIFLUOROMETHYLBENZOYL)-CYCLOHEXANE-1,3-DIONE (NTBC) ON TYROSINE CATABOLISM IN THE MOUSE.

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NTBC is a drug used to treat a rare inborn error of tyrosine metabolism, tyrosinaemia type 1. In the rat, NTBC inhibits hepatic 4-hydroxyphenylpyruvate dioxygenase (HPPD), producing a marked tyrosinaemia which results in injury to the cornea of the eye. We report studies in the mouse where we have examined the effect of NTBC on hepatic HPPD, the extent of tyrosinaemia and whether cornea injury occurs. Administration of a single oral dose of NTBC to mice increased the concentration of tyrosine in the plasma and aqueous humour of the eye. The tyrosinaemia was both time and dose-dependent with a single dose of 10 mg/kg NTBC producing maximal concentrations of tyrosine in plasma of about 1,200 nmol/ml and in aqueous humour of about 2,200nmol/ml 16h after dosing. Analysis of the key hepatic enzymes involved in tyrosine catabolism, following a single dose of 10mg/kg NTBC, showed that HPPD was markedly inhibited soon after dosing and that the activity recovered very slowly. In response to the tyrosinaemia, the activity of hepatic tyrosine aminotransferase was induced about 2-fold, while the activity of hepatic homogentisic acid oxidase was reduced at 4 and 5 days after dosing. Daily oral administration of NTBC at doses up to 160mg/kg/day NTBC/kg to mice produced a maximal tyrosinaemia of about 600-700nmol/ml plasma, showing some adaptation relative to a single dose. Unlike the rat, no treatment-related corneal lesions of the eye were seen at any dose levels up to 6 weeks. Our studies show that NTBC is a potent inhibitor of mouse liver HPPD, which following repeat exposure produces a marked and persistent tyrosinaemia, which does not result in ocular toxicity.

1562 MESOTRIONE: THE RELEVANCE OF TYROSINE IN MAMMALIAN TOXICITY.

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Mesotrione is a triketone developed for use as a herbicide in corn with 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition as the sole mode of action in plants and animals. Administration of mesotrione to rats causes a marked increase in plasma tyrosine. The consequence of this increase is a range of toxicities in subchronic, chronic and reproductive studies in the rat. The purpose of this presentation is to provide evidence that all of these toxicities are produced by elevated plasma tyrosine and are not direct effects of mesotrione administration. The dose response profile of plasma tyrosine closely matches that for HPPD inhibition and also the toxicity dose response for a wide range of endpoints in the rat. Conversely the dose response profile for the chemical in plasma is very different to the above profile and is linear over a wide range of doses. For a large number of HPPD inhibiting triketones of different chemical structures the incidence of one of the toxic endpoints in the rat - corneal opacity - closely correlates with the maximal steady state plasma tyrosine levels measured for each chemical structure. Furthermore, a range of toxicities of mesotrione in the rat were either induced by tyrosine administration alone without the presence of mesotrione or could be markedly exacerbated by co-administration of exogenous tyrosine with mesotrione. This work provides both direct and indirect evidence that the toxicity of mesotrione in the rat is mediated by the amino acid tyrosine.

1563 THE BIOCHEMICAL RESPONSE OF HUMANS TO THE TRIKETONES MESOTRIONE AND NTBC.

M. G. Hall, M. Provan, M. Robinson, J. W. Botham, J. E. Doe, R. W. Lewis and L. L. Smith. *AstraZeneca, Macclesfield, United Kingdom.*

Mesotrione is a triketone developed as a herbicide in corn. NTBC is a structurally related triketone developed as a drug in the treatment of hereditary tyrosinemia type I in humans. The sole biochemical mode of action of these chemicals is identical in both plants and animals and is the specific inhibition of the enzyme 4-hydroxyphenylpyruvate dixygenase (HPPD). The consequence of this inhibition in mammals is an increase in plasma tyrosine concentration. The extent of this tyrosinemia across different mammalian species is determined by the innate activity of the hepatic enzyme tyrosine aminotransferase (TAT). Both HPPD and TAT are enzymes in the catabolic cascade of tyrosine. The purpose of this presentation is to report the biochemical changes in tyrosine catabolism associated with administration of these chemicals to humans and to suggest the relevance of this information in the human risk assessment of mesotrione. TAT activities were measured in rats and mice and compared with the reported levels in humans. The results of the clinical use of NTBC in the treatment of hereditary tyrosinemia type I were reviewed and compared with the results obtained in human healthy male volunteer studies with NTBC and Mesotrione. TAT activities differed across species. The activities in mice and humans are similar and up to 4-fold greater than those levels detected in rats. In patients treated with the drug NTBC the levels of steady state plasma tyrosine does not exceed 800nmol/ml - the level similar to that seen in mice treated with the herbicide mesotrione. In rats similar treatment with mesotrione induces much higher levels of plasma tyrosine at steady state. Human volunteer studies with NTBC and mesotrione have shown NTBC to be 1000-fold more potent in terms of the dose levels required to induce tyrosinemia than the herbicide mesotrione. Even so, the extent of the tyrosinemia following NTBC administration did not exceed 800nmol/ml at steady state. The results indicate that due to the innate levels of TAT, mice and humans respond similarly to HPPD inhibiting triketones and the rat responds differently with much greater levels of plasma tyrosine being achieved at steady state. These biochemical data are important in the extrapolation of toxicology data in experimental animals to man and show that the mouse is the more relevant species for human risk assessment.

1564 THE REGULATORY TOXICOLOGY OF MESOTRIONE: IDENTIFICATION OF THE MOST RELEVANT ANIMAL MODEL FOR HUMAN RISK ASSESSMENT.

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In order to properly assess the potential risk to humans, it is a regulatory requirement that all new chemicals including pesticides undergo a thorough evaluation in a battery of toxicology tests. In this way the intrinsic hazard associated with a chemical can be identified. In relating intrinsic hazard as identified in animal models to those effects likely to occur in humans, certain assumptions have to be made; a central assumption is that the effect of a compound observed in an animal model is relevant and can be used to predict possible effects in humans. The purpose of this work is to provide a regulatory compliant animal database recognising, for mesotrione, the relevance of species differences in toxicity to predict effects in humans. To this end a complete toxicological database is provided for mesotrione which has been extended to cover the most relevant animal model, the mouse. Across a range of species, mesotrione causes no significant toxicity in short term studies. The compound is not neurotoxic and is not genotoxic in short term studies both *in vitro* and *in vivo*. Mesotrione is not oncogenic in the mouse or in the rat and is not a repro-toxicant. The specific toxicities of mesotrione in rats which are absent in mice include corneal opacity, reduced litter size, pup survival and pup renal pathology, and increased incidence of spontaneous degenerative pathology in lifetime studies. Other toxicities of mesotrione occur in both species but occur at much lower dose levels in rats. These include liver, kidney and body weight changes in subchronic studies. Potential developmental effects have been investigated in the rat, rabbit and mouse with mesotrione producing no teratogenicity or other significant effects in any species tested. In chronic bioassays in the dog and mouse the material is of low potency. These data show that mechanistic understanding coupled with regulatory toxicology studies in the most relevant species can provide the optimum database for human health risk assessment.

1565 SPECIES DIFFERENCES IN MESOTRIONE-INDUCED TYROSINEMIA.

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Mesotrione is a triketone herbicide for use in corn with 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition as the single mode of action in both plants and animals. In mammals, HPPD is the second enzyme in the catabolic cascade of the amino acid tyrosine. Inhibition of HPPD results in an increase in plasma tyrosine. A range of toxicities associated with exposure to mesotrione occurs in rats but not mice, mediated by tyrosine. The purpose of this presentation is to report the mechanistic basis for the species difference in toxicity. The specific toxicities of mesotrione in rats which are absent in mice include corneal opacity, reduced litter size, pup survival and pup renal pathology, and increased incidence of spontaneous degenerative pathology in lifetime studies. Other toxicities of mesotrione occur in both species but occur at much lower dose levels in rats. These include liver, kidney and body weight changes in subchronic studies. For example, the no effect levels for liver weight increase in 90 day studies were: male rats 1ppm, female rats 7.5ppm and male and female mice 350ppm. Rats and mice have marked differences in the innate activity of hepatic tyrosine aminotransferase (TAT) the rate limiting enzyme in the tyrosine catabolic cascade. This results in marked differences in the maximum achievable steady-state levels of plasma tyrosine. Each toxicity which is shown in the respective species requires a steady-state tyrosine elevation above a particular threshold for that toxicity. Because of the species differences in innate TAT levels and hence maximum achievable steady-state tyrosine levels there are marked differences in the nature and extent of the toxicities exhibited in each species. In conclusion, although the primary biochemical target of mesotrione is hepatic HPPD, it is the innate activity of another catabolic enzyme (TAT) which determines the species specificity in mesotrione toxicity.

1566 SPECIES DIFFERENCE IN DEVELOPMENTAL CHANGES AFTER TREATMENT WITH MESOTRIONE.

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In mammals, the single mechanism of action of mesotrione, a selective herbicide, is inhibition of the hepatic enzyme, 4-hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme is important in the catabolism of the amino acid, tyrosine. The consequence of prolonged inhibition of this enzyme is an increase in plasma tyrosine levels i.e. a tyrosinemia. Following inhibition of HPPD, the maximal extent of any tyrosinemia is controlled by a second hepatic catabolic enzyme, tyrosine aminotransferase, TAT. Rats have relatively low innate activity of TAT and consequently, on exposure to mesotrione, develop a severe tyrosinemia. The purpose of this work was to compare developmental changes in the rat (low TAT, high tyrosinemia) with the rabbit and mouse (high TAT, low plasma tyrosine) after treatment with mesotrione. Time mated female AP rats, New Zealand White rabbits and AP mice received up to 1000, 500 or 600mg/kg/day mesotrione respectively by oral gavage; Prior to parturition animals were killed and their *uteri* examined for live foetuses and *intra-uterine* deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination. At high doses of mesotrione there were no teratogenic effects in the rat, the rabbit and the mouse. In the rat and the rabbit noteworthy changes were confined to high dose level i.e. reduced foetal bodyweight, at the limit dose in the rat and a low level of whole litter losses in the rabbit at the high dose levels of 250 and 500 mg/kg/day. There were no noteworthy changes in the mouse at any dose level tested. Following treatment with mesotrione at a range of dose levels in the rat and the rabbit, differences from concurrent control were observed in the number of foetuses showing minor skeletal defects. A marginal difference was also observed in the mouse, but only at the highest dose level tested. All of observed skeletal defects were indicative of a small reduction in ossification or a slight shift in the normal and highly variable pattern of ossification in experimental animals. Such changes do not adversely affect post-natal development as shown in multigeneration reproduction studies in the rat and mouse. The changes show a close relationship in their extent to the different innate levels of tyrosine amino transferase across the three species. The weight of evidence strongly suggests that these changes are tyrosine mediated and are not due to a direct effect of mesotrione. Due to the differences in the innate activation of TAT in different species, the mouse is the more relevant species for human risk assessment.

1567 GROSS ANATOMY AND HISTOPATHOLOGICAL CHANGES FROM CHRONIC EXPOSURE TO 2,4-DICHLOROPHENOXYACETIC ACID IN RATS.

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Many forms of 2,4-dichlorophenoxyacetic acid(collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The herbicides have been used extensively in Egypt and many other countries. This study was designed to evaluate the safety of the commercial formulation of 2,4-D in male and female rats. Animals received 2,4-D orally at a daily dose of 20 ppm for 1,2 and 3 months respectively. Animals were observed for clinical symptoms of toxicity. As early as one month, 2,4-D induced weight loss and slow movement in some of the examined animals. Post-mortem examination revealed the presence of congestion with hemorrhagic spots in liver, kidneys, salivary glands and lungs in addition to pneumonic patches in lungs. The stomach and intestine were congested and mucous exudate in their lumina was present. The heart, spleen, brain, thyroid glands, testes, ovaries, fallopian tube and uterus showed congestion with hemorrhagic spots on their surfaces. However, the severity of the symptoms varied with the exposure time. Histopathological examination indicated the presence of telangiectiasis in liver, congestion and hemorrhage in glomeruli; absence of the granuloma in lung with the presence of perivascular round cell infiltration. Sialoadenitis and necrosis of uterine glands with edema and fibroblastic cell proliferation in the subendometrium were also detected.

1568 EFFECT OF 2-METHYL-4-CHLOROPHENOXYACETIC ACID (MCPA) ON THE PLASMA AUC AND URINARY ELIMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN THE DOG.

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The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used for post emergent broadleaf weed control, often in combination with other phenoxy compounds. 2,4-D and MCPA have been shown to undergo similar metabolism and are primarily renally excreted in the dog at a substantially slower rate than in other species. The purpose of this study was to investigate the toxicokinetics of 2,4-D alone and in the presence of a second phenoxy herbicide, 2-methyl-4-chlorophenoxyacetic acid (MCPA). Six female beagle dogs were used in a crossover design study consisting of two treatment groups (A and B) of three dogs. Treatment group A received intravenously administered 0.5 mg 2,4-D/kg b.w. Treatment group B received orally administered 5 mg MCPA/kg b.w. followed 12 hours later by intravenously administered 0.5 mg 2,4-D/kg b.w. Blood and urine were collected at various times during 96 hours post administration and frozen until analysis. Plasma and urine 2,4-D and MCPA concentrations were determined using HPLC with fluorescence detection. The area under the plasma 2,4-D concentration-time (AUC) to the last quantitatable concentration-time point was greater in 5 of the 6 dogs following MCPA co-administration when compared to 2,4-D only administration. The percent of administered 2,4-D found in the urine ranged from 18-57% when 2,4-D was administered alone and from 24-44 % when 2,4-D was given after MCPA administration. The amount of 2,4-D excreted in the urine from 0-96 hours post 2,4-D administration following MCPA co-administration was not statistically different from the amount excreted when 2,4-D was administered alone. When 5.0 mg MCPA/kg was orally administered to beagle dogs, no statistically significant changes in the plasma AUC or urinary elimination of 2,4-D were observed.

1569 EVALUATION OF NEUROTOXICITY OF DIMETHOATE 4E FOLLOWING SHORT-TERM DERMAL EXPOSURE TO SPRAGUE-DAWLEY RATS.

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This study was designed to estimate the no-observed effect level (NOEL) for cholinesterase inhibition and clinical signs following short-term dermal exposure to Dimethoate 4E formulation. Dimethoate 4E (undiluted) was dermally administered (6 hours per day for 5 days) to male and female Sprague-Dawley rats, at doses of 5, 10, 20, 40, and 100 mg active ingredient (ai)/kg/day. A control group received a Blank formulation vehicle. Clinical signs, dermal irrita-

tion, an abbreviated Functional Observational Battery (FOB), and body weights were evaluated. Gross necropsies were conducted and brain weights recorded. Cholinesterase (ChE) activities were evaluated in plasma, erythrocytes, and 3 brain regions (hippocampus, striatum, and cortex) sampled following exposures on Days 3 and 5. There were no treatment-related effects on body weight, brain weights or gross necropsy findings. Dermal irritation in control and dosed groups included desquamation (males and females) and erythema (females only), with a possible dose-relationship for desquamation in males. Clinical signs of ChE inhibition were observed only in four females from the 100 mg ai/kg dose group whose collars came off during the exposure period. Two of the four also showed abnormal findings in the Day 3 FOB. Oral exposure of the four 100 mg ai/kg/day females that lost their collars cannot be excluded. Other FOB observations were unremarkable. The NOEL for clinical signs of toxicity or FOB observations was 100 mg ai/kg/day for males and 40 mg ai/kg/day for females. Dose-related inhibition of ChE activity was seen in this study. The NOEL for ChE inhibition in this study was 40 mg ai/kg/day for males and 10 mg ai/kg/day for females, based on statistically significant inhibition of erythrocyte and regional brain ChE activities at higher dose levels.

1570 DIETHYLDITHIOCARBAMATE CHELATES CATALYZE THE FORMATION OF CATECHOL THIOETHERS.

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Parkinson's Disease (PD) is currently second to Alzheimer's Disease as the most common neurodegenerative disorder in the United States. Epidemiological studies have repeatedly demonstrated an increased risk for PD associated with pesticide exposure. Particularly prominent in recent studies are the diethyldithiocarbamate (DEDC) family of pesticides, including Maneb and Mancozeb. DEDC complexes have been hypothesized to react with catechols, resulting in oxidative stress. The oxidation of catechols to quinones is followed by reaction with GSH to form catechol thioethers. In these studies, varying concentrations of *N*-acetyl-dopamine and *N*-acetyl-cysteine were incubated together at 37°C for 30 minutes with 50 μM $Mn(DEDC)_2$, $Fe(DEDC)_3$ or $Cu(DEDC)_2$. At various time points, 100 μL of the reaction mixture was removed for HPLC detection of the catechol thioether. Under these conditions, no significant formation of the catechol thioether was observed when either $MnCl_2$, $FeCl_3$, or $Cu(NO_3)_2$, or NaDEDC were added alone. However, the $Mn(DEDC)_2$ and $Fe(DEDC)_3$, but not $Cu(DEDC)_2$, catalyzed the formation of the catechol thioether in a linear manner out to 15 minutes. On the contrary, when 3,4-dihydroxyphenylacetate (DOPAC) was substituted as the catechol, no corresponding thioether was formed under these conditions. These experiments show that $Mn(DEDC)_2$ and $Fe(DEDC)_3$ can catalyze both the depletion of low molecular weight thiols, and the formation of catechol thioethers. (Supported by grants ES02611, AG00774, AG16835, and ES/NS10196.)

1571 DEVELOPMENTAL EXPOSURE TO TRIADIMEFOM: BEHAVIORAL AND DOPAMINERGIC EFFECTS.

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Triadimefon (TDF), an antifungal triazole commonly used on fruits, grains and shrubs, has been shown to exert CNS-stimulant effects similar to other known psychomotor stimulants such as amphetamine and methylphenidate in adult rodents. Specifically, TDF targets the dopamine (DA) transporter, blocking DA reuptake. Furthermore, adult exposures have been shown to alter motor activity, stereotypical behaviors, and performance on fixed interval schedules of reinforcement. Little is known, however, about what consequences these stimulant properties could impose on CNS development. Long-Evans male rats were exposed either prenatally, postnatally, or pre- and postnatally to 0, 25, or 50 mg/kg TDF. Prenatal exposure (maternal gavage) occurred from gestational day 6 to 19, and postnatal exposure (i.p.) occurred from postnatal day 4 to 21. DA and DA metabolite levels in the striatum and tyrosine hydroxylase density in striatum and nucleus accumbens were determined at postnatal day 21. The results of these analyses were consistent among all exposure groups. However, locomotor activity on postnatal days 21-23 and 50-52, and response rate on a repeated learning and performance task were significantly lower in the pre- and postnatal 50 mg/kg exposure group compared to controls. These results indicate that developmental exposure to TDF could produce long-term behavioral deficits. It is hypothesized that these effects may be related to changes in DA transporter function. (Supported by ES01247, ES07026, Strong Children's Research Center.)

1572 EFFECTS OF TOXAPHENE ON THE IMMUNE SYSTEM OF CYNOMOLGUS (*MACACA FASCICULARIS*) MONKEYS.

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The immunotoxic effects of toxaphene were investigated in female and male Cynomolgus monkeys. Toxaphene, in glycerol/corn oil, was administered in gelatin capsules at 0.1, 0.4 or 0.8 mg/kg b.w./day seven days/week to 10 healthy young adult female cynomolgus/group while a group of 5 male monkeys received 0.8 mg/kg b.w./day. Control male (5 monkeys/group) and female (10 monkeys/group) ingested glycerol/corn oil only. Testing for immune effects was initiated at 34 weeks of treatment. Statistically significant treatment-related effects included: a trend towards reduced primary and secondary anti-sheep red blood cell (SRBC) titres for immunoglobulin (Ig) M across all post-immunization dates and IgG for days 14 ($p=0.243$) and 21 post immunization in treated females ($p<0.05$); reduced (primary) anti-SRBC titers in treated males ($p<0.05$); a trend towards lower anti-tetanus toxoid titres in treated females for days 14, 21 and 28 post immunization ($p<0.05$) and a trend towards reduced T helper/inducer:T suppressor/cytotoxic cell ratio ($CD4^+$:$CD8^+$) due to reduced $\%CD8^-$ and increased $\%CD4^+$ with increasing dose ($p<0.05$). Increased NK cell activity was observed in the treated males ($p<0.05$). The differences between control and treated groups were not statistically significant for the following parameters: Lymphocyte transformation following mitogen stimulation, delayed type hypersensitivity response, antibody titers to pneumococcus antigens and cortisol levels. Based on pairwise comparisons between the treated and control monkeys the no effect level for adverse immunologic effects was 0.1 mg/kg b.w./day for female and below the 0.8 mg/kg b.w./day level for male monkeys.

1573 TOXAPHENE IS ANTIESTROGENIC IN A HUMAN BREAST-CANCER CELL ASSAY.

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Toxaphene, a complex mixture of chlorinated bornanes, bornenes, and bornadienes, was a heavily used insecticide in the US until its use was restricted in 1982. There are conflicting reports regarding the potential for toxaphene to induce estrogenic responses in human and animals. Therefore we examined the estrogenicity of toxaphene in a human breast-cancer cell assay, the MCF-7 focus assay, which is based on *in vitro* postconfluent cell proliferation and tissue restructuring. Toxaphene was also tested for its ability to bind the estrogen receptor (ER) and to alter the catabolism of E_2 in MCF-7 cell cultures. Results from the MCF-7 focus assay showed that toxaphene alone and in binary combinations with other pesticides was not estrogenic between the concentrations of 0.5 nM and 10 μM, but rather that toxaphene was weakly antiestrogenic (it reduced the number of foci induced by 0.01 and 0.1 nM E_2 by 40 - 50%). Results from the mechanistic studies showed that 1) toxaphene alone or in binary combinations with other pesticides did not bind rhER or ER in MCF-7 cells, 2) toxaphene did not alter the growth rate of MCF-7 cell cultures over 13 days, and 3) toxaphene did not alter the catabolism of E_2. In conclusion, results from the MCF-7 focus assay demonstrate that toxaphene is weakly antiestrogenic rather than estrogenic. (Supported by NIH ES04913.)

1574 *IN VIVO* PROMOTING POTENCY OF TECHNICAL TOXAPHENE, UV-IRRADIATED TOXAPHENE, AND WEATHERED TOXAPHENE.

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The pesticide toxaphene has been classified as possible carcinogenic to humans. Human exposure mainly occurs through the consumption of toxaphene contaminated fish. Due to weathering and biotransformation, the bornane composition of technical toxaphene (TT) differs from the bornane composition in consumption fish. Therefore information on the carcinogenicity and general toxicology of weathered and biotransformed TT would be of major interest. To mimic the weathered toxaphene found in fish, we developed a so-called 'realistic exposure' procedure for toxaphene. This procedure makes use of cod that were exposed to TT. Toxaphene residues that were extracted from cod liver (CLE), were then used in an *in vivo* exposure study with rats to obtain information on its tumor promoting potency. Besides CLE,

we also studied the tumor promoting properties of UV-irradiated toxaphene (UVT) and TT. The carcinogenic potency of CLE, TT and UVT was studied using a medium-term two stage promotion assay, measuring the development of altered hepatic glutathione S-transferase (GST-p) positive foci. In short, 2/3 hepatectomy was performed on female Sprague Dawley rats, ± 6 weeks of age, followed by a single i.p. NDEA injection. Starting 5 weeks after partial hepatectomy, rats were dosed weekly with CLE, TT, or UVT (0.46-12.5, 0.67-18, and 0.33-9 mg/kg/week respectively) by s.c. injection, using corn oil as a carrier. Control animals received corn oil only whereas TCDD (1 mg/kg/week) was administered as positive control. No effect on body and liver weight gain was observed in rats treated either with CLE, TT, UVT, or TCDD. In addition, no liver damage was observed as measured by plasma ALT and AST levels. Moderate induction of GST-p positive foci were observed in livers of TT and UVT exposed rats as compared to TCDD exposed rats. Development of altered hepatic GST-p positive foci in CLE exposed rats is currently under investigation. This new toxicological information may have its consequences for tolerance levels of toxaphene in fish consumption. (This study was supported by a grant from the European Commission [FAIR CT PL.96.3131].)

1575 *IN VITRO* GENOTOXICITY AND TUMOR PROMOTING POTENCY OF TECHNICAL TOXAPHENE, UV- IRRADIATED TOXAPHENE, AND WEATHERED TOXAPHENE.

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Toxaphene, a complex mixture of polychlorinated camphenes, has been used extensively as a pesticide. Due to its lipophilic, persistent, and volatile nature, toxaphene has become widespread globally. Reports on the mutagenic and carcinogenic properties of toxaphene in mammalian test systems, have led to the assumption that toxaphene poses a serious thread to humans. However, data on the risk for humans associated with toxaphene exposure is scanty. Since exposure of humans mainly occurs through consumption of contaminated fish, we studied the *in vitro* genotoxicity and tumor promoting potency of toxaphene residues extracted from contaminated cod (CLE), in addition to technical toxaphene (TT) and uv-irradiated toxaphene (UVT).Weathered toxaphene was obtained by feeding cod toxaphene spiked diets after which livers were collected and extracted for toxaphene residues. Direct as well as indirect genotoxicity was studied employing the umu-assay. The *in vitro* tumor promoting potency was studied by means of inhibition of gap junctional intercellular communication. Hepa 1c1c7 cells were allowed to grow in small disks for 48 hrs after which a small amount of lucifer yellow was injected into single cells and the number of communicating cells counted. 0-24 hrs prior to injection of lucifer yellow, growing medium was replaced by exposure medium spiked with CLE (1-10 mg/ml), UVT or TT (0.5-5, and 1-10 mg/ml respectively). Preliminary results indicate that TT as well as UVT and CLE are only slightly genotoxic in the presence or absence of S9 mix. TT, UVT and CLE already inhibited GJIC after 0.5 hrs up to 50%. After 6 hrs of exposure, GJIC was restored to 80% in the presence of TT and UVT, but not CLE. The highest concentrations of TT, UVT and CLE inhibited GJIC by 60, 20 and 40% respectively after 24 hrs of exposure. These results emphasize the possible risk for humans of consumption of toxaphene contaminated fish.This study was granted by the Europian Community (FAIR CT PL.96.3131).

1576 THE INSECTICIDE DPX-MP062 AND ITS METABOLITE DCJW BLOCK SODIUM CHANNELS IN MAMMALIAN NEURONS.

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DCJW, a metabolite of an oxadiazine insecticide DPX-MP062, is known to block compound action potentials in insect nerve preparations at submicromolar concentrations and to cause a concentration-dependent inhibition of Na^+ currents in cultured insect neurons. However, little is known about the effects of these compounds on the Na^+ channels of mammalian neurons. We compared the effects of DPX-MP062 and DCJW on tetrodotoxin-sensitive Na^+ channels in rat dorsal root ganglion neurons by using the whole-cell patch clamp technique. DPX-MP062 and DCJW blocked Na^+ channels in a voltage-dependent manner. At a holding potential of -80 mV, DPX-MP062 at 1 μM suppressed the Na^+ current by 52%, whereas no suppression occurred at -100 mV. Surprisingly, DCJW at 1 μM was more potent than DPX-MP062 suppressing the Na^+ current by 85% and 50% at holding potentials of -80 mV and -100 mV, respectively. The analysis of the Na^+ channel inactivation curve clearly indicated that both agents shifted the fast and slow inactivation curves in the hyperpolarizing direction resulting in an enhanced block at more depolarized holding potentials. DCJW had an additional blocking mechanism, which contributed to the more potent blocking action of the metabolite. Thus, DCJW is more potent than DPX-MP062 in blocking the mammalian Na^+ channel, and is less potent in blocking mammalian Na^+ channels than insect Na^+ channels. (Supported by NIH grant NS14143.)

1577 ACUTE FISH TOXICITY OF PHOSPHOROTHIONATE-ESTERS DUE TO TWO INDEPENDENT TOXIC EFFECTS ON THE NERVOUS SYSTEM.

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Toxicity of organophosphorus(OP)-esters in fish is generally attributed to their ability to inhibit acetyl-cholinesterase (AChE). For phosphorothionates, metabolic activation to the oxon-analog is required to form the active inhibitor. However, for a test set of 20 phosphorothionates, the measured in-vitro inhibition rates of AChE by the oxon-analogs did not correlate with the observed acute toxicity of the parent compound in guppy (p. reticulata). To understand the mechanism of acute toxicity we tested whether narcosis (general anesthesia), being a second plausible mode of action, governs the acute toxicity of some of the compounds in the test set. Narcosis potency at observed lethal exposure levels was modeled with a critical body burden approach. Nine out of 20 OP-esters in the test set were thereby identified as possible narcotic compounds. For the 11 remaining, non-narcotic compounds a good correlation between acute toxicity and measured in-vitro AChE inhibition rate was found (r^2=0.68). Thus, the interpretation of acute toxicity data of fish may be improved by effect-based clustering of test set chemicals. This is an alternative to the more often used structure-based clustering.

1578 MALE REPRODUCTIVE FUNCTION AND ENDOCRINE PROFILE IN MEXICAN PEASANTS EXPOSED TO P,P'-DICHLORODIPHENYL DICHLOROETHYLENE (P,P'-DDE).

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DDT has been banned during the 1970's in developed countries but is still being used in the developing world for agriculture and malaria control. The main metabolite of p,p'-DDT, p,p'-DDE, is a potent anti-androgen that was shown to affect male reproductive function in rodents. We conducted a pilot cross-sectional study to evaluate possible relationships between p,p'-DDE body burden, androgen status and male reproductive function in a group of 24 peasants (mean age=21 years; range=16-28) from the Chiapas region (Mexico). After informed consent was obtained, a blood sample was collected from each individual from a cubital vein, centrifuged and the serum analyzed for organochlorines by high-resolution gas chromatography with electron capture detectors. Testosterone (total and bioavailable), sex-hormone binding globulin (SHBG), LH and FSH concentrations were also determined in serum samples using ELISA or RIA assays. Sperm quality was assessed in 21 participants using standard andrological tests. Mean p,p'-DDE concentration in serum lipids was 69.7 mg/kg (range=0.6-177.2). Simple correlation analyses (Spearman correlation coefficient) revealed that p,p'-DDE concentration was positively correlated to serum SHBG concentration (r=0.484; p=0.02) and negatively correlated to the bioavailable/total testosterone ratio (r=-0.572; p=0.003). Mean sperm density averaged 146 millions/ml (range=10-650) and sperm volume 2.1 ml (range=0.7-5.0). p,p'-DDE concentration was inversely correlated to both sperm volume (r=-0.450; p=0.04) and sperm density (r=-0.421; p=0.06). These results suggest that p,p'-DDE body burden in this group of young men may alter male hormone status and decrease sperm quality.

1579 TOXICOKINETICS OF METHYL PARATHION AFTER DIFFERENT ROUTES OF EXPOSURE.

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Methyl parathion became a public health concern with its illegal use in private homes. Yet, there are limited data with which to predict the long-term health effects resulting from a pattern of exposure such as occurs in a domestic setting. The objective of these studies was to compare the toxicokinetics of methyl parathion in adult female rats following intravenous, oral and dermal exposure. The toxicokinetics of methyl parathion after intravenous exposure (2.5 mg/kg) best fit a model in which it was distributed between multiple compartments and rapidly eliminated ($T^1/_2$ ~1.5 h). The toxicokinetics of methyl parathion after single oral exposure (2.5 mg/kg) contrasted with those after intravenous exposure. First, methyl parathion concentrations in blood were ≤2% of those after intravenous exposure. Second, there was no discernible peak in blood methyl parathion concentrations. These data indicate a significant 'first pass' effect. After single dermal exposure (25 mg/kg), blood methyl parathion levels increased during the first 6 h and then remained constant for the next 42 h. Levels of methyl parathion in blood after dermal exposure were comparable to those after oral exposure. Despite differences in toxicokinetics and maximal blood concentrations, time-dependent changes in blood cholinesterase activity following intravenous or oral exposure to methyl parathion were similar. A maximal inhibition of 70-80% occurred within 15-30 minutes, and activity recovered within 30 to 48 h. In contrast, after a single dermal exposure (25 mg/kg) to methyl parathion, blood cholinesterase activity declined to a minimum by 21-24 h that was sustained. Repeated dermal exposure to a smaller dose (1 mg/kg/day) of methyl parathion also caused a sustained decrease in blood cholinesterase activity. These data indicate that the toxicokinetics and toxicodynamics of methyl parathion are complex, and the complexity of the model varies with route of exposure. These data also suggest that repeated dermal exposure to methyl parathion, as occurred with the illegal spraying of private homes and businesses, exacerbates its toxicity and increases the potential for long-term adverse health effects. (Supported by ATSDR Grant U50/ATU486513-01.)

1580 RECOVERY OF BLOOD CHOLINESTERASE ACTIVITY AFTER ORAL AND DERMAL EXPOSURE TO METHYL PARATHION.

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Methyl parathion is an organophosphate insecticide which has been used inappropriately to rid private residences of pests. Methyl parathion is bioactivated to methyl paraoxon, and the covalent attachment of methyl paraoxon to the active site of acetylcholinesterase causes inhibition of the enzyme. In the present study, the time-dependent action of oral (2.5 mg/kg) and dermal (25 or 50 mg/kg) administration of methyl parathion on the activity of blood cholinesterase of female Harlan Sprague Dawley rats was examined. Femorally-cannulated rats were given a single dose of methyl parathion, and blood samples were collected over the next 48 h. All samples were diluted immediately in heparinized saline and kept at 0-4°C prior to measurement of cholinesterase activity. After oral gavage, cholinesterase activity decreased by 70-80% within 15 min, but recovered completely 48 h after dosing the animals. In the presence of the butyrylcholinesterase inhibitor Iso-OMPA total cholinesterase activity was decreased, but there was no change in the onset of inhibition or recovery of enzyme activity. In dermally exposed rats, inhibition of cholinesterase activity in the blood was not dose-dependent at 25 or 50 mg/kg, developed more slowly than the inhibition observed after gavage and showed no recovery 48 h after dermal exposure. In blood collected 30 min after gavage of methyl parathion, cholinesterase activity recovered spontaneously *in vitro* in a time- and temperature-dependent fashion. In blood allowed to stand at 37°C, recovery was complete within 6 h. In contrast, only slight recovery occurred in blood stored at 4°C for up to 24 h. These preliminary findings suggest major differences in the pharmacokinetics of methyl parathion as a function of route of exposure. Recovery of cholinesterase after oral exposure can be partially explained by spontaneous recovery and suggests a rapid clearance of methyl parathion. On the other hand, the prolonged inhibition of cholinesterase caused by dermal administration suggests the presence of a depot from which the release of methyl parathion and/or its active metabolite is sustained. (Supported by ATSDR Grant U5O/ATU486513-01.)

1581 GENE EXPRESSION IN HUMAN NEUROBLASTOMA CELLS AND IN NERVES FROM HENS EXPOSED TO NEUROPATHIC ORGANOPHOSPHORUS ESTERS (OPS).

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To test the hypothesis that neuropathic OP compounds could cause early expression of gene products, *in vitro* and *in vivo* models of OP-induced delayed neuropathy (OPIDN) were exposed to neuropathy-inducing compounds. For studies in cell culture, SH-SY5Y human neuroblastoma cells exposed to mipafox, 10^{-4} M or PSP 10^{-6} M for 1,2,4,6,8 and 12 hr before examination for expression of hsp72 by western blotting. This stress response protein was expressed in untreated as well as treated cells. The expression was greater after OP exposure, especially at 6 and 8 hr, but the increases were not dramatic and definitive. Data were more difficult to obtain with tissues from hens exposed to a neuropathy-induced OP, phenyl saligenin phosphate (PSP, 2.5 mg/kg) when fresh-frozen spinal cord sections were immunostained for c-fos, c-jun and GFAP. These results suggest that dramatically increased expression of early markers of cell injury are not easily detected in SH-SY5Y cells and hens following acute exposure to neuropathic OP compounds, with these methodologies. (Supported by US EPA R825356.)

1582 QUANTITATION OF LOW DOSE ORGANOPHOSPHATE BINDING IN MURINE BLOOD AND BRAIN.

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Organophosphate (OP) compounds are a class of commonly used insecticides and are similar to offensive nerve agents. Their principal extracellular targets are soluble plasma esterases and membrane-bound acetylcholinesterase (AChE) on erythrocytes (RBC) and throughout the nervous system. We quantitate persistent [14C]-OP binding to AChE as a probe of chemical synergism at low exposures. We measured the bound levels of 14C-paraoxon (PXN) and 14C-diisopropyl-fluoro-phosphate (DFP) in the plasma, RBC, and brains of CD2F1 mice as functions of time to 1 week after single 100 ng/kg doses, given IP (DFP) and orally in peanut butter (PXN). DFP had an initial blood clearance mean life (t) of 6 hours. Peak protein binding occurs in blood at 24 hours, with RBC containing 5% (on per gram basis) of the plasma 14C levels. Peak brain binding arguably occurs 24 hours later at a level 30% of the RBC peak. OP-AChE complexes in both the brain and RBC show a clearance t>200 hour, significantly longer than the plasma clearance at t=35 hour. Esterase activity assays are inadequate to quantify OP binding at these environmentally relevant doses (<1 μg/kg). Work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract W-7405-ENG-48.

1583 ORGANOPHOSPHORUS COMPOUNDS INDUCE CASPASE-3 ACTIVATION.

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Organophosphorus (OP) compound exposure *in vitro* elicits a predominantly apoptotic response as evaluated by morphological criteria. The activation of caspase-3, a cysteine-aspartate effector protease, was examined in SH-SY5Y human neuroblastoma cells to assess functional attributes of activated cell death programs. For this, SH-SY5Y cells were exposed to the OP compounds paraoxon, parathion, phenyl saligenin phosphate (PSP), tri-ortho-tolyl phosphate (TOTP), and triphenyl phosphite (TPPi) at 10μM to 1mM for 60 to 4320 minutes. The cells were then fixed, permeabilized, incubated with phycoerythrin conjugated anti-active caspase-3 antibodies, and analyzed on an Epics XL-MCL flow cytometer (ex488nm em575nm). All OP compounds induced time and dose-dependent activation of caspase-3 proteases. Nine hour pretreatment with the caspase-3 inhibitor Ac-DEVD-CHO or the caspase-8 inhibitor Ac-IETD-CHO (25μM) decreased protease activation following parathion and TPPi exposure when compared to OP-only controls. Similar pretreatment with the serine/cysteine protease inhibitor phenylmethylsulfonyl fluoride (PMSF, 1mM) had little effect (parathion) or resulted in increased caspase-3 activation (PSP, TOTP). Thirty hour pretreatment with 500nM cyclosporin A decreased caspase-3 activation following incubations with paraoxon, parathion, TOTP, and TPPi. Caspase-3 activation and inhibition indicated these proteases were important in OP-induced apoptosis. Decreased OP-induced caspase-3 activation following caspase-8 inhibition suggested potential apoptotic initiation through TNF or Fas receptors. Caspase-3 activation paralleled apoptotic nuclear condensation and fragmentation induced by

paraoxon, parathion, and TPPi. Caspase-3 was also activated following PSP and TOTP exposure, even though these induced nuclear morphologies not typically considered apoptotic (type-II apoptosis). Discrepancies between morphological estimates of apoptosis illustrate the importance of using additional functional assays (e.g., caspase) for the determination of cell death.

1584 HUMAN PARAOXONASE (PON1) ISOZYMES: QUANTITATIVE ANALYSIS OF ISOZYMES AFFECTING INDIVIDUAL SENSITIVITY TO ORGANOPHOSPHATES.

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Epidemiological evidence and *in vitro* experiments rather than direct testing must be used in human studies to demonstrate a direct relationship between enzymatic deficiencies (or polymorphic variability)and toxicity resulting from exposure to organophosphates (OPs). Ourrecent studies on Gulf War veterans who developed neurological symptoms showed significantly lower levels of the PON1 type Q allozyme than thenon-deployed or deployed healthy controls (Haley, Billecke and La Du,Toxicol & Appl. Pharmacol., 157:227, 1999). Human sensitivity to OP'sdepends upon the particular OP involved, the level and type of PON1 isozyme (Q or R) of the person exposed, and the hydrolytic efficiency ofthat isozyme for that OP compound. The relative order of hydrolytic rates with the Q and R isozymes for representative organophosphates has been determined by both their rates of hydrolysis in serum and by their kinetic characteristics with purified human serum enzymes (Vmax/Km). For the type Q isozyme the order is: sarin, soman, diazoxon, chlorpyrofos oxon and paraoxon. In contrast, the type R isozyme shows the completely reverse preference order. Heterozygous individuals, with various proportions of the two isozymes, can also be evaluated by taking into account the levels of each component isozyme. The latter analysis should be done if one isozyme is considerably more important than the other in protection against OP toxicity. Our finding that the Gulf War veterans developing neurotoxicity were either type R or low in their level of isozyme Q (as heterozygotes or homozygotes) suggests that they were exposed to a toxic compound or some OP that is preferentially inactivated by isozyme Q. The level of the latter isozyme seems to have been critical for protection in that environ-ment. Similar quantitative analyses may be useful inunderstanding "genetic predisposition" and individual variable response to environmental agents, based upon the toxic agent, its metabolicinactivation, and the level of polymorphic allozymes involved in that pathway.

1585 TISSUE PARTITIONING OF PARATHION AND PARAOXON AS ASSESSED BY EQUILIBRATION DIALYSIS.

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A valid physiologically based pharmacokinetic model requires an accurate estimation of partition coefficients which represent the relative distribution of the chemical of concern between the tissues and the blood at equilibrium. While a variety of approaches for estimating partition coefficients are available, determination of partition coefficients for non-volatile chemicals that can undergo metabolism within certain tissues can be problematic. In the present study the technique of equilibrium dialysis was evaluated for assessing the partitioning of the organophosphorus pesticide parathion (*O,O*-diethyl-*O-p*-nitrophenyl phosphorothioate) and its active metabolite paraoxon (*O,O*-diethyl-*O-p*-nitrophenyl phosphate) into various tissues in the mouse. Dialysis of several dilutions of tissue homogenates (brain, liver, heart/lung, fat, and skin) against buffer containing a variety of parathion and paraoxon concentrations (0.5 μM/ml-25 μM/ml) over time (0-150 min) indicated that equilibrium was achieved in about 60-100 min and 20-25 min for parathion and paraoxon respectively at 37°C. While high performance liquid chromatography analyses revealed limited metabolism of both compounds in certain tissues, this loss of parathion and paraoxon had a minimal effect in the partitioning of these compounds from buffer to homogenate as evidenced by achievement of equilibrium.These data suggest that the equilibrium dialysis is a valid approach for assessing tissue partitioning for parathion and paraoxon.

1586 KINETIC INTERACTIONS OF PARAOXON WITH RAT BRAIN ACETYLCHOLINESTERASE.

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Previous studies from this laboratory have indicated that the $appk_i$ for *in vitro* inhibition of mouse brain acetylcholinesterase by the organophosphate paraoxon (*O,O,*-diethyl-*O-p*-nitrophenyl phosphate) changed as a function of oxon concentrations below about 10 nM. Furthermore, studies utilizing mouse brain recombinant monomeric acetylcholinesterase showed similar findings, suggesting that attentuation of paraoxon's phosphorylation of acetylcholinesterase might occur through oxon binding to a peripheral binding site known to occur on acetylcholinesterase. In the present study, incubation of rat brain homogenate with various concentrations of paraoxon and 1% Triton X-100 resulted in an increase of acetylcholinesterase activity (as assessed by the Ellman method) by 20-100%, depending on the experimental conditions, compared to incubations without detergent. Application of a continuous systems computer model, which included the bimolecular inhibition rate constant *ki*, the first order rate constant for reactivation of phosphorylated acetylcholinesterase, paraoxon concentration, and acetylcholinesterase active site concentration, indicated that the presence of 1% Triton X-100 increased the concentration of active sites. These data suggest that changes in $appk_i$ as a function of oxon concentration might result, at least in part, from different pools of enzyme, with respect to paraoxon access, within brain homogenate.

1587 *IN VIVO* AND *IN VITRO* RECOVERY OF CHOLINESTERASE ACTIVITY IN THE RAT BRAIN CORTEX AFTER ORAL ADMINISTRATION WITH METHYL PARATHION.

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The recovery of brain cholinesterase activity *in vivo* and *in vitro*, after acute methyl parathion treatment, was studied in adult Sprague-Dawley female rats. Animals received a single dose of methyl parathion (2.5 mg/kg) by gavage. To examine the *in vivo* inhibition and recovery of cholinesterase activity, animals were sacrificed 30 minutes, 2 hours, 4 hours and 24 hours after methyl parathion administration. There was a 65% decrease in brain cortex cholinesterase activity 30 minutes after methyl parathion treatment compared to activity in brain of untreated rats. Brain cholinesterase activity recovered fully within 24 hours. For the *in vitro* study, animals were sacrificed 30 minutes after methyl parathion administration, and homogenates of the brain cortex were incubated at 4°C, 22°C, or 37°C for 0.25, 2, 4, 6, or 24 hours. At 37°C, cholinesterase activity showed a rapid recovery (from 35% of control values to 60% of control values) during the first 2 hours of incubation and a slower phase of recovery during the next 22 hours. At 22°C, cholinesterase activity recovered more gradually. In homogenates incubated at either 37°C or 22°C, cholinesterase activity was completely recovered by 24 hours. There was no significant recovery in the cholinesterase activity at 4°C. Cholinesterase activity of control animals did not change at any of the tested temperatures. These results indicate a rapid recovery of brain cholinesterase activity *in vivo* and *in vitro* following a single dose of methyl parathion given by gavage. Additional studies are required to identify the mechanisms contributing to enzyme recovery and to determine if they are tissue specific. (Supported by Grant U50/ATU486513-01.)

1588 EFFECTS OF SINGLE DERMAL ADMINISTRATION OF METHYL PARATHION ON BEHAVIOR AND BLOOD CHOLINESTERASE ACTIVITY IN THE RAT.

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Methyl parathion (O,O-dimethyl O-p-nitrophenyl phosphorothioate), an organophosphorous insecticide, has gained notoriety as a consequence of its illegal use in private homes across several states. As a result, at least 10,000 children were repeatedly exposed to methyl parathion by direct contact. As an initial step to identify potential effects of such exposure on behavior studies were performed to correlate blood cholinesterase activity with selected behaviors in adult Sprague-Dawley female rats. Animals were treated with a single dermal application of methyl parathion (6.25 mg/kg or 12.5 mg/kg), doses that correspond to about 10% and 20% of the dermal LD_{50}, respectively. Locomotor activity (open field test), neuromuscular coordination (rota-rod test), and blood cholinesterase activity were each examined before and 48 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks following treatment. Exposure to 12.5 mg/kg methyl parathion resulted in about 80%, 30%, and 20% inhibition of blood cholinesterase activity 48 hours, 1 week, and 2 weeks after treat-

ment. Cholinesterase activity had recovered fully after 3 weeks. Spontaneous locomotor activity, neuromuscular coordination and body weight were significantly decreased in rats exposed to 12.5 mg/kg methyl parathion after 48 hours and recovered after 1 week. Exposure to 6.25 mg/kg methyl parathion resulted in a 50% inhibition of cholinesterase activity 48 hours after the treatment that recovered within 1 week. Spontaneous locomotor activity, neuromuscular coordination and body weight were not affected. Thus, single dermal administration of relatively high doses of methyl parathion can cause both cholinesterase inhibition and behavioral changes. Repeated dermal exposure, as occurred in a domestic setting, may have even more profound and persistent effects. (Supported by Grant U50/ATU486513-01.)

1589 SIMULTANEOUS EXPOSURE TO ENDOSULFAN AND METHYL PARATHION CAUSES SPATIAL LEARNING DEFICITS IN ADULT RATS.

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Endosulfan and Methyl Parathion are widely used by mexican agricultors and chronic exposure to several pesticides is a frequent consequence of application. Simultaneous exposure to Endosulfan and Methyl Parathion may have effects at the nervous system because their targets are the GABAergic and Cholinergic systems, respectively. These systems are the main modulators of neuronal excitability in the cortex and hippocampus. We tested whether low-level, subchronic exposure of adult rats to Endosulfan and Methyl-Parathion disrupts learning in absence of symptoms of intoxication. Endosulfan (Agrevo, Mexico), Methyl-Parathion (Anajalsa, Mexico) and mixtures of both products were administered subcutaneosly to male Wistar rats. Dose-response curves were performed in groups of 3 animals to select NOAEL dosages. Afterwards, 5 groups of 8 animals received 10 days the following doses (NOAEL, mg/kg): saline, Endosulfan: 25, Methyl-Parathion: 2, 2 Endosulfan plus 25 Methyl-Parathion and 25 Endosulfan plus 1 Methyl-Parathion. Markers of hepatic or renal toxicity (alanine aminetransferase, creatinine, glomerular filtration) and acetilcholinesterase inhibition were analyzed in blood and urine. After 7 days of exposure the rats were tested in the water maze. Controls, Endosulfan and Methyl Parathion exposed rats decreased the escape latency from 90±31 s on the first day to 35±11 s on the seventh day. Exposure to mixtures caused significant differences from control and from single-exposure values. Mean escape latency on the first day was 166±14 s and remained 124±14 s on the last day. None of the groups presented hepatic or renal damage, nor weight loss. Acetilcholinesterase inhibition (40%) was the same for all three groups exposed to Methyl Parathion. These results demonstrate that combined exposure to Endosulfan and Methyl-Parathion disrupts spatial learning.

1590 IDENTIFICATION OF THE ACETYLCHOLINESTERASE (ACHE) ADDUCT, AFTER INHIBITION WITH (1*S*,3*S*)-ISOMALATHION, USING MATRIX-ASSISTED LASER DESORPTION/IONIZATION-MASS SPECTROMETRY (MALDI-MS).

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Previous work found a difference in postinhibitory kinetics for ACHE inhibited with (1*R*)- versus (1*S*)-stereoisomers of isomalathion. Moreover, a discrepancy in postinhibitory kinetics for ACHE inhibited with (1*S*,3*R*)- versus (1*S*,3*S*)-diastereomers led to the hypothesis of an unusual mechanism of inhibition and three possible modes of "rapid aging" for enzyme inhibited with (1*S*,3*S*)-isomalathion. This study sought to identify the adduct that renders ACHE refractory towards reactivation when inhibited with the (1*S*,3*S*)-isomer using peptide mass mapping with MALDI-MS, thereby elucidating the mechanisms of inhibition and aging. Electric eel ACHE was inhibited to <10% of control activity with (1*S*,3*S*)-isomalathion. An aliquot was removed and treated with pyridine-2-aldoxime methiodide to verify aging. Peptides from tryptic digests of control and treated ACHE were separated with reverse-phase HPLC. Eluted fractions and unseparated peptides were analyzed with MALDI-MS. Active-site peptide with covalently bound *O*-methyl phosphate was discovered in treated, but not control, samples. This adduct was strikingly different from the *O*-methyl-phosphorothiolate group predicted by the conventional inhibition mechanism. Identities of the modified active site peptide and adduct were confirmed using MALDI-MS in reflectron mode, and peaks representing loss of the adduct as phosphoric/phosphorous acid *O*-methyl ester were observed. The results support the hypothesis that inhibition of ACHE with (1*S*,3*S*)-isomalathion proceeds by the novel mechanism predicted by kinetics and specifies the mode of aging. (Supported by Dow AgroSciences, NIH ES07062, NIH RR00480 and NSF MCB9808372.)

1591 INHIBITORY AND POSTINHIBITORY KINETICS OF ELECTRIC EEL ACETYLCHOLINESTERASE (ACHE) WITH THE CHIRAL PROBE ISOMALATHION.

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Previous work has demonstrated that ACHE is stereoselectively inhibited by isomalathion and a difference exists in the postinhibitory kinetics of enzyme inhibited with (1*R*)- versus (1*S*)-isomers. This study sought to determine if isomalathion reacts with electric eel ACHE in a chiral manner and to assess the viability of the isomalathion stereoisomers as chiral probes of active site topology for ACHE. Electric eel ACHE was inhibited stereoselectively with the order of potency being (1*R*,3*R*) > (1*R*,3*S*) = (1*S*,3*R*) > (1*S*,3*S*). A 61-fold difference was found between the bimolecular rate constant (k_i) for most to least potent isomer. ACHE inhibited with the (1*R*)-isomers rapidly reactivated both spontaneously and in the presence of pyridine-2-aldoxime methiodide (2PAM) with $t_{1/2}$ less than 5 and 2 min, respectively. On the contrary, enzyme inhibited with the (1*S*)-isomers was refractory towards spontaneous and 2PAM-mediated reactivation with $t_{1/2}$ values equal to or greater than 114 and 55 min, respectively. Disparity in rates of reactivation between ACHE inactivated with (1*S*,3*R*)- versus (1*S*,3*S*)-isomalathion was observed, which suggests that a different ACHE inhibitory adduct is formed for each (1*S*)-isomer. Both the inhibitory and postinhibitory kinetic parameters of isomalathion with electric eel ACHE are strikingly different from values previously determined with ACHE from other species. These results demonstrate that electric eel ACHE reacts with isomalathion in a remarkably stereoselective manner and the stereoisomers of isomalathion can function as chiral probes of active site topology. (Supported by Dow AgroSciences, NIH ES07062 and NSF MCB9808372.)

1592 INTRACYTOPLASMIC CISTERNAL HYPERPLASIA IN CHICKEN EMBRYO BRAIN REAGGREGATE CULTURES FOLLOWING EXPOSURE TO THE NEUROTOXIC ORGANOPHOSPHATE PHENYL SALIGENIN PHOSPHATE (PSP).

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Ultrastructural studies of neurodegenerative lesions in organophosphorus ester-induced delayed neuropathy (OPIDN) revealed the presence of proliferation of intra-axonal vesicles and cisterns resembling smooth endoplasmic reticulum (SER) early in the degradative process (Bischoff, Acta Neuropath 9:158; Prineas, J Neuropath Exp Neurol 28:571). The significance of this event is unclear. We report replication of this putative OPIDN lesion in an *in vitro* system. Reaggregate cultures of chicken embryo brain were grown and maintained in a serum free medium (Funk et al., Tox Appl Pharm 124:149) for 28 days. These were exposed to the direct acting organophosphorus delayed neurotoxicant phenyl saligenin phosphate (PSP) at 10-5M for 24 hours and prepared for transmission electron microscopic examination. Cell necrosis was enhanced in treated cultures. Viable cells in these preparations often contained arrays of excess intracytoplasmic vesicles and cisternal structures resembling SER and the intra-axonal structures described by Bischoff and Prineas in early OPIDN. These cisterns were frequently associated with enhanced cytoskeletal filaments, and sometimes with lesions of sub-lethal injury (membranous bodies, lipid droplets). The presence of an *in vitro* system for induction of these cytoplasmic cisterns by a neurotoxic organophosphate provides an opportunity to investigate the possible pathogenetic significance of this alteration. (Supported by USEPA R825356.)

1593 ADENOSINE TRIPHOSPHATE (ATP) CONCENTRATION IN HEN SCIATIC NERVES AFFECTED WITH ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROPATHY (OPIDN).

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Continuing local supply of ATP is critical for anterograde and retrograde axonal transport and other nerve fiber functions (Goodrum and Morell, Neurotoxicology, 1992). To assess the role of alterations of ATP in OPIDN, we evaluated concentrations of that molecule in hen peripheral nerves following exposure to an organophosphate capable of inducing delayed neuropathy. To provide an appropriate model of OPIDN, a single 2.5 mg/kg dose of phenyl saligenin phosphate (PSP) was administered to adult hens. ATP concentrations were determined at days 2, 4, 7, and 14 post-dosing, from five segments (n=5/group) representing the entire length of the sciatic nerve. Initial effects of PSP dosing was seen in the most distal segment of sciatic nerve at day 2, when a transient ATP concentration increase (388±79 pmol/ml/mg vs. control value of 215±23, $p<0.05$) was noted. Subsequently, ATP concentration in this distal segment returned to normal. In all other nerve segments, ATP concentration decreased with time, and was significantly lower than control values on day 14 post-dosing ($p<0.05$). Changes in ATP concentration corresponded to incidence of decreased nerve conduction velocity, beginning 2 days post-dosing ($p<0.01$), and preceded development of clinical neuropathy and axonopathic lesions. These results suggest that variations in sciatic nerve ATP concentration are early events in the development of OPIDN.

1594 PROMOTION AND PROTECTION FROM AN ORGANOPHOSPHATE- INDUCED DELAYED POLYNEUROPATHY (OPIDP) BY MOLINATE: BIOCHEMICAL, CLINICAL AND MORPHOLOGICAL STUDIES.

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Certain esterase inhibitors, including thiocarbamates, carbamates, organophosphinates and sulfonyl fluorides, protect from OPIDP by inhibiting its target, neuropathy target esterase (NTE), when given before the neuropathic organophosphate. They promote OPIDP when given afterwards and this effect is associated with inhibition of an esterase called M200. Hens were treated with technical molinate (purity 95% w/w) (180 mg/kg sc) and 24 hours later inhibitions of brain and peripheral nerve NTE was were (mean±SD, n=3): 92±4 and 86±1% and brain and peripheral nerve M 200for brain and peripheral nerve NTE and 91±5 and 70±10%, for brain and peripheral nerve M200, respectively (mean±SD, n=3). No clinical neuropathy developed and morphological findings on day 21-23 after dosing were normal. Hens treated with DBDCVP (0.4 mg/kg sc) developed a mild OPIDP which was promoted by molinate (180 mg/kg sc), given 24 hours later: the clinical score (0-8 point scale, median, range, n) increased from 1 (0-1) (5) to 3 (2-5) (5) and axonal degeneration was more widespread in sciatic, tibial and peroneal nerves. Hens treated with a higher dose of DBDCVP (1 mg/kg sc) developed OPIDP with clinical score 3.5 (3-6) (4) and widespread axonal degeneration in peripheral nerves. This OPIDP was protected by molinate (180 mg/kg sc) pre-treatment 24 hours earlier [clinical score 0 (0-1) (5) and normal morphological findings]. These biochemical and clinical data are comparable with those previously obtained with a commercial formulation of molinate (Gardiman et al., Toxicologist, 1999, 48:466). Newly presented morphological data are consistent with the clinical and biochemical findings.

1595 AGE-RELATED INHIBITION OF FORSKOLIN-STIMULATED CAMP FORMATION BY CHLORPYRIFOS OXON IN RAT CORTICAL SLICES.

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Chlorpyrifos (CPF) is a common organophosphorus pesticide (OP). Young animals are generally more sensitive than adults to the acute toxicity of CPF. CPF elicits toxicity, following metabolic transformation to the reactive metabolite chlorpyrifos oxon (CPO), via inhibition of acetylcholinesterase (AChE). Recent studies have reported that CPO may also directly activate muscarinic receptors, specifically m2 and/or m4 subtypes coupled to inhibition of adenylyl cyclase. We studied the direct activation of muscarinic receptors and inhibition of adenylyl cyclase/cAMP formation by CPO in frontal cortex from 7, 21 and 90 day-old rats. Fresh cortical slices from each age group were pre-loaded with [3H]adenine and then incubated in the presence or absence of forskolin (FS, 20 micromolar) with CPO (1 nM-1 mM) or the muscarinic agonist oxotremorine (100 micromolar). Atropine was used to demonstrate muscarinic specificity. FS increased (about 100%) cAMP formation in cortical slices from all age groups. CPO inhibited FS-stimulated cAMP formation in a concentration-dependent manner (about 40% maximal), and this inhibitory action was partially sensitive to atropine. CPO was more potent at inhibiting FS-stimulated cAMP formation in neonatal compared to juvenile or adult tissues (IC50s: neonate=15 nM; juvenile=62 nM; adult=158 nM). Oxotremorine also inhibited FS-stimulated cAMP formation to a lesser degree (about 30%) but in a completely atropine-sensitive manner. We conclude that CPO can directly modulate cortical cAMP formation through muscarinic receptor-dependent and independent mechanisms and that the developing nervous system may be more sensitive to these non-cholinesterase actions. (Supported by U.S. EPA R 825811 and University of Louisiana Board of Regents Fund.)

1596 AGE PROFILE OF CARBOXYLESTERASE AND A-ESTERASE ACTIVITIES AND THEIR RELATIONSHIP TO THE TOXICITY OF CHLORPYRIFOS AND PARATHION IN RATS.

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Carboxylesterases (CEs) and A-esterases (AEs) are known to play an important role in the detoxification of organophosphorus insecticides (OPs). AEs (such as chlorpyrifos oxonase and paraoxonase) catalytically inactivate OP-toxic metabolites (oxons) while CEs act as molecular scavengers, stoichiometrically binding to OPs. Earlier studies indicate a relationship between OP toxicity and the developmental profile of these esterases, i.e., higher sensitivity and lower AE and CE activities were noted in younger animals. Little information exists, however on the relative OP sensitivity and biotransformation capacities of aged animals. In the present study, we investigated the relationship between toxicity of chlorpyrifos (CPF) and parathion (PS) with levels and OP-sensitivity of esterases in liver, plasma and lung of neonatal (7 day), juvenile (21 day), adult (3 month) and aged (24 month) rats. Acute maximum tolerated dosages for CPF and PS were estimated (CPF = 45, 127, 279, 279 mg/kg; PS = 2.1, 4.8, 18, 8 mg/kg, sc, in neonatal, juvenile, adult and aged rats, respectively). There were no significant age-related differences in *in vitro* sensitivity of CEs. Levels of esterases in neonatal and juvenile rats were significantly lower than in adult tissues, e.g. neonatal tissues showed 3-11, 5-12 and 2-9 fold less CE, CPF-oxonase and paraoxonase activity, respectively, compared to adults. Only plasma CE was significantly lower (about 50%) in aged compared to adult rats. Reduction in plasma CE may be important in the higher sensitivity of aged rats to PS. (Supported by NIEHS R01-09119 and University of Louisiana Board of Regents Fund.)

1597 AGE-RELATED EXPRESSION OF CORTICAL NICOTINIC AUTORECEPTOR FUNCTION: PARTIAL CHARACTERIZATION AND EVALUATION OF SENSITIVITY TO ORGANOPHOSPHORUS PESTICIDES.

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Nicotinic autoreceptors enhance acetylcholine (ACh) release in the adult central nervous system. We studied the expression of nicotinic autoreceptor function (NAF) in rat cortex from neonatal, juvenile, adult and aged rats. NAF was defined by the difference in the area under the curve (AUC) for [3H]ACh release in cortical synaptosomes stimulated in the presence of atropine (ATR, 100 nM) with buffer containing potassium (9 mM) with or without exogenous ACh (0.01 mM). NAF increased in an age-related manner to adulthood (AUC: 7-day, 6.9±6.0; 21-day, 44.4±5.5; 90-day, 196.3±37.0; 24-month, 172.9±51.5). The effects of ATR, epibatidine (EPI), nicotine (NIC), and mecamylamine (MEC) on NAF were studied. When ATR was omitted from the superfusion buffer, exogenous ACh did not stimulate further release. EPI and NIC, nicotinic receptor agonists (0-0.1mM), had no effect on release. The nicotinic antagonist MEC (0.1 mM), however, inhibited NAF by 79%. The *in vivo* effect of chlorpyrifos (CPF, 280 mg/kg, sc) on NAF in aged rats was also evaluated. Ninety-six hours after treatment with CPF, cortical cholinesterase activity was inhibited about 85% but minimal signs of acute toxicity were noted. NAF was substantially reduced (91%), however. From these preliminary data, high levels of CPF appear to markedly alter this modulatory neurochemical process in aged animals. Together, these data suggest that NAF is mediated by atypical nicotinic receptors (i.e., ATR, NIC, and EPI-resistant but MEC-sensitive) in an age-dependent manner and that this neuromodulatory

process may be sensitive to anticholinesterase exposures. (Supported by NIEHS R01ES09119 and University of Louisiana Board of Regents Fund.)

1598 EFFECTS OF CHRONIC DIETARY AND REPEATED HIGH-LEVEL SPIKE EXPOSURE TO CHLORPYRIFOS ON LEARNING IN RATS.

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The cholinesterase (ChE)-inhibiting compound chlorpyrifos (diethyl 3,5,6-trichloro-2-pyridyl phosphorothionate) is the most widely used organophosphorus (OP) insecticide in the United States. Whereas OPs including chlorpyrifos (CPF) have been associated with impaired cognitive and motor function in both animals and humans, the consequences of chronic dietary exposure to these insecticides has not been evaluated. The present study examined the effects of chronic dietary and repeated high-level spike exposure to CPF on learning a lever-press response and visual discrimination. Adult male Long-Evans rats were fed CPF in their diet at the rate of 0, 1, or 5 mg/kg body weight/day. In addition, half of each feeding group received an oral dose (spike) of CPF (45 mg/kg) every 2 months, resulting in 6 exposure groups: Control-Oil, Control-Spike, Low-Oil, Low-Spike, High-Oil and High-Spike. During initial training (autoshaping) the groups differed in their acquisition of the lever-press response, acquiring the response in the following order: High-Oil, High-Spike, Low-Oil and Control-Oil. The Control-Spike and Low-Spike groups did not learn the response to criterion in 3 sessions of training. There were no effects of CPF on learning a subsequent visual discrimination. These data suggest that repeated spike exposures to CPF may retard learning a motor response, whereas chronic exposure may accelerate it. These effects of CPF are consistent with previous work that linked motor impairment to changes in autoshaping. (This abstract does not necessarily reflect U.S. EPA policy.)

1599 *IN VITRO* AND *IN VIVO* EFFECTS OF CHLORPYRIFOS ON CARDIAC MUSCARINIC RECEPTORS.

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Organophosphorus (OP) insecticides elicit toxicity through inhibition of acetylcholinesterase (AChE). Typically, young animals are more susceptible than adults to the acute toxicity of OPs. Several studies have shown that there may be additional macromolecular targets for some OPs, including muscarinic receptors. Recent reports have focused on the possibility that CPF (or its active metabolite, chlorpyrifos oxon, CPO) can bind directly to muscarinic M2 receptors. The effects of CPO *in vitro* on cardiac muscarinic ([3H]oxotremorine, OXO) receptor binding were evaluated in neonatal (7 days) and adult (90 days) rats. We also compared age-related effects of CPF on AChE activity and OXO binding following either acute or repeated CPF exposures. CPO affected M2 receptor binding similarly in both age groups across all concentrations tested (about 30% maximal displacement at 100 nM). Following acute dosing with equitoxic (LD10) dosages similar levels of AChE inhibition were noted in both age groups but recovery was more rapid in neonates. OXO binding was reduced in neonates at all timepoints (4, 24 and 96 hrs after exposure) but was reduced only at the 24 hr timepoint in adults. With repeated dosing (0-7.5 mg/kg/day for 14 days), similar levels of AChE inhibition were noted between the age groups 4 hrs after the last treatment but more extensive reductions in OXO binding were noted in adult tissues: kinetic analysis suggested changes in affinity. The results suggest that OXO binding may be differentially modulated by CPF in neonates and adults following relatively similar levels of AChE inhibition depending on the nature of the exposures. (Supported by University of Louisiana Board of Regents Support Fund.)

1600 CIRCADIAN VARIATIONS IN CHLORPYRIFOS (CHP)-INDUCED ALTERATIONS IN CORE TEMPERATURE (T_C) AND MOTOR ACTIVITY (MA) IN THE RAT.

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Sponsor: R. C. MacPhail.

CHP is a heavily used organophosphate (OP) pesticide that causes an acute period of hypothermia followed by a delayed fever in the rat. Since T_c and MA display a natural circadian variation, the thermoregulatory and other toxic effects of OP pesticides could be influenced by the time of day of exposure. In this study, T_c and MA were monitored by radio telemetry in 90-day-old, male LE rats. The rats were gavaged with corn oil (n=12) or 30 mg/kg CHP (n=14) in the morning (9am) or in the afternoon (3pm). CHP induced a marked drop in T_c, reaching a nadir by ~4 hr after treatment. However, afternoon CHP exposure resulted in a greater decrease in T_c (-1.57°C) than the morning exposure (-0.4°C). A fever was observed the day after CHP exposure in rats dosed in the afternoon but not in the morning. MA measured the night after CHP exposure was depressed to a similar extent (40%) regardless of the time of administration. We have previously shown that the thermoregulatory response to cholinergic antagonists is greatest during the late afternoon. This may explain why CHP is more efficacious in altering T_c when administered in the afternoon. The nocturnal elevation in T_c of control animals is also thought to play a role in the thermoregulatory effects of CHP. The effects of CHP on MA appear to be independent from effects on T_c. These data suggest that time of day of exposure affects the toxicity of OP pesticides. (This abstract does not necessarily reflect US EPA policy.)

1601 PROLONGED ELEVATION IN BLOOD PRESSURE (BP) IN THE RAT EXPOSED TO CHLORPYRIFOS (CHP).

C. J. Gordon, B. K. Padnos and P. Becker. *USEPA, Research Triangle Park, NC.* Sponsor: R. C. MacPhail.

Organophosphate (OP) pesticides are likely to alter BP because brain stem centers for control of BP utilize cholinergic synapses and the irreversible inhibition of acetylcholinesterase activity by OP's causes cholinergic stimulation in the CNS. This study used radiotelemetric techniques to monitor systolic (S), diastolic (D), and mean (M) BP, pulse pressure (PP=S-D), heart rate (HR), core temperature (T_c), and motor activity (MA) in the male rat treated with the OP pesticide chlorpyrifos (CHP) at doses of 0, 5, 10, and 25 mg/kg (p.o.). 10 and 25 mg/kg CHP led to parallel elevations in S-BP, M-BP, and D-BP within 2 hr after dosing. M-BP increased 15 to 20 mmHg above controls and persisted throughout the night and into the next day. HR decreased slightly in the 25 mg/kg but not in the 10 mg/kg group. T_c was initially reduced by 25 mg/kg and then increased above controls the next day. MA was reduced by 25 but not 10 mg/kg CHP. PP was elevated by 2 to 4 mmHg for 40 hr after exposure to 10 and 25 mg/kg CHP. CHP-induced hypertension developed without changes in T_c, HR, and MA. The elevation in BP without an increase in HR suggests that CHP increases total peripheral resistance and alters the baroreflex control of BP. (This abstract does not necessarily reflect US EPA policy.)

1602 FIPRONIL MODULATION OF GABA(A) RECEPTORS IN RAT DORSAL ROOT GANGLION NEURONS.

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The insecticide fipronil is known to act on the GABA system. To elucidate the mechanism of fipronil interaction with the GABA system, patch clamp experiments were performed using rat dorsal root ganglion neurons in primary culture. Bath application of fipronil with an infrequent short GABA test puff was used to examine the onset of block of resting GABA receptor. Fipronil blocked the currents in a concentration-dependent manner with an IC_{50} of 1.3 μM. The onset of block was slow. For example, the time constant was ~60 sec in the presence of 10 μM fipronil. Co-application of 10 μM GABA and 10 μM fipronil was used to examine the onset of block of activated GABA receptor. In this experiment, fipronil was found to accelerate the current decay with a time constant of ~10 sec. These results suggest that the affinity of the GABA receptor for fipronil increases by channel opening. However, the steady-state fipronil block was independent of the degree of receptor activation. The suppressive action of fipronil on the GABA(A) receptor is deemed directly responsible for the symptoms of poisoning in animals characterized by hyperactivity and convulsions. (Supported by NIH grant NS14143 and a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.)

1603 EXAMINATION OF THE INCREASED OCCURRENCE OF DYSTOCIA AND STILLBIRTHS OBSERVED IN A REPRODUCTIVE BIOASSAY WITH AN EXPERIMENTAL CYANAMIDE (YRC 2894).

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Both an increase in stillbirths as well as signs of dystocia (difficult labor) were noted during the 1st generation of a 2-generation reproductive bioassay with [3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]-cyanamide (YRC 2894), a chemical agent with insecticidal properties. To develop data that could contribute to a mechanistic understanding of the YRC 2894-induced alterations in the two reproductive endpoints, a modified 1-generation reproductive study was designed, which included a pre-mating phase, a gestation phase, and a post-partum phase. General toxicological endpoints as well as non-routine hormonal and histo-pathological assessments were made at 9 weeks pre-mating, gestation Days 18 and 21, and at 2 days post-partum. Exposure was carried out via the diet at constant concentrations of either 0 or 800 ppm YRC 2894. It was hypothesized, insofar as dystocia has been associated with a hormonal inbalance in the rat, that a link may exist between chemical induction of metabolic activity in the liver and hormonal regulation of reproductive processes. Three specific areas of interest involved circulating levels of estrogen as well as the measurement of uterine and cervical levels of glutathione and prostaglandin. In addition to a moderate decline in body weight gain, YRC 2894 induced a marked increase in both absolute and relative liver weight. Centrilobular hepatocytomegaly, microsomal enzyme induction, and proliferation of the smooth endoplasmic reticulum (SER) were also noted in the liver; microscopic analysis of the pituitary, hypothalamus, ovary, and adrenal showed no change. Though prostaglandin and reduced glutathione measurements were unremarkable, elevations in circulating estradiol, progesterone, corticosterone, and luteinizing hormone were measured at either two or all three of the sampling phases of this study (pre-mating, gestation day 18 or 21, lactation day 2). Estrogen and progesterone receptor populations in the uterine cytosolic and nuclear fractions remained unchanged. All other hormones evaluated (T_4, T_3, TSH, oxytocin, prolactin and FSH) were unaltered. These data suggest that YRC 2894 is promoting, via its action at the liver, some form of interference with the capacity of the animals to regulate steroidal homeostasis.

1604 TOLERANCE AND SENSITIZATION TO WEEKLY NICOTINE EXPOSURES ON THE MOTOR ACTIVITY OF RATS.

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Motor activity was examined in adult female Long-Evans rats in a photocell device during daily (M-F) 30-min sessions. Following adaptation to the testing routine the rats were divided into six groups of eight that were designated to receive either nothing (non-injected control), saline vehicle, or 0.3, 0.6, 1.2 or 1.8 mg/kg nicotine, s.c. (in 1 ml/kg) five-min prior to a session. Nicotine (or vehicle) was administered at weekly intervals for four weeks. Initially, nicotine produced either no effect on horizontal activity or a decrease (30%) at the highest dose, but a substantial dose-related decrease in vertical activity. Weekly dosing produced tolerance to nicotine's effect on vertical activity, and increases (ca. 100%) in horizontal activity at all doses. Baseline activity levels determined throughout the period of weekly dosing did not differ between dose groups. Alterations in motor activity due to weekly nicotine were still obtained after three weeks without nicotine. Finally, when all rats received 0.3 mg/kg, prior controls (non-injected, vehicle-injected) displayed the same effect of nicotine obtained initially in the 0.3-mg/kg group, which differed from that obtained in all prior nicotine dose groups. Systematic changes in the behavioral effects of insecticidal nicotinic agonists may therefore occur with episodic (weekly) exposures. ()This abstract does not necessarily reflect US EPA policy.)

1605 AN APPROACH TO NEUROLOGICAL/BEHAVIORAL TESTING WITHIN THE LONG-TERM RODENT STUDY.

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A new component of recently adopted chronic toxicity test guidelines (OPPTS 870.4100 and 870.4300) is the enhanced neurological / behavioral assessment. We have developed an approach to accommodate the requirements of this assessment, without compromising our ability to achieve the core goals of the basic guideline chronic rat study. First, our approach assumes that both a chronic study (1-year) and a subchronic (3-month) neurotoxicity screen (OPPTS 870.6200) will be conducted as part of the development of a toxicological data package for a given chemical. Secondly, the chronic neurological assessment then takes the form of a modified subchronic assessment, consisting of key elements that can be practically incorporated into the study design of the chronic rat bioassay. Specifically, during months 6 and 12 of the 12-month chronic rat study, a representative sample (10 animals/sex/dose) is subjected to a functional observational battery which includes observations in the home cage, during handling, and in an open field, as well as various reflex/physiologic observations and measurements. Observations in the home cage and open field include assessments of motor activity. In addition, all animals (~25/sex/level) are observed weekly outside the home cage in a standard arena. At termination, 5 rats/sex/dose are selected for perfusion and collection of neural tissues for microscopic evaluation. Representative sections of the tissues from control and high dose animals are evaluated; tissues in which compound-related neuropathology is detected are examined at lower doses, as necessary, to establish a no-observed-effect level. Based on both scientific and practical considerations, landing foot splay, aerial righting, and motor activity evaluated in a maze have been excluded. Overall, this approach provides a sound neurotoxicological assessment that integrates well with the conduct of the chronic rat study.

1606 DETERMINATION OF HEXACHLOROBENZENE IN RODENT BLOOD AND TISSUE.

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Hexachlorobenzene (HCB) is an environmental contaminant that was selected for evaluation by NIEHS. To determine internal dose, methods were developed and validated to measure the concentration of HCB in blood, fat, liver and lung taken from rats used in toxicology studies. Tissue was extracted with a mixture of acetonitrile/hexane and the extracts analyzed by gas chromatography with either electron capture (ECD) or mass spectrometric (MSD) detection, depending upon the concentration of HCB. Fat samples required additional clean-up using alumina solid-phase extraction cartridges. Chromatographic conditions were developed to resolve HCB from the internal standard and matrix components. Separation was obtained using a 5% phenyl capillary column and a thermal gradient from 180°C to 275°C. ECD was used for blood samples with HCB concentrations from 5000 to 20 ng/mL and fat samples from 400 to 50 ng/g. MSD was used for blood samples with HCB concentrations from 400 to 1 ng/mL and liver and lung samples from 10,000 to1.25 ng/g. A method validation (MV) showed weighted (1/y) regression equations for data were linear, ($r > 0.99$) with relative errors < than 10%, blood (ECD); 12%, blood (MSD); 10%, lung; 11%, liver; and 9%, fat. Relative standard deviations for triplicate determinations at the lowest concentrations were 2.3%, blood; 1.2%, lung; 1.5%, liver; and 3.5%, fat. Limits of detection were 0.07 ng/mL, blood; 0.05 ng/g, lung and liver; and 5.2 ng/g, fat. These methods are suitable for the analysis of HCB in the range of 1 to 10,000 ppb in the tissues examined as demonstrated by the MV and recoveries >80% obtained upon analysis of spiked tissue samples.

1607 DETERMINATION OF HEPTACHLOR AND METABOLITES IN RODENT TISSUE.

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Heptachlor (HEP) is a persistent environmental contaminant that was selected for evaluation by NIEHS. To determine internal dose, methods were developed and validated to measure the concentration of HEP and its metabolites (epoxides a & b) in rat blood, milk, fat, liver, and whole pup samples taken from rats used in toxicology studies. Tissue was extracted with an acetonitrile/ethyl acetate mixture and extracts analyzed using gas chromatography with electron capture detection. Fat samples required additional clean-up using alumina solid-phase extraction cartridges. Chromatographic conditions were developed to resolve HEP, epoxide a, epoxide b, and internal standard from each other and matrix components. Separation was obtained using a 5% phenyl capillary column with a thermal gradient from 180°C to 275°C. A method validation (MV) showed weighted (1/y) regression equations for data for all three analytes were linear ($r > 0.99$) over the concentration ranges studied: 10 to 500 ng/mL, blood; 5 to 180 ng/mL, milk; 5 to 180 ng/g, whole pup;

25 to 150 ng/g, liver; and 100 to 1000 ng/g, fat with relative errors were less than 9%, blood; 15%, milk; 14%, pup; 14%, liver; and 16%, fat. Relative standard deviations for triplicate determinations at the lowest concentrations were 4.6%, blood; 7.5%, milk; 8.4%, pup; 14.9%, liver; and 9.9%, fat. Limits of detection were found to be 1 ng/mL, blood and milk; 1 ng/g, pup; 10 ng/g, liver; and 40 ng/g, fat. Confirmation of the identity of analytes found in study samples was provided by gas chromatography with mass spectrometric detection. These methods are suitable for the analysis of HEP in the range of 5 to 1000 ppb in the tissues examined as demonstrated by the MV and recoveries > 80% obtained upon analysis of spiked tissue samples.

1608 STATISTICAL PERFORMANCE OF MATHEMATICAL CORRECTIONS FOR THE INVERSE RELATIONSHIP BETWEEN QT INTERVAL AND HEART RATE.

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Pharmacodynamic prolongation of ventricular repolarization (QT Interval) has the potential liability of inducing polymorphic ventricular tachycardia (Torsades de Pointes), ventricular fibrillation and death. An inverse relationship exists between changes in heart rate and the QT interval, which poses a dilemma for studying drugs that affect heart rate. Of the various mathematical formulae that have been proposed as a correction for this problem, 4 of these ($aoRR^{(1/2)}$, $aoRR^{(1/3)}$, $aoRR^{(1/4)}$ and aolog RR) were evaluated in the present study. In addition, an analysis of covariance (ANCOVA) of QT interval was evaluated using heart rate as the covariate. The statistical power of each of these models was determined for a 10% change from control. This magnitude of difference is similar to that actually observed in a study of PNU-142093, a $5HT_{1D}$-serotonin receptor agonist known to cause QT Prolongation. Electrocardiograms were collected by radiotelemetry using a lead II configuration from unrestrained, cynomolgus monkeys. A statistical power of 80% is considered good. Of the 4 correction formulae, Fridericia's formulae $aoRR^{(1/3)}$ was the best, with a power of 62% and 95% for 4 and 8 animals/group, respectively. Bazett's formulae $aoRR^{(1/2)}$ was close to Fridericia's with a power of 60% and 95% for 4 and 8 animals/group, respectively. The quartic root $aoRR^{(1/4)}$ and log formulae aolog RR had lower power, between 45% and 55% for 4 animals/group and 85% and 92% for 8 animals/group, respectively. Based on these analyses, either Fridericia's or Bazett's formulae could provide a corrected QT interval for analysis of variance. However, the analysis of covariance of QT would provide the best model for detecting QT prolongation with a power of 72% and 99% for 4 and 8 animals/group, respectively.

1609 CARDIAC TOXICITY OF NITROGEN TETROXIDE: QTC PROLONGATION IN EXPOSED PATIENTS.

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Exposure to dinitrogen tetroxide (N2O4) has been shown to precipitate the onset of reactive airway dysfunction syndrome (RADS), and neurocognitive symptomatology. In addition, exposure to other airborne toxins and pollutants have been implicated in an increased number of ectopic cardiac beats as a result of parasympathetic neurotoxicity. This study examined additional cardiac effects after exposure to dinitrogen tetroxide; specifically, QTc prolongation in the Bogalusa, LA cohort of patients. Thirty-eight (38) patients who were exposed to the N2O4 gas cloud were graded symptomatically as mild, moderate, or severe by one of the investigators. Each subject underwent a 24-Hour Holter recording. The QT interval was measured for each normal ECG complex from the Holter recording. The QT interval data were then removed to an off-line computer for additional analysis. QTc interval measurements were calculated for each beat using Bazett's formula. Mean QTc intervals, the percentage of beats greater than 450 milliseconds (pQTc450), and the percentage of beats greater than 500 milliseconds (pQTc500) were calculated for each Holter recording. These data were compared to a group of 19 normal subjects who also underwent Holter recording. The overall cohort showed a significantly prolonged QTc interval calculation in each of the three measurements compared to normals. Moderately symptomatic patients had a significant increase in all of the calculations compared to mildly symptomatic. Severely symptomatic patients had significantly increased QTc calculations compared to moderately symptomatic. Patients exposed to N2-04 had a significant increase in the QTc interval measurements. Increases in the QTc interval have been linked to an increase in syncopal episodes and sudden cardiac death. In light of this information, patients exposed to toxins of this type should undergo additional studies, among those, 24-Hour Holter recordings to have their QTc interval measured.

1610 A GUINEA PIG ANIMAL MODEL FOR EVALUATION OF QTc INTERVAL PROLONGATION.

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The QT interval represents the entire duration of ventricular systole and is measured from the end of the QRS complex to the end of the T wave. The normal QT interval varies with heart rate and numerous formulae and data are available to obtain the corrected interval time (QTc). There is a brief moment in the middle of repolarization, during the T wave in the ECG, when some myocardial fibers are refractory and others are not. This has been called the vulnerable period. A variety of agents can prolong this vulnerable period and include but are not limited to: β adrenergic antagonists, carbamazepine, cyclic antidepressants, erythromycin, nonsedating antihistamines and antidysrhythmics. This activity can lead to peracute death. We have modified the Hey anesthetized guinea pig model to evaluate the potential of test articles to prolong the QTc interval. Five to nine week old, 481 - 790 gram guinea pigs/Elm(HA) were apportioned into four test groups, each group consisting of 6 animals. Guinea pigs were anesthetized, placed on a ventilator and a catheter inserted into the jugular vein for i.v. administration of test articles(TA). Subdermal pin electrodes were placed in each limb to record a Lead II electrocardiogram. Animals were stabilized for 30min. prior to administration of TA. TA were administered via i.v. infusion over a 30 min. period at a dose level of 5 mg/kg. The vehicle (10%DMSO/90% PEG 200) was administered over 30 min. at 1ml/kg. ECG recordings were made at -30, -20, -10 and 0 minutes prior to the start of the i.v. infusion, at one minute intervals during the infusions and 5, 10, 15, 20, 25 and 30 minutes after the termination of the infusions. Means from three predose recordings were used as baseline data and means from five post-dose recordings were used to determine TA effect. Heart rate was significantly decreased by the vehicle (-13.6%), norastemizole (-13.6%) and sotalol (-8.0%). These changes were not considered biologically relevant. Sotalol, vehicle and norastemizole did not have any effect on the QTc interval. As expected, the reference compound terfenadine significantly ($p<0.01$) prolonged the QTc interval (+27%) and reduced the heart rate (-40%).

1611 A CANINE ANIMAL MODEL FOR THE EVALUATION OF QTc - INTERVAL PROLONGATION.

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The QT interval represents the entire duration of ventricular systole and is measured from the end of the QRS complex to the end of the T wave. The normal QT interval varies with heart rate and numerous formulae and data are available to obtain the corrected interval (QTc). There is a brief moment in the middle of repolarization, during the T wave in the ECG, when some myocardial fibers are refractory and others are not. This has been called the vulnerable period. A variety of different agents can prolong this vulnerable period and include but are not limited to: β adrenergic antagonists, carbamazepine, cyclic antidepressants, erythromycin, nonsedating antihistamines and antidysrhythmics. This activity can lead to peracute death. We have developed an anesthetized canine model to evaluate the potential of a test article to prolong the QTc interval. Seven to thirteen month old dogs (Marshall) weighing between 6 - 11 kg were distributed equally between four test groups, with two males and two females in each group. Animals were given Brevital® (10 mg/kg, i.v.) as a preanesthetic and then maintained on a stable anesthetic plane with a mixture of oxygen and isoflurane (2.5-4%). Effects on the cardiovascular system were determined by changes in arterial blood pressure (systolic, diastolic and mean), heart rate and a Lead II electrocardiogram. Following stabilization, baseline values for each parameter were established over a minimum time of ten minutes. Vehicle or reference standard was administered as a continuous infusion over a fifteen minute period. Arterial blood pressure and electrocardiograms were evaluated every 60 sec. during infusion and at five minute intervals for a minimum of 30 minutes following the completion of the dose. Additional ECG recordings of AVR, AVL, AVF, I, II, III, RV_2 and V_{10} were performed prior to the initial treatment, at the conclusion of each dose and at 10, 20 and 30 min following dose administration completion. At doses of 4 mg/kg and 10 mg/kg, dl-Sotalol prolonged the QTc interval by 23% and 43%, respectively. At this high dose, dl-Sotalol slightly reduced systolic (16%), diastolic (27%) and mean (23%) arterial blood pressures and heart rate (37%). The QTc prolongation observed with dl-Sotalol is expected for this Class III antiarrhythmic agent.

1612 QUINOLONE QTC PROLONGATION AND HEMODYNAMICS IN AN ANESTHETIZED DOG MODEL.

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Sparfloxacin is a quinolone antibiotic known to prolong the QT interval corrected for heart rate (QTc) whereas ciprofloxacin and trovafloxacin have not been shown to prolong QTc. These compounds were tested in an anesthetized dog model to see if the model could differentiate between quinolones with demonstrated clinical effects on QTc versus those with no known clinical effects. The model consists of beagle dogs (6-15 kg, 6-12 months) which are artificially ventilated with a mixture of oxygen and isoflurane to maintain anesthesia. Dogs were cannulated in the femoral vein for compound administration and in the opposite vein for blood sample collection. The femoral artery on the blood collection side was cannulated for blood pressure measurement. Electrodes were installed for recording Lead II electrocardiogram. Blood pressure, heart rate and Lead II ECG were monitored. QT interval was determined from ECG recordings taken prior to dosing and at the end of each infusion and QTc was calculated as QT - [0.087 x (60/heart rate - 1)]. Ciprofloxacin, trovafloxacin mesylate (as alatrovafloxacin) and sparfloxacin were infused at 0, 5, 25 and 50 mg/kg as sequential infusions (2.5 ml/kg over 30 minutes each for a total of 10 ml/kg over 120 minutes). Four dogs were used per compound. Sparfloxacin prolonged QTc at all doses (16, 88 and 105% from vehicle) whereas ciprofloxacin and trovafloxacin only prolonged QTc at 50 mg/kg (19% from vehicle). Heart rate was decreased at all doses in all dogs with sparfloxacin, at 25 and 50 mg/kg with trovafloxacin in 3 dogs and ciprofloxacin in 2 dogs. Blood pressure was decreased at all doses in all dogs with trovafloxacin and at 25 and 50 mg/kg with ciprofloxacin in 3 dogs and sparfloxacin in 2 dogs. One sparfloxacin dog had an increase in blood pressure at all doses. These results demonstrate that sparfloxacin prolongs QTc in the dog similar to clinical observations. Ciprofloxacin and trovafloxacin that have no known clinical effects on QTc had significantly smaller changes in the dog.

1613 INFLUENCE OF THE RESPIRATORY SINUS ARRHYTHMIA ON THE QT INTERVAL LENGTH AND ON THE QT/RR RELATIONSHIP IN CONSCIOUS BEAGLE DOGS.

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The length of the QT interval is generally considered to be directly dependent on the length of the preceding cardiac cycle. In dogs, unlike man, respiratory sinus arrhythmia induces considerable variation in the length of contiguous cardiac cycles. This study was performed to describe the relationships between the instantaneous cardiac cycle length or average heart rates and the length of the QT interval. The relationships between the instantaneous cardiac cycle length (RR) or the average heart rate (Fm) and the length of the QT interval were studied from ECG records obtained in 36 male and 35 female beagle dogs aged six to seven months. These ECGs were performed as pretest routine investigations during toxicology studies. Quantification of the parameters was performed manually on hardcopy traces (50 mm/s; 1 cm/mV; 10 seconds) of lead II ECGs. Fm ranged from 66 to 186 beats per minute. The QT duration remained relatively constant despite great variation in contiguous RR intervals resulting from respiratory sinus arrhythmia. There were no differences between male and female animals. We found that for this data, derived from normal non-treated animals, Van de Water's approximation formula gave the best correction of QT as a function of average heart rate and Bazett's formula was the least suitable.

1614 EFFECTS OF COCAINE ALONE OR COCAINE PLUS GBR-12909 ON CARDIOVASCULAR HEMODYNAMIC AND ELECTROGRAPHIC PARAMETERS IN CONSCIOUS UNRESTRAINED BEAGLES.

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To eliminate anesthesia, restraint and investigator presence as confounding factors in an assessment of potential cardiovascular interactions of cocaine and other modulators of the sympathetic nervous system, we conducted studies in chronically instrumented, conscious, unrestrained Beagles in dog runs using telemetry (ITS®) and a programmable infusion pump (Cadd-Prizm®). Eight dogs were instrumented with aortic and left ventricular pressure transducers (Konigsberg®), flow probes (Triton®) on the pulmonary artery to monitor cardiac output and ECG leads (base/apex placement) to obtain a lead II electrocardiogram. Animals pretreated with 0, 1 or 10 mg/kg of GBR-12909 given orally in gelatin capsules were dosed 3 hrs later with 0.56, 1.7 or 3.0 mg/kg of cocaine delivered through a vascular access port with the programmable infusion pump. Cocaine alone dose-dependently increased systolic, mean and diastolic blood pressures, heart rate, cardiac output, and left ventricular contractility (dP/dt), peak and end diastolic pressures. In contrast, increases in total peripheral resistance calculated as the quotient of systemic pressure and cardiac output were less with the high dose than with the middle and low doses, due to a disproportionate increase in flow with the high dose. GBR-12909 (1 or 10 mg/kg) did not alter any of these hemodynamic parameters either before or after the low and middle doses of cocaine. The effects of cocaine on electrographic parameters were slight and were generally dose dependent. They included a decrease in the PR interval, an increase in QRS duration, an increase in the corrected QT interval (Bazett's), and were not altered by pretreatment with GBR-12909. In conclusion, this study revealed a divergence in the pressure-flow relationship with the high dose of cocaine that was not previously seen in anesthetized or sling-restrained dogs. Further, 1 and 10 mg/kg of GBR-12909 given orally had no effect on any of the cardiovascular parameters either before of after cocaine. (Supported by NIDA contract N01DA-6-8068.)

1615 A NEW PERSPECTIVE FOR IDENTIFYING POTENTIAL CARDIAC SENSITIZERS.

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The purpose of this investigation is to construct a mathematical model for predicting the onset of cardiac sensitization using ouabain, a digitalis glycoside known to provoke ventricular arrhythmia. Both the dog (conscious and anesthetized) and the pig (anesthetized) were used as animal models. Physiologic and electrocardiographic measurements were taken during the baseline period and after each dose of ouabain. The model was based on logistic regression because of the binary nature of the data. Logistic regression allows the observer to predict the probability of developing an arrhythmia for a given value of the observed parameter. Five parameters were significant predictors of arrhythmia in dog, and three were significant in the swine. The study demonstrates that a mathematical model can be constructed to predict the onset of ouabain induced arrhythmia, in both the dog and the swine, and that there are similarities in the two animal models. However, efforts to reduce the number of parameters to a single term showed colinearity among simple pair wise combinations. At the moment the data suggest the QT interval to be the most promising of all the parameters for predicting cardiac sensitization regardless of species.

1616 NORMAL ELECTROCARDIOGRAPHIC VARIANTS IN UNANESTHETIZED MACAQUES.

J. W. Richig. *Anilab, Inc., Englishtown, NJ.* Sponsor: D. M. Schiavo.

Nonhuman primates are commonly used in toxicology and biomedical research. The electrocardiogram is one of the most important evaluations performed on macaques (cynomolgus or rhesus monkeys) prior to and during drug safety evaluation studies. Electrocardiograms are performed in order to ascertain what, if any, effects pharmaceutical agents may have on cardiac conduction. However, prior to such studies electrocardiographic evaluations are performed to ascertain whether a monkey is electrocardiographically suitable for use on a study. It is through these pre-study screens that it has come to light that normal variants exist in these monkeys. These normal variants are listed as follows: sinus arrhythmia, an occasional atrial or ventricular premature complex, low-voltage QRS complexes, right bundle branch block (incomplete or complete) and tall T waves. In addition, these variants can be found in combinations of two or more together. Monkeys exhibiting these variants have been found to be clinically normal and healthy, and thereby suitable for use on study.

1617 HEART RATE VARIABILITY IN HEALTHY- AND MONOCROTALINE-TREATED RATS DURING EXPOSURE TO LOWERED AMBIENT OXYGEN.

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Heart Rate Variability (HRV) is reported to predict adverse clinical cardiac outcomes. While HRV has been used in a number of recent animal studies, little is known about its toxicological significance. The present study describes initial observations of HRV indices in healthy rats (male, 60-day, Sprague-Dawley) and rats with monocrotaline (MCT; 60 mg/kg)-induced pulmonary hypertension during both normoxic and hypoxic conditions. Frequency domain indices were defined as follows: very low frequency power (VLF) = 0.0033-0.2 Hz; low frequency power (LF) = 0.2-0.6 Hz; high frequency power (HF) = 0.6-3.0 Hz; total power (TP) = 0.0-3.0 Hz. Time domain indices and fractal domain approximations were also determined. Animals were exposed to decreasing ambient oxygen (O_2) levels (16.75% and 12.5%, each for 5 min periods) while anesthetized with urethane. Continuous electrocardiograms (ECG) from each rat were recorded to magnetic tape; RR-interval series were extracted from the recorded ECG signal by computer and analyzed by a modified fast Fourier transform algorithm. Conscious MCT-treated rats displayed significantly lowered HF and TP values compared to saline-treated rats. Decreased O_2 tended to cause an increase in VLF and LF domains in all rats under anesthesia, while TP and HF values decreased. A few MCT-treated rats displayed severe decreases in HF during 16.75% O_2, while the response to 16.75% O_2 in healthy animals was mild or absent. TP consistently decreased during hypoxic exposures in healthy and MCT-treated rats. These initial findings demonstrate HRV alterations caused by experimental stressors, as well as by an existing cardiopulmonary pathology. (This abstract was funded in part by EPA/UNC T901915 research training grant and does not represent EPA policy.)

1618 A CARDIOVASCULAR RADIOTELEMETRY STUDY OF A SUBCUTANEOUS DOSE OF APOMORPHINE HCL IN BEAGLE DOGS.

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Apomorphine is a dopamine D1/D2 receptor agonist with a subcutaneous T_{max} ~ 15 to 30 min. In the current study, 5 male beagle dogs were surgically instrumented with implantable telemetry for monitoring ECG, BP, and/or to measure left ventricular end diastolic pressure (LVEP). All dogs were injected with vehicle once daily for 3 days followed by 0.04 mg/kg of apomorphine for 3 days, then a washout period, followed by 0.04 mg/kg of apomorphine preceded by 500 mcg/kg of Domperidone® (a peripheral D2 antagonist), then a washout period followed by 500 mcg/kg Domperidone® alone. Cardiovascular evaluations included: BP, HR and ECG. To evaluate the effect on baroreceptor function and vagal tone, HR was calculated from beat to beat ECGs during head-up and head-down maneuvers. Vomiting and lethargy resulted only with apomorphine. Following apomorphine treatment, the head-up maneuver produced a significant increase in the HR relative to control (i.e., no inhibition of sympathetic tone). The head-down maneuver resulted in decreased HR which was not different from control (i.e. no potentiation of vagal tone). BP values were significantly lower ($p < 0.01$) following apomorphine compared to vehicle. The maximum decline in BP over the first 3 hours was 10 mmHg. The decrease in BP was not associated with a change in HR or LVEP (one animal). Domperidone® reversed all clinical and cardiovascular changes. The decline in BP was due to peripheral D2-mediated increased venous capacitance or arteriolar dilatation.

1619 AN ACUTE INTRAVENOUS STUDY OF A LONG HALF LIFE FORM OF LEPTIN IN THE CONSCIOUS BEAGLE DOG.

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Leptin is a protein hormone, produced by adipose tissue, involved in the regulation of body fat. Recombinant leptin administered to normal animals results in rapid loss of body weight primarily due to body fat reduction. Additionally, leptin has been reported to effect other physiological parameters, including the cardiovascular system. This study was conducted in conscious beagle dogs to evaluate the acute toxicity and cardiovascular effects of a long half life form of leptin following a single bolus IV injection. All animals dosed at 5, 25 and 100 mg/kg experienced a dose-related loss in body weight for 14 days following treatment. Males dosed at 100 mg/kg sustained this body weight loss for 28 days (28% of baseline). Additionally, hemodynamic parameters were evaluated continuously for 48 hours following treatment. In the majority of animals, including controls, heart rate tended to increase immediately following treatment, returning to baseline ranges within ~ 40 minutes. Blood pressure changes were more variable, with mild increases usually coinciding with the increased heart rate. Clinical signs, indicative of a Type-1 hypersensitivity reaction (i.e. red skin, swollen extremities) were seen in all animals, including controls, within 20 minutes of treatment. The formulation for the long half life form of leptin contains small amounts of Tween 20 (0.01%). To investigate if the clinical signs and hemodynamic changes were related to the Tween concentrations, dogs received either 0.01 or 0.1% Tween 20 in PBS by IV bolus. Both doses of Tween produced clinical signs and hemodynamic changes as expected, with the effects at 0.1% more pronounced. In summary, this long half life form of leptin was well tolerated in the dog at doses up to 100 mg/kg. An expected and reversible loss in body weight was seen, as well as slight hemodynamic changes related to Tween 20.

1620 THE USE OF PROPOFOL AND ALFENTANIL AS A NOVEL ANAESTHETIC REGIME FOR CONDUCTING CARDIOVASCULAR AND RESPIRATORY SAFETY PHARMACOLOGY STUDIES IN THE ANAESTHETISED BEAGLE DOG.

I. Hallett, C. Fuller and A. Esmail. *Covance Laboratories Ltd, Harrogate, United Kingdom.* Sponsor: J. Norris.

An assessment of the pharmacological effects of novel chemical entities (NCEs) on the cardiovascular and respiratory system of a non-rodent species is considered as an essential pre-clinical test prior to Phase 1 clinical trials. Furthermore, this is recognised as an important test in the Japanese Guidelines for nonclinical testing of drugs. This test is most commonly performed in the anaesthetised laboratory beagle dog which can be instrumented under anaesthesia for monitoring arterial blood pressure, left ventricular pressure, systemic blood flow, ECG, and respiratory parameters. However, many of the anaesthetic regimes currently employed for this purpose have profound effects on many of the parameters measured, or may effect the ability of the anaesthetised preparation to respond to any pharmacological challenge. Furthermore, some anaesthetics may depress respiration to such an extent that it becomes neccessary to ventilate the animals artificially, thus preventing the measurement of any respiratory parameters. At Covance, Harrogate, we have devised an intravenous anaesthetic regime using a mixture of the anaesthetic, propofol, and the sedative, alfentanil, which can be administered by continuous infusion. Using this anaesthetic regime gives us a stable preparation, capable of breathing spontaneously, to which drugs can be administered over the course of several hours while haemodynamic and respiratory recordings are taken. Using a continuous intravenous infusion of propofol at a rate of 0.14 to 0.18 mg/kg/min, supplemented by an intravenous infusion of alfentanil of 2 to 3 mg/kg/min, in a group of 4 dogs we were able to maintain the group mean arterial blood pressure between 79 to 86 mmHg, the heart rate between 72 to 79 bpm, mean femoral artery blood flow between 110 to 126 mL/min, respiratory rate between 22 to 31 cycles/min, and tidal volume between 37 to 54 mL over 150 minutes of continuous anaesthesia.

1621 THE USE OF KETAMINE AND PROPOFOL AS A NOVEL ANAESTHETIC REGIME FOR CONDUCTING CARDIOVASCULAR AND REPIRATORY SAFETY PHARMACOLOGY STUDIES IN THE ANAESTHETISED CYNOMOLGUS MONKEY.

A. Esmail, C. Fuller, H. Prior and I. Hallett. *Covance Laboratories, Harrogate, United Kingdom.* Sponsor: J. Norris.

An assessment of the pharmacological effects of new chemical entities (NCEs) on cardiovascular and respiratory system of a non-rodent species is an essential pre-clinical test prior to Phase 1 clinical trials. The test is commonly performed in the laboratory beagle. However, the non-human primate is increasingly used as an alternative species, especially when the metabolism of the test compound in the primate is more comparable to that in man. Some anaesthetic regimes currently used have profound effects on many of the parameters measured, or may depress respiration necessitating artificial ventilation. We have devised an intravenous anaesthetic regime, suitable for cardio-

vascular and respiratory measurements following test compounds in anaesthetised cynomolgus monkey. Cynomolgus monkeys, of either sex, were pretreated with ketamine (21-23 mg/kg). Anaesthesia was induced with propofol (5-8 mg/kg iv) and maintained with a continuous infusion of propofol (0.42-0.48 mg/kg/min). Animals were instrumented to measure cardiovascular and respiratory parameters. Using this model, we were able to obtain stable anaesthesia for several hours. Typical values and ranges for the cardiovascular and respiratory parameters were as follows: Mean Arterial Blood Pressure 75-82 mmHg, heart rate 174-184 beats/min, RR interval of the ECG 330-346 ms, QT interval 205-213 ms, QTc interval 354-361 ms, Respiratory rate 54-60 cycles/min, Tidal volume 5.9-6.2 ml, Minute Volume 320-364 ml, Peak inspiratory flow 26-30 ml/s, peak expiratory flow 26-29 ml/s. This regime gives us a stable preparation, capable of breathing spontaneously, to which drugs can be administered over the course of several hours while haemodynamic and respiratory recordings are taken.

1622 CARDIOVASCULAR EFFECTS OF FUMONISIN B_1 IN MILK-FED CALVES.

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Fumonisins are a group of mycotoxins produced primarily by *Fusarium verticillioides*, a fungus that commonly contaminates corn. Fumonisin ingestion leads to altered sphingolipid biosynthesis and dose-dependent increases in serum and tissue sphinganine and sphingosine concentrations in all species. Fumonisin B_1 (1 mg/kg, IV, daily) caused lethal pulmonary edema in pigs within 4 days, probably due to sphingosine-mediated left heart failure. Pigs died when plasma sphinganine and sphingosine concentrations were ≥2.2 and 1.0 μM/L, respectively. We therefore examined whether fumonisin B_1 induced similar cardiovascular effects in calves. Ten milk-fed male Holstein calves aged 7 to 14 days were instrumented to obtain blood and cardiovascular measurements. Treated calves (n = 5) were administered fumonisin B_1 at 1 mg/kg, IV, daily for 7 days and controls (n = 5) were administered 10 ml 0.9% NaCl, IV, daily. All calves were euthanized on day 7. In treated calves, serum sphinganine concentration was increased from day 3 onwards (day 7, 0.158±0.129 μM/L; baseline 0.006±0.002 μM/L) and serum sphingosine concentration was mildly increased from day 3 onwards (day 7, 0.029±0.022 μM/L; baseline, 0.014±0.008 μM/L). Heart rate, cardiac output, stroke volume, mean arterial pressure, mean pulmonary artery pressure, pulmonary artery wedge pressure, central venous pressure, plasma volume, base-apex electrocardiogram, arterial P_{O2}, and systemic oxygen delivery were unchanged in treated and control calves. Fumonisin treated calves developed a metabolic acidosis (arterial blood pH, 7.27±0.11; base excess, -9.1±7.6 mEq/L), but all survived for 7 days. We conclude that calves are more resistant to fumonisin B_1 toxicity than pigs. This resistance is probably due to an increased ability of the calf to metabolize or excrete sphinganine and sphingosine. Alternatively, fumonisin may not inhibit sphingolipid biosynthesis to the same extent in calves as it does in pigs.

1623 ABCIXIMAB PHARMACODYNAMICS ARE UNAFFECTED BY ANTECEDENT THERAPY WITH OTHER GPIIB/IIIA ANTAGONISTS IN NON-HUMAN PRIMATES.

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Tirofiban or eptifibatide, small molecule GPIIb/IIIa antagonists, are used for the medical stabilization of patients with unstable angina. If an unplanned percutaneous coronary intervention is necessary, conversion from tirofiban or eptifibatide to abciximab, a monoclonal antibody Fab fragment that blocks GPIIb/IIIa and alphav/beta3, may be desirable. The purpose of this study was to determine if the pharmacodynamics, pharmacokinetics and safety profile of abciximab were affected by prior treatment with either tirofiban or eptifibatide in non-human primates. Cynomolgus monkeys (either sex) were surgically instrumented with venous vascular access ports for continuous infusions and blood sampling prior to study start. At the time of study, animals were administered an intravenous bolus plus 18 hour infusion of saline (n=4), tirofiban (n=4) or eptifibatide (n=4). At the end of the 18 hour infusion, a bolus plus 12 hour infusion of abciximab was immediately initiated. Inhibition of platelet aggregation was measured using the bedside Rapid Platelet Function Analyzer (RPFA, Accumetrics) at a variety of time points during and after both infusions. In addition, GPIIb/IIIa receptor occupancy and abciximab pharmacokinetics were also measured. Abciximab pharmacodynamics and pharmacokinetics were not affected either during or after abciximab administration by prior treatment with tirofiban or eptifibatide. In addition, there were no unexpected adverse findings associated with the crossover therapy. Thus, abciximab retains a predictable magnitude and duration of effect despite antecedent therapy with small molecule GPIIb/IIIa antagonists.

1624 THE SYSTEMIC EFFECTS OF RHVEGF WHEN ADMINISTERED TO MALE CYNOMOLGUS MONKEYS BY INTRAVENOUS INFUSION (IV) OR INTRAMUSCULAR INJECTION (IM).

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Recombinant human vascular endothelial growth factor (rhVEGF) is a basic, heparin-binding, homodimeric protein paracrine growth factor that is secreted by a number of tissues and acts to promote angiogenesis. rhVEGF has been proposed as a therapeutic for coronary artery disease and peripheral arterial disease. The two studies discussed here were conducted to characterize systemic toxicity in monkeys from high dose IV infusion or IM injection. Both studies employed radiotelemetry, and the maximum tolerated dose (MTD) in each study was defined as a 20%-50% drop in mean arterial pressure (MAP) from baseline MAP. In the IV study, four monkeys were infused for 4 hours at various doses to determine the MTD. Once determined, two monkeys were dosed at the MTD (0.54 mg/kg/min) to characterize PK parameters. In a separate IM study, three monkeys were given escalating doses of rhVEGF into a caudal thigh muscle, with at least 72 hours between doses, to the MTD (1440 mg/kg). To determine PK parameters, an additional IM dose was administered 72 hours later at the MTD. Rapid and significant decreases in MAP and increases in heart rate were observed at the MTDs in both studies. While the PK profiles of both studies showed similarities, the IM study produced additional clinical signs of scrotal swelling and facial edema. It is unclear if these differences were route-specific or due to differences in study design. Tolerance of rhVEGF for both studies at the MTD was limited by the substantial drop in MAP.

1625 A TIME COURSE STUDY OF GENE EXPRESSION PATTERNS AND PATHOLOGY IN THE HEARTS OF RATS TREATED WITH DOXORUBICIN HYDROCHLORIDE.

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Doxorubicin-induced cardiomyopathy is a limiting factor in the use of this anthracycline anti-neoplastic agent in cancer patients. Doxorubicin dose levels might be increased and its efficacy improved if the mechanisms of doxorubicin toxicity were more fully understood and if protective measures could be established to protect the heart during treatment. The increasing use of gene expression array technology may permit the detection of underlying mechanisms susceptible to manipulation. This study was undertaken to establish the gene expression patterns induced by a weekly toxic dose of doxorubicin hydrochloride (4mg/kg ip) in the rat heart and to follow the sequential changes of gene expression that occur leading to the eventual morphologic endpoint of dilated cardiomyopathy. Clontech Atlas and Stress Arrays (~700 genes) were used to evaluate mRNA expression from whole hearts from animals treated for 2, 7 and 43 days. Gene expression on d2 after initiation of treatment indicated an early stress pattern consistent with activation of hypertrophic and insulin signaling pathways(MAPK/ERK kinase kinase), increased mitochondrial protein trafficking (HSP60), increases in protein degradation (polyubiquitin) and in genes associated with chromosome rearrangements and DNA damage (helicase II and GADD45). By day 43, the pattern of gene expression had changed and reflected a huge down regulation cAMP-response element binding protein 1 and thus of cyclic AMP responsive genes and an increase in p53 responsive genes (waf 1) and DNA repair genes (homologues to RAD genes of yeast). Histologic samples were taken from all organs and clinical and histopathological endpoints were compared to the accompanying patterns of gene expression. Study design and knowledge of the physiologic and pathologic status of the whole animal are essential when interpreting such patterns of gene expression.

1626 IMMUNOGOLD LOCALIZATION OF METALLOTHIONEIN AND SUBCELLULAR PROTECTION AGAINST DOXORUBICIN TOXICITY IN TRANSGENIC MOUSE HEARTS.

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Previous studies using a cardiac-specific metallothionein (MT)-overexpressing transgenic mouse model have demonstrated that MT provides protection against cardiotoxicity of doxorubicin (DOX). The present study was undertaken to examine the relationship between subcellular distribution of MT and its protective action among organelles in the heart of transgenic mice. Transgenic mice containing high levels of cardiac MT (about 40 fold) and non-transgenic littermate controls were treated with DOX in a dose of 15 μg/kg via ip. Four days after the treatment, animals were anesthetized and heart tissues were removed for analyses. Electron microscopy and immunogold staining of the transgenic myocardial tissues after incubation with monoclonal mouse anti-MT antibody identified that MT was localized in nucleus, myofibers and cytoplasm. DOX induced cytoplasmic vacuolization and severe damages in myofilaments and nucleus in non-transgenic myocardium. The most predominant injury, however, ocurred in mitochondria, including striking size and shape changes, focal swelling and loss of cristae. These damages were rarely found in the DOX-treated transgenic myocardium. In particular, the internal morphology of mitochondria maintained essentially normal, although MT was not localized in this compartment. This study thus demonstrates that while subcellularly localized action of MT is important, it also plays a significant role in protection against oxidative injury by DOX in remote organelles. (Supported in part by NIH grants CA68125 and HL59225, American Heart Association EI Award (9640091N), and Jewish Hospital Foundation, Louisville, Kentucky.)

1627 PREVENTION OF VERAPAMIL-INDUCED MYOCARDIAL DEPRESSION *VIA* AN EXPERIMENTAL VERAPAMIL-SPECIFIC IGG.

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Verapamil-related fatalities have steadily increased resulting in approximately 30 deaths reported to the American Asssociation of Poison Control Centers in 1998. Current treatment focuses on reversal of post-binding effects of verapamil. Our laboratory has produced an ovine verapamil-specific IgG (VSIgG) that could be used as a therapeutic aid in preventing verapamil toxicity. Determine the dose response of verapamil toxicity towards left ventricular papillary muscles isolated from rats and the preventative effect of verapamil-specific IgG towards verapamil toxicity in a premixed preparation. The study was approved by the Institutional Animal Care and Use Committee. Following carbon dioxide narcosis, left ventricular papillary muscles were dissected from the hearts of male Sprague-Dawley rats (350-410g) isolated in 30 mM 2,3-butanedione monoxime-Tyrode buffer. Muscles were suspended in a oxygen perfused 30 ml Tyrode buffer bath at 37.5 degrees Celsius, stimulated at 1 Hz, and equilibrated for 90 minutes. Baseline tension was increased until Lmax was reached; isometric forces were monitored using a force displacement transducer; changes in developed tension were expressed as percent reduction from initial developed force at 15 minutes post-treatment. Concentrations of verapamil for the dose response experiment ranged from 31-1020 nM. In the VSIgG trial phase 0.1 g simethicone was added to each bath to prevent protein foaming and the following treatments were used: 510 nM verapamil, 510 nM verapamil + 1x VSIgG, and 510 nM verapamil + 1x non-specific ovine IgG. Results: Based on the calculated dose-response curve a verapamil concentration of 510 nM reduced developed tension by a mean of 53.7% (range 50-56%). VSIgG + verapamil had a mean reduction of 15.8% (+13.3%) compared with the verapamil-only mean of 31.1% (+11.2%). No statistically significant difference was shown between the non-specific ovinve IgG + verapamil and the verapamil-only treatments. The use of rat left ventricular papillary muscles proved a useful model in studying the effect of VSIgG in preventing verapamil toxicity *ex vivo*. With the production of more VSIgG further experiments could be performed to test the ability of VSIgG to reverse the effects of muscles already intoxicated with verapamil in the same model.

1628 ANTI-APOPTOTIC EFFECT OF METALLOTHIONEIN CONTRIBUTES TO ITS INHIBITION OF ISCHEMIA/REPERFUSION-INDUCED INFARCTION IN MOUSE HEARTS.

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Using a Langendorff-perfused mouse heart model, we have previously demonstrated that metallothionein (MT), a potent antioxidant, functions in cardiac protection against ischemia/reperfusion-induced infarction. The present study was undertaken to investigate possible mechanisms by which MT functions in this cardioprotection. Because apoptosis is a constant feature of myocardial damage following ischemia/reperfusion and reactive oxygen radicals are inducers of apoptosis, this study focused on effects of MT on myocardial apoptosis induced by ischemia/reperfusion. Transgenic mice with high levels of cardiac MT (40 fold) and non-transgenic controls were subjected to a left anterior descending coronary artery occlusion for 30 min followed by reperfusion. Four hrs after reperfusion, the area at risk and infarct size were assessed by Evens blue dye and triphenyltetrazolium chloride staining with computerized planimetry using an image analysis software program. Heart tissue within the area of risk was also collected and processed for a TUNEL assay to determine myocardial apoptosis. In addition, immunohistochemical method was applied to detect caspase-3 activities within the area of risk. Mitochondrial release of cytochrome c was examined by Western blot after mitochondria were separated from cytosol. Ischemia/reperfusion markedly induced infarction in non-transgenic myocardium. This occurrence was significantly suppressed in the transgenic myocardium. Corresponding to this effect, ischemia/reperfusion caused remarkable apoptosis within the area of risk in non-transgenic myocardium, which was also inhibited in the transgenic myocardium. The anti-apoptotic effect of MT correlated with its inhibition of mitochondrial release of cytochrome c and activation of caspase-3. These results thus demonstrate that MT inhibition of ischemia/reperfusion-induced infarction can at least partially be attributed to its anti-apoptotic effect through inhibition of cytochrome c-mediated pathway. (Supported in part by NIH grants CA68125 and HL59225, American Heart Association EI Award (9640091N), and Jewish Hospital Foundation, Louisville, Kentucky.)

1629 PHOSPHODIESTERASE TYPE III (PDE III) INHIBITOR INDUCES VASCULITIS PRECEDED BY APOPTOSIS IN MESENTERIC VESSELS AND LYMPHOID TISSUES IN RATS.

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PDE inhibitors have been shown to cause vasculitis in nonclinical toxicology studies with numerous species. Due to the lack of mechanistic details and predictive monitorable biomarkers the clinical relevance of this toxicity is uncertain. Previously, we have found vasculitis associated with necrosis and apoptosis of endothelial cells (EC) and smooth muscle cells (SMC) and with enhanced expression of ICAM-1 and von Willebrand Factor on EC in mesenteric vessels of rats 24 hrs after treatment with the PDE III inhibitor, SK&F 95654 (±-5-methyl-6-[4-(4-oxo-1,4-dihydropyridin-1-yl)phenyl]-4,5-dihydro-3(2H)-pyridazinone). The present study further defined the course of apoptosis in the mesenteric vessels and lymphoid tissues within this time frame. Sprague-Dawley rats received a single injection of 100 mg/kg of SK&F 95654 (s.c.) and were euthanized at 1, 2, 4, 6, 8, 12, 24 hrs and 2 weeks later. Control rats were given DMSO (s.c.). TUNEL assay was used to identify apoptotic cells. Apoptosis was detected in EC and SMC of mesenteric vessels and mononuclear cells in lymphoid tissues (mesenteric lymph nodes, bronchial-associated lymphoid tissue, spleen, and bone marrow). Apoptosis of circulating lymphocytes and monocytes in the lumina of mesenteric vessels was also detected. Electron microscopy confirmed the occurence of apoptosis. Apoptosis began as early as 1 hr. A time dependent increase in apoptosis reached its highest incidence at 4 hrs, gradually decreased at 6-12 hrs, and disappeared at 2 weeks. Apoptosis of mononuclear cells in peripheral blood was confirmed by flow cytometry using Annexin V-FITC assay. In the SK&F 95654-treated rats, the percentage of Annexin V binding of mononuclear cells rose to 6.4±1.9 at 1 hr, increased to 7.4±2.9 at 4 hrs, and declined to 4.4±0.7 at 24 hrs. These values were significantly different ($p<0.05$) from a value of 2.3±0.6 in control rats. These results suggest that apoptotic cell death of EC and SMC may play an important role in the pathogenesis of SK&F 95654-induced vascular toxicity in rats and that this response was also observed in peripheral blood leukocytes.

1630 EFFECT OF LEAD ACETATE AND CADMIUM CHLORIDE ON ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION.

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Both epidemiological and animal studies suggest a positive correlation between chronic exposure to lead and hypertension. Similarly, cadmium exposure is also linked to increase in blood pressure in animals. The mechanisms involved in the heavy metal-induced hypertension is not clear. It is well established that endothelium derived nitric oxide serves as a potent vasodilator and could play an important role in the regulation of vascular tone and blood pressure. To our knowledge, effect of lead as well as cadmium on endothelial nitric oxide synthase (eNOS) expression is unknown. It is hypothesized that lead and cadmium exposure could decrease the expression of endothelial nitric oxide synthase. Using bovine aortic endothelial cells (BAECs) as the model system, we investigated the effect of lead or cadmium on endothelial nitric oxide synthase mRNA, and protein levels as well as enzyme activities. Exposure of endothelial cells to lead acetate (0 to 10 μg/ml) or cadmium chloride (0 to 1.0 μg/ml) caused no morphological changes as observed by phase contrast microscopy. Individual exposure of BAECs to high concentrations of lead (10 μg/ml) and cadmium (1.0 μg/ml) caused 53.5% and 81.8% increase in eNOS mRNA levels, respectively, by Northern analysis. Similar increase in eNOS protein content was found by lead and cadmium treatment, by Western analysis. The eNOS activity, as measured by the conversion of labeled arginine to labeled citrulline, was significantly elevated by lead (8.93±1.16 vs 14.05±2.76 pmoles citrulline/mg protein/min) but not by cadmium (8.93±1.16 vs 10.68±1.04 pmoles citrulline/mg protein/min) treatments. Additionally, measurement of endothelin-1 by ELISA indicated that lead or cadmium treatment had no effect on endothelin-1 secretion. Contrary to our hypothesis, these results suggest that lead- or cadmium-induced hypertension may not be related to decrease in eNOS expression or increase in endothelin-1 levels. Further studies are necessary to completely understand the mechanisms of lead and cadmium-induced hypertension.

1631 PLASMA CARDIAC TROPONIN T LEVELS IN THE SPRAGUE-DAWLEY RAT AS AN INDICATOR OF CARDIOTOXICITY.

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Chronic abuse of the alkaloid emetine in ipecac syrup can result in cardiac toxicity. We investigated the effects of emetine cardiac toxicity on plasma cardiac troponin T (cTnT), plasma creatine kinase-MB (CK-MB), EKGs, body weights and histopathology in Sprague-Dawley rat. Male rats weighing 150-174 g were randomized to 3 groups, and were injected ip with 2 mg/kg or 4 mg/kg of emetine diHCl daily or with equivalent volumes of saline. Parameters were measured at 0, 20, 30, and 40 days of emetine treatment. The animals were anesthetized with methoxyflurane, and 400 ml of blood was collected by retro-orbital puncture. Results showed significant increases above control for cTnT activity ($p<0.05$) in the 4 mg/kg animals. CK-MB levels remain unchanged in all groups. The homology of SD rat cTnT was similar enough to the mouse monoclonal cardiac troponin T (used in human kits) for detection. During the study, the control rats continued to grow, while the emetine rats lost weight. At the end of the study the heart rates in the treated animals were less than the controls. The QRS-waveforms generally were less complex in the treated animals. Exposure to emetine left notable ventricular scarring as confirmed by histopathology. In this model, the elevations in cTnT correlated with EKG and histopathology results. These results suggest that the use of cTnT for detecting cardiac toxicity is more sensitive than CK-MB and that it may prove useful for the *in vivo* evaluation of cardiotoxicity in general.

1632 CARDIAC HYPERTROPHY IN MICE LACKING THE ARYL HYDROCARBON RECEPTOR.

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a common environmental contaminant, has been shown to cause cardiac malformations such as ventricular-septal defects (VSDs), aortic arch anomalies, and general hypertrophy in the developing chick. These effects are believed to be mediated via the aryl hydrocarbon receptor (AhR), a bHLH/PAS protein, which binds TCDD with high affinity. Upon TCDD binding, AhR dimerizes with the aryl hydrocarbon receptor nuclear translocator protein (ARNT), binds xenobiotic responsive elements, and upregulates a battery of xenobiotic metabolism genes, including P4501A1. In response to hypoxia, ARNT also heterodimerizes with hypoxia-inducible factor 1-alpha (HIF1a), and facilitates transcription of hypoxia-responsive genes such as vascular endothelial growth factor (VEGF). This suggests a possible competition for ARNT between TCDD and hypoxic stress responses within the developing embryo. The purpose of this investigation was to use mice with a genetic deletion of AhR to dissect the roles of AhR/ARNT and HIF1a/ARNT signaling pathways in cardiogenesis. Mice lacking AhR show a marked cardiac hypertrophy, which begins before birth, and typically survived less than one year. At birth neonate heart weights for the knockouts were 0.79% of the total body weight, compared to 0.68% in controls ($p<0.001$). At six months of age, the knockout hearts were 0.82% of the total body weight, compared to0.67 for controls ($p<0.005$). Histological examination of the hearts revealed a thickening of the ventricular wall and septa, as well as an enlarged diameter of the coronary vasculature, consistent with over-stimulation of HIF1a signaling. This data suggests that AhR/ARNT signaling may have a role in normal cardiogenesis, and disruption of this normal function may lead to characteristic heart malformations.

1633 INHIBITION OF FATTY STREAK FORMATION AND LIPID OXIDATION BY LOW DOSES OF ACETAMINOPHEN IN HYPERCHOLESTEROLEMIC RABBITS.

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Acetaminophen inhibits LDL oxidation *in vitro*. To investigate the antioxidant and the potential anti-atherosclerotic properties of acetaminophen *in vivo*, rabbits were placed on a 1% cholesterol diet for 12 weeks, with 0 or 1 mg/ml of acetaminophen in the drinking water. The dosed animals received average doses of 28 mg/kg/d, which is lower than doses used therapeutically in humans. At the time of sacrifice, blood was collected for measurements of lipids, renal and hepatic functions, and for isolation of LDL. The aortas were removed, opened longitudinally, stained for lipid, photographed, and the total fatty streak areas determined by planimetry. Aorta fatty streaks are expressed as areas of fatty streaks relative to total aortic areas. Acetaminophen attenuated fatty streak accumulation significantly (0.32+/-0.05 vs. 0.58+/-0.06; $P<0.001$). No effects of acetaminophen were observed in total plasma cholesterol, triglycerides, BUN, or creatinine levels or in ALT/AST activities. LDL isolated from the acetaminophen-treated rabbits showed no differences in rates of Cu-catalyzed oxidation (TBARS) *in vitro*. However, the levels of 7-oxysterols in the LDL isolated from the acetaminophen-treated animals were lower than in the animals not dosed (7a-OH, 0.58+/-0.12 vs. 2.31+/-0.62; 7b-OH, 0.32+/-0.07 vs. 1.01+/-0.26; 7-keto, 0.41+/-0.14 vs. 3.86+/-1.72 nmol/mg LDL protein, $p<0.05$). The data demonstrate that low therapeutic doses of acetaminophen can diminish fatty streak formation and inhibit accumulation of oxysterols in circulating LDL. The attenuation of the levels of the cytotoxic oxysterols in circulating LDL, in conjunction with the inhibition of an important early step in atherogenesis, suggests that oxidation of circulating LDL may contribute to atherosclerosis.

1634 DIFFERENTIAL EFFECTS OF BENZO[A]PYRENE AND 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ON CELL ADHERENS JUNCTION AND ACTIN CYTOSKELETAL PROTEINS IN HUMAN UTERINE RL95-2 CELLS.

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This study investigated potential mechanisms whereby benzo(a)pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may contribute to uterine disease pathology using the human endometrial adenocarcinoma RL95-2 cell line. The effects of BaP and TCDD were evaluated on the level and localization of the adherens junction proteins β-catenin, cadherin and vinculin, as well as the cytoskeletal protein actin. RL95-2 cells were cultured for 48 hours in the presence of 10 nM TCDD, 10 μM BaP or 0.1% DMSO vehicle. Western immunoblot analysis showed the presence of 92 kDa β-catenin, 120 kDa cadherin, and 130 kDa vinculin proteins in cell membrane preparations and whole cell lysates of control cells. In BaP-treated cells, membrane preparations exhibit a significant 80% decrease in β-catenin and 38% decrease in cadherin protein levels, while vinculin levels were unchanged compared to control cells. In contrast, TCDD treatment had no effect on the level of β-catenin, cadherin or vinculin in isolated membranes or whole cell lysates. Further immunocytochemical studies used the fluorescent-labeled peptide phalloidin to visualize filamentous actin. In control cells, actin filaments were localized in a continuous subcortical layer, whereas BaP-treated cells show aggregation of subcortical actin. Thus evidence from Western blot analysis and immuno-

cytochemistry indicate that BaP, but not TCDD, is associated with selective alterations in the membrane localization of key adherens junction and actin cytoskeletal proteins in RL95-2 cells. These data indicate that BaP and TCDD have different effects on uterine cells. (Supported by NIEHS Superfund Grant 07375.)

1635 OXIDATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR EFFECT ON GAP JUNCTION INTERCELLULAR COMMUNICATION.

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Polycyclic aromatic hydrocarbons (PAHs) and ozone oxidized PAHs have been reported to suppress gap junction-mediated intercellular communication (GJIC). However, toxic effects of ozonated PAHs were attributed only to the least polar of a liquid chromatography (LC) fraction. We tested the hypothesis that aqueous solutions of benzo[a]pyrene (BaP), pyrene, and other PAHs subjected to short temporal ozone exposure, would cause toxicity in the GJIC assay that would correlate with oxidation of the polycyclic rings as observed by liquid chromatography/mass spectrometry (LC/MS). Following ozonation of a 5 mg/l BaP (19.6 μM) aqueous mixture (containing 0.5% acetonitrile) at varying time intervals, the products were isolated using a solid phase extraction technique and reconstituted in DMSO for dosing in a rat liver cell line (Clone 9). Treatment with 5μg/ml of ozonated BaP resulted in a decrease in GJIC which was inversely proportional to the amount of ozonation. After 1 min (7 mmol) ozonation of BaP, there was a 90% reduction in GJIC compared to controls, whereas after 5min (35 mmol) the GJIC was the same as controls. For each ozonation time the decrease in GJIC was the same regardless of exposure duration (i.e. 1 min through 16 hr) in the Clone 9 cells. Products of pyrene ozonation exhibited an opposite pattern of toxicity. At 0.5 μg/ml the products from 1 and 2 min of ozonation showed an 18 and 37% reduction in GJIC (respectively), however 3, 4, 5, and 6 min of ozonation produced products which were still lethal to the cells. LC/MS, using atmospheric pressure chemical ionization (APCI), revealed numerous mass fragments above 252 (MW of BaP) following 1 min of ozonation but almost none after 5 min. The data indicates the reduction in GJIC may result from the oxidation of polycyclic rings and not from the cleaved oxidized chains. (Supported by NIH P42 ES04917, USDA 9703230, and TAES H6215.)

1636 COMPARISON OF *IN VITRO* BINDING AND GENE EXPRESSION OF BENZO[A] PYRENE AND TWO HYDROXYLATED BENZO[A]PYRENE METABOLITES MEDIATED THROUGH THE ESTROGEN RECEPTORS ALPHA AND BETA, AND LACK OF *IN VIVO* EFFECTS ON MOUSE UTERINE WEIGHT OR LACTOFERRIN mRNA EXPRESSION.

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The ability of benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon, to bind to the alpha and beta forms of the estrogen receptor (ER) and to produce *in vitro* and *in vivo* estrogenic responses was assessed. While B[a]P did not significantly compete with tritiated 17-beta-estradiol ([3H]E2) for binding to the human ER alpha (bacterially expressed fusion protein consisting of GST and the DEF domains of the receptor) or human ER beta (full-length) *in vitro*, two hydroxylated B[a]P metabolites showed strong differential binding to the alpha and beta receptor isoforms. 3-hydroxy- and 9-hydroxy-B[a]P competed fully with [3H]E2 for binding to hER beta, with IC50 values of 49 nM and 1.0 mM respectively. However with hER alpha, concentrations of up to 10 mM caused only 60% and 30% displacement of [3H]E2 respectively. In transiently transfected MCF-7 human breast cancer cells, B[a]P, 3-hydroxy- and 9-hydroxy-B[a]P were similarly potent in causing human ER alpha- and mouse ER beta-mediated reporter gene expression (EC50 values 0.3 - 2 mM). Furthermore, at a concentration of 10 mM all three compounds in both the hER alpha and mER beta systems induced reporter gene expression to levels equal to that of E2 at 10 nM, with one exception: B[a]P in the hER alpha system induced levels only 25% of those of E2. Three daily doses as high as 10 mg/kg oral B[a]P or 20 mg/kg subcutaneous 3- or 9-hydroxy-B[a]P did not significantly increase uterine wet weight or lactoferrin mRNA expression in C57BL/6 or DBA/2 mice. These results suggest that the alpha and beta isoforms of the ER have a different capacity to bind and interact with B[a]P and its major hydroxylated metabolites *in vitro*. However, these compounds do not cause increased uterine weight or lactoferrin expression in the mouse.

1637 THE K-REGION, 8,9-DIHYDRODIOL OF DIBENZO[A,L]PYRENE IS A POTENT MORPHOLOGICAL CELL TRANSFORMING AGENT IN THE APPARENT ABSENCE OF STABLE COVALENT DNA ADDUCTS.

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K-region dihydrodiols of polycyclic aromatic hydrocarbons (PAHs) have been considered to be detoxification products. We previously reported on the metabolic activation of the K-region dihydrodiol of dibenzo[*a,l*]pyrene (DB[*a,l*]P), *trans*-DB[*a,l*]P-8,9-diol, to intermediates that form polar DNA adducts. This suggested that this K-region dihydrodiol might serve as an intermediate along an alternate route of metabolic activation of DB[*a,l*]P and possibly lead to genotoxic effects. We now report on the comparative genotoxic effects of *trans*-DB[*a,l*]P-8,9-diol and DB[*a,l*]P in transformable mouse embryo C3H10T½C18 (C3H10T½) fibroblasts. The C3H10T½ mouse embryo morphological transforming activities of these PAHs were conducted using concentration-response studies. At concentrations of 31 nM and above, both *trans*-DB[*a,l*]P-8,9-diol and DB[*a,l*]P produced significant (and similar) numbers of Type II and III foci per dish, and numbers of dishes with Type II&III foci. Concomitant cytotoxicity studies revealed a reduction in colony survival of approximately 25% up to 200 nM for both PAHs. DNA adducts of *trans*-DB[*a,l*]P-8,9-diol and DB[*a,l*]P in C3H10T½ cells were analyzed by a ^{32}P-postlabeling-TLC/HPLC method. Adducts were not detected in the DNA of C3H10T½ cells treated with *trans*-DB[*a,l*]P-8,9-diol at a concentration that induced morphological cell transformation. The DNA of DB[*a,l*]P-treated cells did bear adducts of deoxyadenosine and deoxyguanosine derived from the fjord-region diol epoxides of DB[*a,l*]P. These results indicate that *trans*-DB[*a,l*]P-8,9-diol has intrinsic genotoxic activity equal to that of DB[*a,l*]P, based on morphological cell transformation of mouse embryo cells. This activity is apparently not associated with the formation of observable stable covalent DNA adducts. These results suggest that under appropriate conditions *trans*-DB[*a,l*]P-8,9-diol may serve as an intermediate in the genotoxicity of DB[*a,l*]P. (This abstract does not necessarily reflect US EPA policy.)

1638 DIFFERENTIAL PROTECTION BY RAT UDP-GLUCURONOSYL TRANSFERASE UGT1A7 AGAINST BENZO[A]PYRENE-3,6-QUINONE- VERSUS BENZO[A]PYRENE-INDUCED CYTOTOXIC EFFECTS IN HUMAN LYMPHOBLASTOID CELLS.

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UDP-glucuronosyltransferase 1A7 (UGT1A7) is a polyaromatic hydrocarbon (PAH)-inducible UGT with activity towards various benzo(a)pyrene [B(a)P] metabolites. To investigate the influence of rat UGT1A7 on B(a)P-induced cytotoxicity, human lymphoblastoid L3 cells were transfected with either pMF6 (control expression vector), p167Dtk2 (microsomal epoxide hydrolase expression vector) or p167Dtk2-1A7 (epoxide hydrolase/UGT1A7 co-expression vector), and the cell populations were compared for sensitivity to B(a)P-induced effects. B(a)P inhibited cell proliferation and decreased relative cell survival of p167Dtk2- and p167Dtk2-1A7-cells to a similar extent. Metabolism studies using [^{3}H]-B(a)P revealed increased formation of glucuronide conjugates of B(a)P-4,5-diol, 3-OH- or 9-OH-B(a)P and an unidentified metabolite by p167Dtk2-1A7 cells but the presence of unconjugated metabolites suggested that glucuronidation capacity may be limited. No differences between p167Dtk2 and p167Dtk2-1A7 L3 cells were observed in the growth inhibitory effects of 3-OH-B(a)P or B(a)P-7,8-diol, but p167Dtk2-1A7 expressing cells were found to be less sensitive to B(a)P-3,6-quinone-induced effects on cell proliferation and relative cell survival. The effect was also observed in AHH-1 lymphoblastoid cells expressing UGT1A7 without epoxide hydrolase. The UGT1A7-expressing AHH-1 cells were also less sensitive to growth inhibition by B(a)P-1,6-quinone and B(a)P-6,12- quinone. Flow cytometric analysis of vehicle and B(a)P-3,6-quinone-exposed cell populations showed an association between UGT1A7 expression and resistance to B(a)P-3,6-quinone-induced apoptosis and loss of cell viability. These data suggest that UGT1A7 may be preferentially active towards B(a)P-quinones and that UGT1A7 may represent the PAH-inducible UGT activity previously implicated in protection against toxic redox cycling by B(a)P-3,6-quinone.

1639 EXPRESSION OF CYTOCHROME P-450 1A1 (CYP1A1) AND DNA ADDUCT FORMATION IN RAT LIVER AND LUNG SLICES INCUBATED WITH BENZO[A]PYRENE (BAP) IN DYNAMIC ORGAN CULTURE.

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The cytochrome P-450 (CYP450) dependent monooxygenase system is the primary route by which xenobiotics and environmental contaminants are detoxified in mammals. However, polycyclic aromatic hydrocarbons (PAHs) are one class of compounds that are activated by CYP450s to mutagenic, carcinogenic, and toxic metabolites. BaP is a prototypical PAH which is metabolized by the subsequent actions of CYP1A1 and epoxide hydrolase to its ultimate carcinogen BaP-7,8-diol-9,10-epoxide (BPDE). Furthermore, BaP can enhance its own metabolism by binding to the aryl hydrocarbon receptor, inducing expression of genes containing xenobiotic response elements, including CYP1A1. Precision cut rat liver and lung slices maintained in dynamic organ culture were utilized as an *in vitro* model to assess expression of CYP1A1 and DNA adduct formation under various conditions. Rat liver and lung slices were incubated for 4 or 24 hrs in medium containing 0, 1, 10, or 80 μM BaP. The *in vitro* expression of CYP1A1 was measured by competitive reverse transcriptase polymerase chain reaction (RT-PCR). Rat liver slices incubated for 24 hrs with 1, 10, or 80 μM BaP contained 8.09×10^5, 2.49×10^6, and 1.58×10^7 molecules of CYP1A1 mRNA, respectively. CYP1A1 mRNA in rat liver slices incubated for 24 hrs without BaP was below the limit of detection. DNA was isolated from tissue slices and DNA adduct formation was measured by ^{32}P-postlabeling. Rat liver slices incubated for 24 hrs with 0, 1, 10, or 80 μM BaP contained 0.02, 0.05, 0.32, and 2.43 fmoles adduct/μg DNA, respectively. Thus, the induction of CYP1A1 mRNA by BaP correlates with the formation of BaP-DNA adducts. Furthermore, the adduct levels correlate directly with the concentration of BaP. The preliminary results suggest this is a useful model for the detection of CYP450 expression and the quantitation of DNA adduct formation under these conditions and may be suitable for the examination of other tissues and species, including humans.

1640 TRANSIENT ANEMIA INDUCED IN NZB/WF1 MICE TREATED WITH BENZO[A]PYRENE.

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The NZB/WF1 mouse is an autoimmune model for systemic lupus erythromatosus (SLE) and it is derived from the cross of the New Zealand Black (NZB) and the New Zealand White (NZW). The NZB parent develops an autoimmune hemolytic anemia, which can be fatal. In previous studies, no changes in autoimmune SLE parameters (serum total IgG, or IgG titers to double stranded DNA, DNP-HSA, or laminin) were observed in BaP treated NZB/WF1 mice. However, hematological characteristics of anemia and an increase in spleen weight was observed in mice treated with 20 mg/kg BaP dermally. The objective of this study was to determine if the observed anemia was autoimmune-related. NZB/WF1 female mice, 7 weeks of age, were exposed dermally to BaP (0.02, 0.2, 2, 20 mg/kg) daily for thirty days. Vehicle mice were dermally exposed to acetone/olive oil (9:1). A significant increase (40-90%) was seen in spleen weight in the 20mg/kg BaP treated groups, however, evaluation of spleen cellularity demonstrated that the elevated spleen weight was not due to an increase in spleen leukocytes. At this dose level there was a significant decrease in erythrocytes (17%), hemoglobin (7%), and hematocrit (8%) and an 11% increase in both mean corpuscular volume and mean corpuscular hemoglobin. No significant effects were observed in spleen weights or hematological parameters at doses less than 20 mg/kg. In animals treated with 20 mg/kg for 30 days and then maintained for 50 days, hematological values returned to normal and did not differ from the vehicle controls. Furthermore, serum from anemic animals did not differ from control serum in its ability to lyse NZB/WF1 erythrocytes. These results suggest that BaP administered dermally at a dose of 20mg/kg has the potential to produce transient anemia in NZB/WF1 mice, which is not autoimmune mediated. (Supported in part by NIEHS Contract ES 55387.)

1641 BENZO[A]PYRENE METABOLITES MODULATE SP1 DEVELOPMENTAL EXPRESSION PROFILES IN RATS SUBSEQUENT TO AEROSOL EXPOSURE.

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Control male and female and timed-pregnant rats were dosed via nose-only exposure for four hours to acute doses of B(a)P : carbon black aerosol. A subgroup of the control animals was sacrificed at 30, 60, 120, and 240 minutes post-exposure and blood, lung, liver, testis, and ovaries were removed and analyzed for B(a)P metabolites. Nuclear extracts from various brain regions from pups that resulted from exposure of the timed-pregnant dams on gestation day 15 were analyzed by electrophoretic mobility shift assay on postnatal days (PND) 3, 5, 10, 15, 20 and 30. The results demonstrate a dose-dependent increase in B(a)P metabolite bioavailability in reproductive tissues. Tissue metabolite levels were maximal at 60 minutes post exposure with females exhibiting a 5-fold statistically significant increase ($p < 0.05$) compared to males within the 1.0 mg/m^3 exposure group. At 240 minutes post exposure, 95% clearance from tissues was observed when compared to the 30-minute post exposure group ($p < 0.05$). Sp1 cortex and cerebella developmental expression profiles from pups exposed transplacentally to B(a)P : carbon black aerosol exhibited a dose-dependent ($p < 0.05$) premature peak of Sp1-DNA binding on PND 3. The results suggest a possible neurotoxic molecular mechanism involving the 7β, 8α dihydroxy-9α, 10α-epoxy 7, 8, 10-tetrahydro-benzo(a)pyrene metabolite augmenting gene expression through modulation of the function of transcription factors. (Supported by Cooperative Agreement MHPF/ATSDR U50/ATU398948, NIH G12 RRO 3032, and NIEHS 00287.)

1642 DIETARY 3-METHYLCHOLANTHRENE (3MC) INDUCES CYP1A AND CONVERSION OF BENZO[A]PYRENE-7,8-DIHYDRODIOL TO METABOLITES THAT BIND DNA IN CATFISH INTESTINE.

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The intestine is both a target organ and site of uptake of environmental carcinogens. Dietary exposure of catfish to 3MC (10 mg/kg diet) prior to use in an *in situ* intestinal perfusion affected the extent of biotransformation, but not systemic uptake of [^{3}H]-benzo(a)pyrene-7,8-dihydrodiol (BaP-7,8D), introduced at 20μM and 40μM. Portions of the mucosa collected from *in situ* isolated intestinal preparations were homogenized and used to prepare subcellular fractions. DNA was isolated from the nuclear fraction and the radioactivity measured. AHH activity of intestinal microsomes was determined with a fluorescence assay and CYP1A content was detected by Western immunoblots, using a scup monoclonal antibody, and quantitated relative to the signal from rat CYP1A. DNA adducts were found in all samples. In controls, 0.8 to 2.6 pmol BaP-7,8-D molar equivalents bound per mg DNA, with no difference between fish exposed to 20 or 40 μM BaP-7,8D. The extent of DNA binding was higher in samples from 3MC-exposed fish. Fish exposed to 40 μM BaP-7,8D bound 42.5 ± 18.2 pmol/mg DNA(mean±S.E., n=5) than the 20 μM group 9.22±2.86 pmol/mg DNA, n=5. CYP1A was undetectable in intestinal microsomes from control fish but was present in all 3MC-exposed fish, 20±4 pmol/mg protein, n=10. AHH activity was low in control fish, 2.7±0.4 pmol 3OHBaP/mg protein, n=12 and induced in 3MC-exposed fish, 26.9±4.1, n=9. These results indicate the importance of CYP1A in activation of BaP-7,8D to DNA-reactive metabolites, and suggest the possibility that other CYP contribute to the formation of reactive metabolites in intestine. (Supported in part by NIEHS/NIH grant ES05781.)

1643 CHEMISTRY OF FINE PARTICLES, PAHs AND ORGANIC FREE RADICALS (OFRS) IN SOOT FROM COMBUSTION OF 1,3 BUTADIENE AT PETROCHEMICAL PLANTS AND REFINERIES.

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Production, processing, containment, and transport of C2-C8 hydrocarbon streams at refineries and petrochemical plants gives rise to losses of volatile organic chemicals (VOCs). These materials typically are flared, and this generates soot particles over a broad size range (0.002 - >5μm). Work in our laboratories shows that flaring of common VOCs such as acetylene, butane,

toluene, ethyl benzene, and styrene can give rise to substantial amounts of soot and other partially combusted products (up to 1% w/w). Working with 1,3-butadiene (a major component of petrochemical VOC mixtures to be flared) as a model, we found that its soot contains a broad and unique array of polycyclic aromatic hydrocarbons (PAHs). These included several known and suspected human carcinogens and high molecular weight PAHs (mass 300 - >1000 amu) in substantial amounts (e.g., 1000-5000 cigarette equivalents of (benzofluoranthenes+benzopyrenes) + perylene per g substrate burned; 2040 µg benzo(a)pyrene per g soot). When NOx were present during or after combustion, nitro- and polynitro- phenols, toluenes, and PAHs were found in the mixture in similar amounts. Further, the soot particles were found to have strong solid state free radical character in ESR analyses (i.e., g=2.0024; approx. 4% spin/weight). This free radical was stable for more than a year in the solid state, and extractable into solvents (toluene, DMSO) where it was stable for days. Butadiene soot extract also is electrochemically active at electrodes (+1.2 V vs. SCE) and in solution where it oxidized dissolved ascorbate. These and other data are offered in support of ongoing toxicological studies of these mixtures in normal human cell lines (q.v., sister presentation of C. Kennedy, et al.).

1644 EFFECTS OF PCB MIXTURES AND CONGENERS ON THE BILIARY EXCRETION OF THYROXINE.

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Polychlorinated biphenyl (PCB) mixtures and congeners effectively reduce circulating concentrations of thyroxine (T_4). This is thought to occur because of their ability to induce the UDP-glucuronosyl transferases that conjugate T_4 and the subsequent excretion of the glucuronide into bile. To determine whether there is a good correlation between the ability of PCBs to reduce T_4 and increase the biliary excretion of T_4 glucuronide, PCB congeners 95 (16 mg/kg), 99 (16 mg/kg), 118 (16 mg/kg) and 126 (40 µg/kg); Aroclors 1242 (32 mg/kg) and 1254 (32 mg/kg), and TCDD (3.9 µg/kg) were administered *via* gavage to male Sprague-Dawley rats for seven days. Twenty-four hours after the last dose, the femoral artery and vein and the common bile duct were cannulated. Following administration of $[^{125}I]T_4$, bile was collected at 30-min intervals for two hrs. Blood was collected at the mid-point of each bile collection period. Urine was also collected at two hrs. The total excretion of T_4 and its metabolites was quantified by gamma spectrometry, followed by HPLC analysis. All seven treatments decreased the serum concentration of T_4. Of the congeners, PCB 99 and 118 produced the largest decreases in serum T_4 concentration, whereas PCB 95, PCB 126 and TCDD had the least effect. None of the seven treatments had a marked effect on the urinary excretion of T_4 and its metabolites. In contrast, biliary excretion of T_4 glucuronide after administration of TCDD, PCB 118 or Aroclor 1254 was increased 6 to 8-fold, PCB 126 produced a 3 to 5-fold increase, whereas PCB 95 and PCB 99 produced less than a doubling. TCDD, one of the treatments that had the least effect on decreasing the concentration of T_4 in serum, increased the biliary excretion the most. In contrast, PCB 99, one of the congeners that decreased the serum concentration of T_4 the most, had the least effect on its biliary excretion. Therefore, there does not appear to be a good correlation between the ability of PCBs to decrease plasma T_4 concentration and increase its biliary excretion. (Supported by NIH grant ES-08156 and ES-05022, and EPA grant R826297.)

1645 ANTIESTROGENICITY OF CLARIFIED SLURRY OIL AND TWO CRUDE OILS IN A HUMAN BREAST-CANCER CELL ASSAY.

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Clarified slurry oil (CSO), a refinery stream produced by processing crude oil, and two crude oil samples, Belridge Heavy Crude Oil (BHCO) and Lost Hills Light Crude Oil (LHLCO) were examined for their estrogenic and antiestrogenic properties in a human breast-cancer cell (MCF-7) assay. The MCF-7 focus assay is based on postconfluent cell growth and tissue restructuring measured as the development of multicellular layers or foci. Oil samples were prepared in DMSO resulting in extraction of virtually all of the aromatic hydrocarbons including the 3-7 ring polycyclic aromatic compounds (PACs) comprising 62.2% of the CSO, 9.0% of the BHCO, and 2.0% of the LHLCO by total weight. None of the three samples were estrogenic in the MCF-7 focus assay. In contrast, all of the samples were antiestrogenic, i.e. they inhibited the development of foci induced by 1.0 nM 17β-estradiol (E_2). The potency of the samples was correlated with the percent of PACs they contained. Radiometric analysis of the catabolism of $[^3H]E_2$ in MCF-7 cell cultures demonstrated that all three samples resulted in an increase in the catabolism of E_2. It is suggested that PACs in the samples induced enzymes in MCF-7 cells that catalyzed the catabolism of E_2, accounting for at least part of the observed antiestrogenicity. (Supported by NIH ES04913 and Mobil Business Resources Corporation.)

1646 CHARACTERIZATION OF ASPHALT FUME GENERATION SYSTEM.

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A dynamic asphalt fume generation system (Heritage Research Group, Indianapolis, IN) was modified to provide consistent test atmospheres emulating road asphalt paving conditions. Characterization of the asphalt fume test atmospheres included (1) identification and characterization of chemical composition of polycyclic aromatic hydrocarbons (PAHs) by gas chromatography-mass spectrometry (GC-MS) of asphalt fume fractions collected by TPFE filter (0.5µm pore size at a flow rate of 1.0 liters/min), XAD-2, charcoal, and cold trap (-190°C, liquid N_2); (2) determination of the asphalt fume generation system uniformity of the aerosol distribution within the generator and exposure chamber; (3) gravimetric analysis (4) use of a photoionization detector (PID) for the vapor phase and a light scattering aerosol monitor for on-line monitoring of chamber concentration; and (5) detection and minimization of interferences from insulation and gasket materials within the system. The asphalt was initially preheated in an oven to 170°C, pumped to a large bitumen kettle, which maintained the asphalt temperature at between 150-170°C and then transferred to the generator (inlet temperature of 145-150°C). The fume was then conducted from the generator to an exposure chamber through a heated transfer line. Positive chemical ionization GC-MS using methane as the reagent gas and a 30 meter, 5% phenyl fused silica capillary column was used to characterize the fume. A total ion chromatography of PAHs was obtained with an initial temperature 50°C increased at 5°C/min to 310°C. The results indicated that asphalt fume chemical group characterization can be achieved using a combination of sampling methods with GC-MS detection. The generator output was both qualitatively and quantitatively very consistent when run 3 to 3.5 hrs/day for 5 consecutive days (CV < 20%). The results demonstrate that reproducible, consistent road paving type asphalt fumes can be generated for inhalation toxicity studies. (This work was supported, in part, by NIEHS/NTP.)

1647 THE AHR AND CYP1B1 AS TARGETS FOR PEPTIDE/TUMOR-SPECIFIC CANCER IMMUNOTHERAPY.

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Recent advances in the definition of requirements for antigen presentation suggest novel strategies for inducing tumor-specific CTL with peptides derived from tumor-associated antigens. The demonstration here of extremely high *AhR* and *CYP1B1* mRNA levels in human myeloma, ovarian carcinoma, and breast carcinoma cell lines indicates that the AhR and the AhR-regulated CYP1B1 enzyme may be two such proteins. Peptide-MHC class I binding algorithms were used to identify human AhR and CYP1B1 peptides predicted to bind the product of the relatively common HLA-A201 allele. Of 3 AhR and 3 CYP1B1 nanomers predicted by the algorithms, 2 derived from each protein bound HLA-A201 with relatively high affinity. To test their immunogenicity, these peptides were loaded onto HLA-A201 dendritic cells (DC) and CD40 ligand (CD40L)-activated B cells and presented to autologous $CD8^+$ T cells. $CD8^+$ T cell lines were generated following an initial stimulation with peptide-pulsed DC (with IL-7) and repeated stimulations with peptide-pulsed CD40L-activated B cells (with IL-7 and IL-2). These putative CTL lines specifically lysed autologous CD40L-B cells pulsed with cognate but not unrelated peptides. Furthermore, both AhR peptide- and CYP1B1 peptide-specific CTL lines lysed human myeloma and/or carcinoma cell lines expressing both HLA-A201 and high levels of AhR and CYP1B1, respectively, but did not kill tumors expressing little or no AhR/CYP1B1 or tumors expressing a different HLA-A allele. These results encourage the generation of AhR- and CYP1B1-specific CTL for adoptive immunotherapy of AhR^{high} and $CYP1B1^{high}$ cancers.

1648 COMPARATIVE RESPONSIVENESS OF LONG EVANS RATS VERSUS C57BL/6J MICE GIVEN TCDD-LIKE AND PHENOBARBITOL-LIKE PCB (POLYCHLORINATED BIPHENYL) CONGENERS.

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PCBs decrease thyroxine (T4) in the rat by induction of uridine diphosphate glucuronosyl-transferases (UDPGTs) via two different mechanisms; activation of the aryl hydrocarbon receptor (AhR) (PCB126), or a phenobarbitol-like mechanism (PCB153). This study compared the sensitivity of rats and mice to PCB induced hypothyroxemia. Eight-week old male and female C57BL/6J mice, and 25 day-old Long Evans rats were orally dosed with PCB126 (0.03-300.0 μg/kg/day, and 0.03-100.0 μg/kg/day, respectively) or PCB153 (0.3-90.0 mg/kg/day, 0.9-90 mg/kg/day) in 10 ml/kg corn oil vehicle for four consecutive days. Trunk blood and livers were collected one day post-dosing. Total serum T4 was measured via radio-immunoassay. Liver microsomal CYP1A1(EROD) and CYP2B2(PROD) were determined as biomarkers for AhR and Pb-like activities, respectively. T4-glucuronide was determined in both the rat and mouse. PCB153 decreased T4 (up to 80%) in both the rat and the mouse, while PCB126 did not effect T4 in the mouse, but decreased T4 (up to 50%) in the rat. PCB153 produced a 10-fold induction of PROD activity in the rat, and a smaller 3-4 fold induction in the mouse. EROD activity increased by approximately 20 fold in rats and mice given PCB126. These data suggest species differences in induction of glucuronosyltransferase activity between mice and rats. The lack of effect of PCB126 on T4 in mice suggests a lower sensitivity or altered responsiveness compared to rats. (This abstract does not necessarily reflect the policy of NCSU or the US EPA.)

1649 EFFECTS OF CO-EXPOSURE OF BENZENE AND RADIATION (YTTRIUM-90) ON HEMATOPOIESIS IN CBA/CA MICE.

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The hematopoietic system may be sensitive to the effects of low-dose chemical mixtures and ionizing radiation. Exposure to benzene or radiation causes acute bone marrow toxicity and increased incidence of acute myelogenous leukemia in humans. Based on common target tissues and similar biological response it is proposed that biological interactions between benzene and radiation are plausible. Male CBA/Ca mice (5/group) were utilized. Mice were orally administered benzene, by gavage, at doses of 0, 30, 100 and 300 mg/kg as single doses for 5 consecutive days (5 doses/animal). Mice were also given a single ip dose of Yttrium-90 (Y-90) a beta emitting bone-seeking radionuclide at doses of 0, 250, 500 or 1250 μCi/kg and the mice were held for 7 days. The results from these initial studies were used to set dose levels for the mixed exposure. For the co-exposure, mice were dosed benzene alone (all doses), Y-90 alone (1250 μCi/kg) or benzene + Y-90 (100 & 300 mg/kg + 1250 μCi/kg). Y-90 was dosed on day 1, and benzene exposure started on day 3 through day 7 as previously described. Hematological evaluations of bone marrow and peripheral blood were conducted. For all treatment groups, a dose-dependent decrease in the volume of packed red cells and total nucleated cells was observed and the response was greater following co-exposure. Co-exposure to benzene + Y-90 increased the erythroid cells relative to benzene alone (149-171%), but the increase was less than seen with Y-90 (85-89%). In contrast, co-exposure elevated the myeloid cells (119-134%) relative to Y-90 alone, but was less than or comparable to benzene (79-106%). Both benzene and Y-90 alone decreased the lymphocyte cells, however, only high dose co-administration enhanced the inhibition to 24% and 27% of the Y-90 and benzene doses, and 17% of control. The individual responses are consistent with previous results with benzene or radiation on hematopoiesis. However, the biological response following co-exposure is more complex since benzene and Y-90 induce different shifts in the myeloid and erythroid cells. Future studies are needed to elucidate the biological implications of these interactions.

1650 A COMPARISON OF THE CELISA ASSAY AND THE CLONOGENIC COLONY ASSAY IN DETERMINATIONS OF MYELOTOXICITY.

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A comparison of the CELISA™ assay and the clonogenic colony assay in determinations of myelotoxicity. Bone marrow is extremely sensitive to the toxic side effects of chemotherapeutic drugs. Bone marrow toxicity is often a limiting factor in the dosing and duration of chemotherapy. Discovery and elimination of toxic compounds early in the drug discovery process is a high priority. Myelotoxicity is currently assayed through use of the clonogenic "colony assay". The extremely low throughput nature of the colony assay precludes its use in high throughput toxicity testing. A high-throughput CELISA assay of hematopoiesis based on the quantification of differentiation-specific cell surface markers now allows high-throughput analysis of the effects of experimental drugs on hematopoiesis. The CELISA assay is a 96-well-based suspension culture system for human hematopoietic progenitor cells which quantifies the commitment and differentiation of CD34+ cells to specific lineages. The expression of maturation and lineage markers on the cells in culture is measured by use of a time-resolved fluorescence immunoassay. The expression of three different lineage markers can be measured: CD11b for myeloid cells, platelet glycoprotein IIb/IIIa (or CD41a) for megakaryocytic cells, and glycophorin A for erythroid cells. The assay is set up specifically to examine more than one lineage as there are numerous examples of lineage-specific effects. Quantification of the CELISA assay requires only 60 minutes as opposed to the lengthy process of manually counting colonys required by the colony assay. Results of direct comparisons of the CELISA and colony assays document a direct correlation between data from these two assays. These results indicate that the CELISA assay system can substitute for the clonogenic colony assay for screening potential myelotoxic agents and affords researchers the opportunity to perform high-throughput screening for myelotoxicity.

1651 TREATMENT OF RATS WITH CLOZAPINE DECREASES THE NUMBER OF CD34+ BONE MARROW CELLS.

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Clozapine therapy is associated with a relatively high incidence of agranulocytosis (~0.8%). Neutrophil precursors in the bone marrow appear to be the target because these cells are markedly decreased in patients with clozapine-induced agranulocytosis. We have previously demonstrated that a reactive metabolite of clozapine covalently binds to neutrophils in humans and to bone marrow cells in rats *in vivo* even though neither the persons nor the rats that were studied developed agranulocytosis. In this study we examined the bone marrow of rats that had been treated with clozapine (65 mg/kg/day for 6 weeks) using a fluorescent antibody against CD34 and a fluorescence activated cell sorter (FACS). CD34 is a marker for bone marrow precursor cells. We found that this clozapine treatment decreased the fraction of CD34+ cells from 7% to 3% ($P < 0.005$), but such treatment does not cause a significant decrease in the number of peripheral neutrophils. This indicates that clozapine has a significant effect on precursor cells in the bone marrow, and the lack of an effect on peripheral neutrophil numbers suggests that the bone marrow can generally compensate for these changes. We propose that the covalent binding of clozapine to bone marrow cells and the decrease in the number of these cells expressing CD34 in bone marrow are precursors to the development of agranulocytosis; however, additional steps, which do not occur in most patients or animals, must be required. (Supported by a grant from the Medical Research Council of Canada.)

1652 ROLE OF MEK KINASE PATHWAYS IN HEMATOPOIESIS.

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Mitogen actived protein (MAP) kinase inhibitors are under development as potential antiinflammatory and anticancer agents. A role for MAP kinase signal transduction pathways in hematopoiesis has been proposed, therefore, a study was conducted to assess the potential myelotoxic effects of MAP kinase inhibition. Bone marrow from C57BL/6 mice was cultured for 9 days in the presence of erythropoietin, IL-3 and IL-1. Specific inhibitors of the P42/44 MAP kinase pathway (UO126, SL327 and PD 098059; MEK inhibitors), an inactive analogue of UO126 (SM015), an inhibitor of P38 MAP kinase

(SK&F 86002), and of p70/S6 kinase (rapamycin) were added to primary bone marrow cultures at 1 or 10μM, and the effects on erythroid and myeloid colony growth and differentiation were evaluated. To confirm the presence of an active P42/44 MAP kinase pathway in proliferating hematopoietic cells, ERK phosphorylation was assessed. It was found to be rapidly induced (5 min) in bone marrow cells by a cocktail of IL-1, IL-3 and erythropoietin as shown by phosphoimaging and western immunoblot analysis. UO126, SL327 and PD 098059 inhibited ERK phosphorylation in order of potency from most (>95%) to least (approximately 50%), but had no effect on erythroid or myeloid colony growth. SM015 did not alter signal transduction or *in vitro* hematopoiesis. The p38 MAP kinase inhibitor had a mild potentiating effect on erythropoiesis but no effect on myelopoiesis. Rapamycin resulted in a marked reduction in erythroid and myeloid colony formation, confirming an essential role for signals transduced through P70/S6 kinase in hematopoiesis. Both major MAP kinase pathways (P42/44 and P38) were shown to be largely redundant for *in vitro* hematopoiesis, thus inhibitors of MAP kinase pathways were, therefore, considered unlikely to be myelotoxic.

1653 HEMATOTOXICITY OF DITHIOCARBAMATES ON CD34+ HUMAN BONE MARROW CELLS.

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Dithiocarbamates (DTC), an important class of therapeutic and industrial chemicals, have been alternatively suggested to be either beneficial or toxic to the hematopoietic system. DTC have also been shown to be potent inhibitors of the transcription factor, nuclear factor kB (NF-kB) in a variety of cell types. Previous studies in our laboratory have demonstrated that NF-kB is required for normal clonogenic response in CD34+ human bone marrow cells (Pyatt et al., 1998). Therefore, we investigated the potential of two DTC, dimethyl- and diethyl- DTC, to directly alter clonogenic response of primary human CD34+ bone marrow cells *in vitro*. Our results demonstrate that DTC suppresses cytokine-dependent colony formation in human CD34+ bone marrow cells at concentrations between 0.1-0.5 μM. Inhibition of colony formation by DTC is not likely to be the result of cytotoxicity, because DTC-induced apoptosis only occurs at doses significantly higher than those required for colony inhibition. Surprising, experiments reveal that DTC does not inhibit cytokine activation of NF-kB in CD34+ bone marrow cells. These results indicate that extremely low concentrations of DTC suppress growth factor-dependent clonogenic response of CD34+ bone marrow cells *in vitro* which may explain the hematotoxicity observed in previous clinical trials with DTC. DTC induced hematotoxicity also appears to occur via a mechanism that is independent of NF-kB inhibition. (This research project was supported in part by the Chemical Manufacturers' Association.)

1654 HEMATOTOXICITY OF THE CHINESE HERBAL MEDICINE, TRIPTERYGIUM WILFORDII HOOK F IN CD34+ HUMAN BONE MARROW CELLS.

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T2, a chloroform/methanol extract of the herb Tripterygium wilfordii Hook f, has been used in the treatment of autoimmune and inflammatory diseases by the Chinese for many years. Recent experimental evidence has confirmed that T2 has potent anti-inflammatory and immunosuppressive activity and a FDA approved clinical trial is ongoing in the US to explore the efficacy of T2 in the treatment of rheumatoid arthritis. Despite the potential therapeutic benefits of T2, there is ample documentation that T2 is toxic, targeting among other things the hematopoietic system, and it's use has resulted in cases of leukopenia, thrombocytopenia and aplastic anemia. This investigation was undertaken to characterize the *in vitro* effects of T2 on primary human CD34 positive bone marrow cells. Our results demonstrate that T2 has a potent inhibitory effect on the clonogenic response of human bone marrow cells to exogenously added hematopoietic growth factors. This inhibition occurs at concentrations well below those required for immunosuppression and may represent a serious consequence for patients treated with this drug. The inhibition of colony formation by T2 is not the result of direct cytotoxicity nor increased apoptosis and indicates a functional suppression of hematopoiesis. Additional experiments demonstrate that T2 also alters transcriptional regulation in bone marrow cells by inhibiting Nuclear Factor-kB (NF-kB). This transcription factor is found in CD34+ bone marrow cells and has been recently shown to be a requirement for colony formation. These results demonstrate that therapeutic concentrations of T2 exert a significant hematotoxic effect by inhibiting growth factor response in CD34+ bone marrow cells and suggest that inhibition of NF-kB may play a role in the blood dyscrasias encountered with the use of this drug.

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Chronic exposure to benzene has been associated with hematotoxicity including the development of acute myelogeous leukemia (AML). Clonal cytogenetic aberrations are thought to play an important role in the pathogenesis of AML developing secondary to drug or chemical exposure. Recent studies have implicated the inhibition of topoisomerase II alpha, an essential nuclear enzyme that catalyzes the interconversion of various forms of DNA, as a mechanism to explain benzene-induced cytogenetic aberrations. While chemotheraputic modalities that include topoisomerase poisons are also known to be leukemogenic, the factor determining the potential to produce cytogenetic abnormalities is dependent on the mechanism of inhibition (catalytic inhibition versus cleavable-complex stabilization). The purpose of this study was to determine the mechanism by which topoisomerase II alpha is inhibited by the following benzene metabolites: benzoquinone, 1,2,4-benzenetriol, 4,4-biphenol, and hydroquinone. In a DNA cleavage/relaxation assay where topoisomerase II alpha is exposed to different benzene metabolites prior to analysis of enzyme activity, we observe inhibition of topoisomerase II alpha with the above benzene metabolites at 30 mM, 100 mM, 100 mM, and 1.0 mM respectively. However, no stabilization of cleavable complex formation is formed and, in fact, an antagonistic effect on etoposide-stabilized cleavable complex formation is observed. Our data suggests that these benzene metabolites are actually catalytic inhibitors of topoisomerase II alpha and therefore are not likely to contribute to the development of AML through this mechanism. This project was suppored in part by NIH grant ES06258.

1656 FLOW CYTOMETRIC ANALYSES OF MAGNETICALLY-ENRICHED BONE MARROW LINEAGE NEGATIVE CELLS REVEAL TCDD-MODIFIED HEMOPOIETIC STEM CELL DIFFERENTIATION PROFILES.

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Treatment of C57BL/6 mice with the environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) elicits altered bone marrow hemopoietic cellular potentials and markedly-reduced T-lymphoid reconstituting activity. The latter has been hypothesized to play a role in TCDD-induced thymic atrophy. To investigate hemopoietic cellular targets responsible for reduced prothymocyte capacity, lineage-negative (lin$^-$) bone marrow stem cells from adult male C57BL/6 mice were magnetically enriched, stained and assessed in terms of cell surface Thy-1, Sca-1, Sca-2, HSA, c-Kit, CD34, CD38 and IL7Rα expression by flow cytometry 8 days following a single dose of 30μg/kg TCDD. The data reveal reduced lin$^-$ Sca-1$^{-/lo}$ c-Kit$^-$ -defined cells as well as reduced lin$^-$ Thy-1$^{-/lo}$ Sca-1$^{-/lo}$ -defined cells contained within the bone marrow small lymphoblast stem cell compartment. In contrast, the bone marrow granular cell compartment contained an increased complement of lin$^-$ Sca-1$^{-/lo}$ c-Kit$^+$ -defined cells and lin$^-$ Thy-1$^{-/lo}$ Sca-1$^{-/lo}$ -defined cells. At the day 8 timepoint, CD34$^-$ / CD38$^+$ long-term repopulating stem cell subsets were elevated overall, while multipotent, CD34$^+$/CD38$^-$ short-term repopulating subsets were numerically depressed. Taken together with numerically reduced lin$^-$ c-Kit^{lo} Sca-1^{lo} Thy-1$^{-/lo}$ IL-7Rα^{lo} expressing cells classified as common lymphoid progenitors, these data suggest a stem cell mobilization following TCDD treatment with a myelopoietic precedence over lymphopoiesis. These data further indicate that proliferation and/or differentiation processes of hemopoietic stem cells are affected by TCDD and that these effects contribute to a reduced capacity of bone marrow to generate pro-T lymphocytes.

1656 FLOW CYTOMETRIC ANALYSES OF MAGNETICALLY-ENRICHED BONE MARROW LINEAGE NEGATIVE CELLS REVEAL TCDD-MODIFIED HEMOPOIETIC STEM CELL DIFFERENTIATION PROFILES.

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Treatment of C57BL/6 mice with the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elicits altered bone marrow hemopoietic cellular potentials and markedly-reduced T-lymphoid reconstituting activity. The latter has been hypothesized to play a role in TCDD-induced thymic

atrophy. To investigate hemopoietic cellular targets responsible for reduced prothymocyte capacity, lineage-negative (lin-) bone marrow stem cells from adult male C57BL/6 mice were magnetically enriched, stained and assessed in terms of cell surface Thy-1, Sca-1, Sca-2, HSA, c-Kit, CD34, CD38 and IL7Ra expression by flow cytometry 8 days following a single dose of 30mg/kg TCDD. The data reveal reduced lin- Sca-1-/lo c-Kit+ -defined cells as well as reduced lin- Thy-1-/lo Sca-1-/lo -defined cells contained within the bone marrow small lymphoblast stem cell compartment. In contrast, the bone marrow granular cell compartment contained an increased complement of lin- Sca-1-/lo c-Kit+ -defined cells and lin- Thy-1-/lo Sca-1-/lo -defined cells. At the day 8 timepoint, CD34- / CD38+ long-term repopulating stem cell subsets were elevated overall, while multipotent, CD34+/CD38- short-term repopulating subsets were numerically depressed. Taken together with numerically reduced lin- c-Kitlo Sca-1lo Thy-1-/lo IL-7Ralo expressing cells classified as common lymphoid progenitors, these data suggest a stem cell mobilization following TCDD treatment with a myelopoietic precedence over lymphopoiesis. These data further indicate that proliferation and/or differentiation processes of hemopoietic stem cells are affected by TCDD and that these effects contribute to a reduced capacity of bone marrow to generate pro-T lymphocytes.

1657 DENDRITIC CELLS: TARGETS FOR, AND MEDIATORS OF IMMUNOTOXICITY AND ALLERGY.

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Dendritic cells (DC) are a family of bone marrow-derived cells that are found in small numbers in lymphoid organs and in those tissues that come into closest contact with the external environment, including the epidermis, gastrointestinal tract and respiratory epithelium. The main functions of DC are the recognition, internalization, processing and presentation of antigen and the stimulation of T lymphocyte responses. Indeed DC are mandatory antigen-presenting cells for the effective induction of primary immune responses. It is clear that DC play pivotal roles in the initiation and regulation of allergic responses, including those provoked by chemical sensitizers. The participation of these cells in allergic responses is orchestrated by chemokines and cytokines that together control the movement, migration and functional activity of DC. The growing appreciation of the contributions of DC to allergic responses has facilitated consideration of their use in the design of alternative methods for sensitization testing. There is increasing evidence also that DC provide targets for immunotoxic agents. These cells play an important part in the development of immunosuppression caused by exposure to ultraviolet B irradiation and, moreover, DC have been implicated in the immunotoxicity associated with certain chemicals, including TCDD.

1658 DENDRITIC CELLS IN IMMUNOBIOLOGY AND PATHOLOGY.

S. Knight. *Imperial College School of Medicine, Middlesex, United Kingdom.* Sponsor: I. Kimber.

Antigens do not activate T lymphocytes directly. Instead they are acquired in peripheral tissues, as either free antigen or associated with damaged or dying cells, by dendritic cells (DC). These DC have the specialized properties of acquiring and processing antigen. To then initiate a primary immune response the cells must mature and migrate to draining lymphoid tissues where DC lose their ability to process antigen, but become effective at clustering with and activating resting T lymphocytes. The maturation, migration and changing functions of DC are therefore defining events in the stimulation of immunological responses. There exists heterogeneity among DC. Different lineages related to lymphoid or myeloid differentiation pathways have been identified and these include cells that are able to interact with T lymphocytes or B lymphocytes. Both the type of antigenic stimulation and the cytokine milieu influence the maturation pathways followed by DC. For example, an immunosuppressive mouse retrovirus switches DC from interleukin 12 to interleukin 4 production, accompanied by the loss of T lymphocyte priming activity and possibly represents the basis for compromised immune function. Autocrine pathways may reinforce the function of DC. These cells are able to transfer antigens to other DC and the distribution of antigenic material between cells may be an important aspect of immune stimulation. The influence of antigens and toxic insults on the functional characteristics of DC may be a critical determinant of immune-mediated pathology.

1659 ALTERATION OF DENDRITIC CELL CYTOKINE SECRETION BY TOTAL BODY UV EXPOSURE.

S. E. Ullrich. *MD Anderson Cancer Center, Houston, TX.* Sponsor: I. Kimber.

The UV radiation present in sunlight is the primary cause of skin cancer, the most common form of human neoplasia. In addition, UV radiation is immune suppressive and the immune suppression induced by UV exposure has been identified as a major risk factor for skin cancer induction. One consequence of whole body UV-exposure is differential activation of T helper subsets. The ability of antigen presenting cells from UV-irradiated mice to present to T helper (Th) 1 cells is severely depressed, whereas presentation to Th2 cells is normal. We tested the hypothesis that altered antigen presentation to Th1 and Th2 cells results from a modulation of IL-12 secretion by dendritic cells. The secretion of biologically active IL-12p70 by dendritic cells isolated from UV-irradiated mice is suppressed. In addition, secretion of IL-12p40 homodimer, a naturally occurring IL-12 receptor antagonist is increased. We suggest that secretion of IL-12p40 homodimer by dendritic cells isolated from UV-irradiated mice, coupled with suppressed IL-12p70 secretion may help to explain suppressed Th1 cell activation following UV-irradiation. Because Th2 cells require IL-4 and not IL-12 as their growth factor, UV-induced suppression of IL-12p70 and increased secretion of IL-12p40 homodimer should not affect the activation of Th2 cells. Moreover, because we know that cytokines such as prostaglandin E2 and IL-10 suppress the production of biologically active IL-12, we suggest that the cytokines induced following UV exposure may be involved in the modification of IL-12 secretion by dendritic cells isolated from UV-irradiated mice. This leads to suppressed cell mediated immune reactions and permits the out growth of UV-induced skin tumors.

1660 DENDRITIC CELLS AS A POTENTIAL TARGET FOR THE IMMUNOTOXICITY OF TCDD.

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Primary T cell-mediated immune responses are highly susceptible to suppression by TCDD exposure, yet direct effects of TCDD on T cell functions have been difficult to demonstrate. Since the activation of naive T cells has been shown to be mediated, perhaps exclusively, by DC, they represent a potential target for TCDD immunotoxicity. Initial studies have examined the influence of TCDD exposure on splenic DC phenotype and function in the absence of antigenic stimulation in C57B1/6 mice. Results showed that DC from TCDD-treated mice expressed higher levels of several constitutively-expressed costimulatory molecules, including B7-2, CD40, ICAM-1 and CD24. In contrast, the expression of LFA-1 was significantly reduced. These effects were dose-dependent, occurred within 48 hr, and persisted for at least 14 days. Interestingly the number of DC recovered from TCDD-treated mice was significantly decreased 7 and 14 days after exposure. When DC from TCDD-treated mice were incubated with allogeneic T cells, the proliferative response of the T cells and cytokine production was increased. IL-12 production was also increased in comparison to cells from vehicle-treated mice. Taken together, these results suggest that TCDD provides an activation stimulus to DC in the absence of antigen. This inappropriate stimulation may inhibit the ability of DC to process and present antigen or may lead to their premature deletion. Similarly, the decrease in LFA-1 expression may interfere with the ability of DC to migrate to the T cell areas of the lymphoid tissue. These possibilities are under investigation. (Supported by NIH grants ES003966 and ES00040.)

1661 DENDRITIC CELLS AND THE INDUCTION AND REGULATION OF ALLERGIC RESPONSES TO CHEMICALS.

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In non-lymphoid tissues dendritic cells (DC) can be regarded as sentinels of the immune system having responsibility for sampling the external antigenic environment. In the skin this function is performed by Langerhans cells (LC) which form a semi-contiguous network within the epidermis. In response to skin sensitization (or other forms of dermal trauma) a proportion of local LC, many of which bear high levels of antigen, are mobilized to leave the epidermis and migrate from the skin, via afferent lymphatics, to draining lymph nodes. During this migration LC are subject to functional differentiation such that by the time of their arrival in peripheral lymph nodes they have acquired the properties of mature DC and are able to present antigen effectively to responsive T lymphocytes. There is increasing evidence that the mobilization, migration and differentiation of LC are initaited and directed by epidermal

cytokines. The maturation of LC from antigen processing to antigen presenting cells is effected by granulocyte/macrophage colony-stimulating factor (GM-CSF) acting in concert with interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α). The initiation of migration is dependent upon the receipt by LC of two independent cytokine signals, one provided by TNF-α, an inducible product of keratinocytes, and the other by IL-1β, a constitutive product of LC themselves which is upregulated further in response to skin sensitization. Collectively, these cytokines provoke changes in LC phenotype, including the altered expression of adhesion molecules necessary for the release of LC from the skin and their movement through the tissue matrix to lymph nodes. The directed migration of LC from the skin to lymph nodes is facilitated further by chemokines expressed locally. Perturbation of cytokine or chemokine signalling results in the modulation and/or inhibition of allergic sensitization.

1662 DENDRITIC CELLS AND THE DEVELOPMENT OF ALTERNATIVE STRATEGIES FOR SKIN SENSITIZATION TESTING.

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Dendritic cells (DCs) are professional antigen presenting cells which are widely distributed in the body and are key for initiating T cell mediated immune responses, including their role in allergic contact dermatitis (ACD). Methods for culturing human DCs from peripheral blood (PB) have been developed by several laboratories. We evaluated the ability of PB-derived DCs to present chemical haptens to sensitized T cells in an *in vivo* lymphocyte proliferation assay. Immature DCs (iDCs) were generated from PB of patients sensitive to dinitrochlorobenzene (DNCB) using standard GM-CSF/IL-4 protocols iDCs were treated with a water soluble form of the test allergen (dinitrobenzenesulfonic acid [DNBS]), or an unrelated contact allergen (squaric acid [SA]), then cultured with 105 autologous T cells. Mature DCs (mDCs) were generated from iDCs with the addition of TNF-α IL-1β, IL-6 and PGE_2. Compared to hapten treated iDCs, mDCs treated with DNBS stimulated a greater proliferative response in sensitized T cells at responder: stimulator ratios of 50:1 and 100:1. No proliferation was observed in T cells cultured with SA treated DC demonstrating the specificity of the proliferative response. These results indicate that mDCs are more efficient antigen presenting cells than iDCs for the chemical hapten DNBS. Finally, because of the limited number of DCs obtained from PB, we have successfully induced a bone marrow derived $CD34^+$ myeloid leukemia cell line (KG-1) to a DC phenotype and morphology. KG-1 treated 3-days GM-CSF, IL-4, TNF-α, SCF, TPO, and Flt3-ligand remain negative for lineage makers (CD3, CD14,CD16, CD19, CD20, CD56) and express Class I MHC (HLA-A,B,C), Class II MHC (HLA-DR, HLA-DQ), CD54, CD11a, CD11b, CD11c, CD83, CD86 and CD1a. Functionally, KG-1 derived DCs are capable of phagocytosing latex micropheres and inducing a potent allogeneic T-cell response in a mixed lymphocyte reaction. We conclude that the study of DCs offers potential for the development of new skin sensitization test methods.

1663 INTRODUCTION.

R. Tennant. *NIEHS, Research Triangle Park, NC.*

A plethora of technological and conceptual advances in molecular biology and medicine, genetics and genomics, and related research has opened significant opportunities for development of an abundance of new therapies. These opportunities provide a challenge to the assessment of the safety and efficacy of these new candidate therapies. This symposium will provide a review of the current status of toxicology biomarkers in drug development and clinical safety assessments. Particular emphasis is on the demonstration that biomarkers can serve as early predictors of adverse effects and on the development of technological approaches to find out more and better toxicology biomarkers.

1664 CONCORDANCE OF PHARMACEUTICALS TOXICITY IN HUMANS WITH ANIMAL DATA.

H. M. Olson[1], G. Betton[2], A. Monro[1], K. Thomas[3] and D. Robinson[3]. [1]*Pfizer, Inc., Groton, CT,* [2]*AstraZeneca Pharmaceuticals, Macclesfield, United Kingdom and* [3]*ILSI-HESI, Washington, DC.*

This investigation describes the procedure for compiling data and summarizes the results of a multinational pharmaceutical company survey to better understand concordance of the toxicity of pharmaceuticals in humans with that in animals. The main aim of this project is to examine the usefulness of animal studies to identify toxicity biomarkers for pharmaceutical-associated human toxicity (HT), and to identify opportunities to improve anticipation of HT. The database was developed from a survey which tabulated significant HTs for pharmaceuticals identified during clinical development, and determined whether animal toxicity studies did or did not identify target organ biomarkers for the relevant HTs. Data collected included: codified compound therapeutic category, the type (organ system) of HT that was identified, and the duration of animal studies in which the corresponding toxicity was either first identified or not observed. To-date the survey includes input from 10 pharmaceutical companies with data compiled from approximately 171 compounds with more than 230 human toxicity events reported. At a recent HESI project workshop, which included representatives from academia, industry and regulators, the results showed the true positive concordance rate for HT of 73% for rodent plus non rodent species with non rodents being predictive in 65% of HTs and rodent in 45%. The results support the utility for identifying HTs in non-clinical studies of one month's duration. These survey results support the value of *in vivo* studies to predict for many significant HTs associated with pharmaceuticals, while identifying those toxicities requiring improved methods.

1665 RESEARCH NEEDS AND APPLICATIONS OF TOXICITY BIOMARKERS IN THE EVALUATION OF NOVEL THERAPEUTICS.

G. J. Downing. *National Institutes of Health, Bethesda, MD.* Sponsor: J. C. Barrett.

High throughput technologies are enabling production of large numbers of new chemical entities to be evaluated as target-specific candidate therapies. Improved safety evaluation methods are needed to provide preclinical and clinical toxicity testing in an efficient and timely fashion. The use of biological markers, or biomarkers, defined as characteristics that are objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention, is one approach to enhance toxicity testing. Mass spectroscopy, cDNA microarray, protein expression, and imaging technologies are examples of basic discovery tools that may serve as platforms to evaluate predictive biomarkers of toxicity. These fundamental research discovery tools now provide opportunities to develop highly efficient assay systems to establish specific and sensitive indicators of cellular and molecular injury. For example, biomarkers may represent components of cell signaling pathways for apoptosis, growth factors, cytokines, and chemokines, and xenobiotic metabolic pathways. Establishment of the linkage of biomarkers to clinical features of toxicity is critical to their application in drug safety testing. Harnessing new technologies for toxicology testing will be expedited by cooperative efforts among government, universities, and private industry partners. These interactions will facilitate the development of a technology and information-based infrastructure that will support the standardization of testing methods, validation of assay systems, establishment of databases, and development of statistical analyses that are needed to better characterize toxicity biomarkers. Collaborations through public-private partnerships are proposed to address growing needs in safety assessment of new therapeutics using toxicity biomarkers.

1666 DETERMINATION OF SERUM CARDIAC TROPONIN-T AS A BIOMARKER OF ANTHRACYCLINE CARDIOTOXICITY.

S. E. Lipshultz. *University of Rochester Medical Center, Rochester, NY.* Sponsor: R. Tennant.

Anthracyclines, important agents for the treatment of childhood cancer, are associated with progressive cardiotoxicity that is differentially expressed in most long-term survivors (LTS). >50% of LTS of childhood cancer have been treated with anthracyclines. Echocardiographic abnormalities are noted in 60% of these patients 6 years after therapy and is progressive in 75% of these patients. The heart is a major source of morbidity and mortality for LTS. The identification of myocardial injury during therapy should allow individualization of anthracycline therapy and evaluation and implementation of cardioprotective and therapeutic strategies. We employed a multidisciplinary, multispecies approach to determine whether serum cTnT is a biomarker of early and late anthracycline cardiotoxicity. In rats, we found cTnT elevations correlated with cumulative anthracycline dose and myocardial histologic scores of anthracycline cardiotoxicity. The specificity of cTnT elevations for myocardial injury was confirmed by cTnT immunohistochemical staining (J Clin Oncol 1999;17:2237-2243). In children, the level and length of cTnT ele-

vations following anthracycline therapy correlated with cumulative dose (Proc ASCO 1999;18:568A). The level of cTnT elevation after therapy correlated with echocardiographic abnormalities 9 months later, suggesting that early cTnT levels are useful biomarkers of cardiotoxicity at intermediate follow-up (Circulation 1997;96:2641-2648). The utility of cTnT levels during anthracycline therapy to predict late cardiotoxicity in LTS is under investigation. In summary, the use of a multidisciplinary multispecies approach has accelerated the investigation of cTnT as a useful biomarker for late anthracycline cardiotoxicity.

1667 TOXICOGENOMICS IN SAFETY ASSESSMENT.

R. D. Tyler. *Glaxo Wellcome, Inc., Research Triangle Park, NC.*

Most toxicities that are not lethal within minutes are likely to have some impact on gene expression, be a result of gene expression, or both. Some of the gene expression changes (GECs) that occur as toxicity develops are expected to be unique to the mechanism of toxicity (e.g., free radical production, inhibition of cellular respiration). Some GECs are expected to be unique to the type of toxicity, e.g., apoptosis, nongenotoxic carcinogenicity, but common amongst mechanisms that cause the same type of toxicity. Other GECs are expected to be adaptive in response to changes in such things as blood pressure, nutrition, etc. Determination of GECs that reliably indicate the above would allow development of gene expression based biomarkers of toxicity. The most valuable GECs will probably be critical "core" genes that have been conserved during evolution. These GECs will be shared amongst species, greatly improving interspecies comparisons and extrapolations. If gene expression analyses in discovery and toxicology studies identify GECs indicative of toxicity and *in vitro* studies with human and animal cell lines establish that the human gene homologues behave as the animal genes do, the GECs can be used as biomarkers of toxicity in clinical trials and patients. These biomarkers will be especially valuable when they occur in easily assessable tissues, e.g., lymphocytes, and for early detection of chronic toxicity. Although functional and/or morphologic evidence of chronic toxicities may take weeks to years to develop, characteristic GECs likely occur in hours or days. Identification of these GECs would enable early detection of the chronic toxicity, markedly shortening necessary animal studies and protecting clinical trial subjects and patients.

1668 ARSENIC: CARCINOGENIC MECHANISMS, RISK ASSESSMENT AND THE MAXIMUM CONTAMINANT LEVEL (MCL).

K. T. Kitchin[1] and B. Beck[2]. [1]*USEPA NHEERL, Research Triangle Park, NC and* [2]*Gradient Corporation, Cambridge, MA.*

This workshop will provide an up-to-date overview on key issues related to cancer risk assessment of arsenic: carcinogenic mechanisms; application of mechanistic information to risk assessment models; and the development of the MCL for arsenic in drinking water. The two presentations on mechanisms will focus on the possible role of methylated arsenic species as mediators of carcinogenesis. Dr. Kitchin will briefly summarize proposed carcinogenic mechanisms for arsenic and will address the possible role of methylated arsenic metabolites, in particular dimethylarsinic acid (DMA), in carcinogenesis. Dr. Fukushima will present current (and controversial) studies of DMA as a rodent multi-organ tumor promoter and a rat urinary bladder complete carcinogen. The next two presentations will describe risk assessment models for use in quantifying exposures and risks of arsenic. Dr. Mann will describe a physiologically based pharmacokinetic (PBPK) model for inhaled and oral exposures to arsenic, evaluating the validity of this model under different exposure conditions. A biologically-based dose-response model for arsenic based on understanding of arsenic's mode-of-action will be described by Dr. Clewell. Finally, Dr. Abernathy will address the important issue of setting an MCL for arsenic, describing not only the toxicologic and risk issues, but also issues of feasibility and implementation. It is expected that this workshop will be of interest to a broad range of scientists, including basic researchers, risk assessors, and regulators. (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1669 PROPOSED CARCINOGENIC MECHANISMS FOR ARSENIC.

K. T. Kitchin. *USEPA NHEERL, Research Triangle Park, NC.*

Arsenic is a human carcinogen in skin, lung, liver, urinary bladder and kidney. In contrast, there is no experimental animal model of inorganic arsenic carcinogenesis. Proposed mechanisms/modes of action for arsenic carcinogenesis include but are not limited to clastogenic effects, mutation, oxidative stress, gene amplification, altered DNA methylation, cell proliferation, promotion, effects on the progression stage, inhibition of DNA repair and interaction with important cellular proteins. At this time, there is not a scientific consensus on the mechanisms/modes for arsenic carcinogenesis. In regulating human exposure to arsenic, this uncertainty in arsenic's carcinogenic mechanisms/modes makes the risk assessment and extrapolation model selection process considerably more difficult. Recently, dimethylarsinic acid (DMA) has showed both promotional and complete carcinogenic activity in rats and mice. Therefore, arsenate, arsenite, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were administered to rats to determine their biochemical effects. In our laboratory, DMA at 387 mg/kg caused DNA damage in rat lungs, but not in rat liver. Neither arsenate or arsenite damaged rat DNA. Sodium arsenite at doses of 1.6, 8.2 and 24.6 mg/kg increased rat hepatic ornithine decarboxylase activity by 40 to 140%. DNA damage and ornithine decarboxylase induction are interpreted as indicators of initiation and promotion of cancer, respectively. Based on recent experimental findings, for carcinogenesis, methylation of inorganic arsenic may be a "toxification" pathway. The arsenic metabolite DMA may be or may generate an active promoting/carcinogenic chemical(s). (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1670 RODENT CARCINOGENICITY OF DIMETHYLARSINIC ACID, AN ORGANIC ARSENIC METABOLITE.

S. Fukushima, H. Wanibuchi, S. Yamamoto, M. Wei and E. I. Salim. *Osaka City University Medical School, Osaka, Japan.*

Dimethylarsinic acid (DMA) is a major metabolite of inorganic arsenic compounds in most mammals. Promotional effects of DMA were investigated using a rat multi-organ bioassay. The rats were pretreated with 5 initiating carcinogens and then subsequently administered DMA at various concentrations in the drinking water. Significantly increased tumor induction due to promotion of carcinogenesis by DMA was observed in rat urinary bladder, kidney, liver, and thyroid gland. Further experiments with two-stage rat urinary bladder and liver model systems showed that DMA enhanced tumor development in both organs. In a recent complete carcinogen study, male rats received DMA at doses of 0, 12.5, 50 and 200 ppm in the drinking water for 104 weeks. Rat urinary bladder carcinomas were observed in 6 of 31 animals at 50 ppm and in 12 of 31 at 200 ppm. Thus, the results indicate that DMA is a complete carcinogen in rat urinary bladder. In addition, p53 heterozygous knockout and C57BL/6T wild type male mice were given DMA at doses of 0, 50 and 200 ppm in the drinking water for 80 weeks. DMA induced malignant lymphoma in wild type mice. Carcinogenicity of DMA was evident by significantly earlier induction of tumors in both mouse genotypes. Overall, the results indicate that DMA enhanced the development of spontaneous tumors in both types of mice. In conclusion, DMA is a rodent carcinogen and promoter which may be related to the human carcinogenicity of arsenics.

1671 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THE FOUR MAJOR ARSENIC METABOLITES IN ANIMALS AND MAN.

S. Mann[1] and P. O. Droz[2]. [1]*Le Borgeau, Carrouge, Switzerland and* [2]*Lausanne University, Lausanne, Switzerland.* Sponsor: K. T. Kitchin.

The absorption, distribution, metabolism and excretion of arsenate (As(V)), arsenite (As(III)), methylarsonate (MMA) and dimethylarsinate (DMA), the four major metabolites of inorganic arsenic were used to develop a physiologically based pharmacokinetic (PBPK) model. Model routes of arsenic intake were respiratory for dust and fumes and oral for drinking water. Tissue distribution is calculated based on physiological and physico-chemical parameters: blood perfusion, tissue volumes, diffusion coefficients, membrane characteristics, and tissue affinities. Oxidation-reduction reactions between As(V) and As(III) are described by first order kinetic reactions in plasma, and methylation reactions are based on Michaelis-Menten kinetics in the liver. Numerical values are obtained from physiological references and by fitting

the model to *in vivo* studies. Validation of the human arsenic model was carried out by comparison of the results obtained with the human PBPK model to urinary excretion data obtained after exposure of workers to As2O3 and a community exposed to arsenic via drinking water. The PBPK model allows prediction of biological indicator concentrations in human urine (AsV, AsIII, MMA, and DMA). Scenarios are presented for estimation of biological arsenic concentrations under various conditions: for example, exposure of smelter workers to aerosols, workers exposed to coal fly ash and a community exposed to arsenic via drinking water. Finally, the uses and the limitations of the PBPK model will be presented.

1672 REQUIREMENTS FOR A BIOLOGICALLY-REALISTIC CANCER RISK ASSESSMENT FOR INORGANIC ARSENIC.

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Considerable controversy has centered on whether there is epidemiological evidence of a "threshold" for the carcinogenic effects of inorganic arsenic (InAs)i, or at least of a highly nonlinear dose-response. Saturation of metabolism in the dose-range associated with tumors does not appear to be adequate to produce a major impact on the dose-response for carcinogenicity. If there is a strong nonlinearity, it results from the nature of the carcinogenic mechanism(s) of InAsi. A biologically realistic cancer risk assessment for InAsi would require a quantitative description of the dose of active arsenic species in target tissues, the interactions between the active arsenic species and tissue constituents, and the manner in which these interactions result in tumor formation in multiple organs in humans, but not in experimental animals. Although InAsi has only infrequently been associated with tumors in animal studies, it has repeatedly been shown to act as a co-mutagen *in vitro* and as a co-carcinogen *in vivo*. InAsi is clastogenic, producing chromatid aberrations, but does not produce point mutations at single gene loci. Of particular interest, InAsi has been shown to inhibit repair of DNA single-strand breaks, a possible mechanism for its observed co-mutagenicity and co-carcinogenicity. We propose a co-carcinogenic mode of action in which InAsi acts primarily on intermediate cells deficient in cell cycle control at a late stage in a pre-existing carcinogenic process. This interaction enhances genomic fragility and accelerates conversion of pre-malignant lesions to more aggressive, clinically observable tumors. An indirect effect of InAsi on DNA repair is consistent with the expectation of a nonlinear dose-response rather than the linear dose-response traditionally assumed for mutagenic carcinogens. However, defining the exact nature of this tumor dose-response will require further experimental data on the dose-response for the cellular effects of InAsi.

1673 THE ARSENIC (AS) MAXIMUM CONTAMINANT LEVEL (MCL) PROPOSED BY THE USEPA.

C. O. Abernathy, A. Kapadia, J. Bennett, W. Miller, A. Schulman, J. Wu and I. Dooley. *USEPA, Washington, DC.*

The Safe Drinking Water Act, as amended in 1996, directed EPA to propose an arsenic regulation by January 1, 2000. The proposed arsenic regulation includes a maximum contaminant level goal (MCLG), an MCL (which can be measured), a list of feasible treatment techniques for large systems and three size categories of small systems, and a health risk reduction and cost analysis (HRRCA) which identifies costs and benefits likely to occur with each MCL considered. EPA will use the National Research Council report's bladder cancer risk assessment, with an estimated point of departure at the 1% excess cancer risk in humans of approximately 400 :g/L, as the basis for proposing an MCLG. Linear regression analysis of EPA water study data yielded a practical quantitation limit (PQL) of 3 µg/L, which provides a lower bound on the MCL. EPA's national occurrence estimate provided exposure data for the HRRCA. Many treatment factors affected EPA's compliance cost analysis, which was stratified into system size and source types. These include treatment performance, costs, operating complexity, residuals issues, and effects of contaminants on choice of treatment. (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1674 GENE EXPRESSION ARRAY TECHNOLOGY REVEALS UNDERLYING MECHANISMS OF MITOTIC ARREST AND APOPTOSIS INDUCED IN RAT MESOTHELIAL CELL CULTURES BY POTASSIUM BROMATE.

L. M. Crosby[1], K. S. Hyder[2], A. B. DeAngelo[3], L. Yoon[4] and K. T. Morgan[4]. [1]*North Carolina State University, Raleigh, NC,* [2]*Clontech Laboratories, Inc., Palo Alto, CA,* [3]*US Environmental Protection Agency, Research Triangle Park, NC and* [4]*Glaxo Wellcome, Inc., Research Triangle Park, NC.*

Use of gene expression array technology to elucidate mechanisms of toxicity is becoming widespread, but meaningful interpretation of results obtained remains a challenge. We report that potassium bromate (KBrO3), a known rat mesothelial, kidney and thyroid carcinogen and oxidative stressor, produces gene expression changes in mesothelial cells *in vitro* concordant with morphological and biochemical changes. An F344 rat mesothelial cell line was exposed to 6 mM KBrO3 (RNA extraction followed by expression analysis using Clontech Rat Atlas and Rat Stress Arrays and TaqMan; GSH analysis) under standard culture conditions. After 30 min and four hr, when GSH was severely depleted, morphologic analyses showed apoptosis to be significantly increased. Notably, mRNA expression of TNF, FasL and iNOS were also significantly elevated at four hours. Quantitative morphologic evidence of mitotic arrest at four and 12 hr agreed with the observed decreased expression of cyclins B1, C, D1, D2, D3 and E and increased PCNA, Waf1, cyclin G, and GADD45. The oxidative stress-inducible genes HMOX1, NMOR1were highly expressed in KBrO3-treated cells, indicating redox imbalance may play a key role in KBrO3-induced disturbances of the cell cycle. Other changes observed in the >700 genes on these platforms, consistent with the above conclusions, demonstrate (a) the challenge associated with the interpretation of such rich data sets, and (b) that such data represent the beginning and not the end of this research approach. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1675 UPREGULATION OF FAS LIGAND IN VARIOUS TISSUES FOLLOWING TCDD ADMINISTRATION.

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Previous studies from our laboratory demonstrated that 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces apoptosis in thymocytes of C57BL/6 wild-type but not Fas-deficient C57BL/6- lpr/lpr or Fas ligand (L)-defective C57BL/6- gld/gld mice. In the current study, we tested whether TCDD would increase the expression of Fas and/or FasL. When C57BL/6 wild-type mice were injected with 50mg/kg body weight TCDD, there was a significant upregulation of FasL but not Fas in the thymus 1 or 5 days after treatment, as detected by semi-quantitative RT-PCR. On day 5, FasL but not Fas expression also increased in the spleen, lymph nodes, liver, lungs and testes. The upregulation of FasL in the thymus was detected at lower concentrations of TCDD including 0.1,1 and 10 mg/kg body weight. When the organs from wild-type, lpr and gld mice treated with 50mg/kg body weight of TCDD were histopathologically analyzed, on day 5, no significant changes in the lungs, lymph nodes, spleen and testes were observed. The thymus of all three strains exhibited hypocellularity after TCDD exposure. TCDD-treatment also induced hepatocellular vacuolation in wild-type and lpr strains, and to a lesser extent in the gld strain. The TCDD-treated wild-type and gld strains also exhibited multifocal inflammatory cell foci in the liver, which was lacking in the lpr strain. These data indicate that Fas-FasL interactions play an important role in TCDD-induced toxicity. (Supported in part by grants from NIH ES 09098, HL 058641 and AI 01392, Sigma Xi and EPA.)

1676 OKADAIC ACID INDUCED APOPTOSIS AND RESISTANCE IN HT 1080 HUMAN FIBROSARCOMA CELLS.

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Phosphorylation and dephosphorylation are key cellular events that regulate important signaling pathways such as apoptosis and cell cycle progression. Okadaic acid (OA) is an inhibitor of protein phosphatase (PP)-2A and PP-1 and is mitogenic in normal cells but may induce apoptosis in some tumor cells. This study examined the association between p53 protein expression and OA-induced apoptosis in normal wild type (wt) expressing cells and mutant (mt) tumor cell lines. OA at 10-30 nM and the positive apoptotic control, H2O2 at 100-300 µM, both produced DNA ladder formation in wt p53

expressing HT1080 fibrosarcoma cells but not in a chemically-derived mt p53 HT1080 line (6TG c5) or in the p53 null Saos-2 cell line. However, OA-induced apoptosis in HT1080 cells could be prevented by stimulating mitotic pathways by 24 hr pretreatment with IGF-1. In normal wt p53 fibroblasts (IMR-90), only the positive control, H2O2, produced DNA ladders while OA had no apoptotic effect. Western blots from HT1080 cells show that OA up-regulates expression of p53, p21-Waf1, and pro-apoptotic Bax, but decreases Bcl-2, suggesting a biochemical shift favoring apoptosis. We also created an OA-resistant HT1080 subline (OA-R) after 4 months of OA exposure. OA-R cells were resistant to OA-induced apoptosis and showed increased p53 and bcl-2 expression as well as P-glycoprotein compared to parent HT1080 cells. These results suggest OA-apoptosis requires wt p53 expression but may be selective for certain tumor phenotypes like HT1080 fibrosarcoma cells. Furthermore, OA-induced reversal of apoptosis by acute mitogen treatment or by chronic OA exposure suggest an alteration of the p53 pathway or induction of p-glycoprotein can circumvent the effect.

1677 POTENTIAL REGULATION OF MEKK3 KINASE ACTIVITY BY CASPASE-CLEAVED BCL-XL.

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The anti-cancer drug, etoposide, induces apopotosis. However, the signaling proteins involved in this process are unclear. Since the Jun kinase pathway has been implicated in apoptosis signal transduction, our studies have focused on MEKK3, Mitogen-Activated Protein (MAP) Kinase/Extracellular Signal-Regulated Kinase (ERK) Kinase Kinase) which is a protein kinase that activates Jun kinase. The proteins that regulate MEKK3 are not known and the yeast two hybrid system was used to identify such proteins Two known proteins were isolated that interact with MEKK3 in the yeast two hybrid system, 14-3-3epsilon and Bcl-xL. Subsequent experiments have shown that only the caspase-cleaved form of Bcl-xL interacts with MEKK3. One of the functions of the Bcl-xL and 14-3-3epsilon proteins is to maintain cell survival or inhibit apoptosis. An interaction between MEKK3 and these proteins suggests that MEKK3 regulates the apoptotic process. Recently, proteolytic cleavage of Bcl-xL by caspases has been demonstrated in cells treated with apoptotic stimuli such as etoposide or IL-2 deprivation. The hypothesis of our studies is that caspase-dependent cleavage of Bcl-xL produces a protein, Delta 61 Bcl-xL (amino acids 62-233), that functions as an intracellular activator of MEKK3 kinase activity. A consequence of MEKK3 activity is phosphorylation of a downstream kinase, JNKK (Jun Kinase Kinase) and ultimately translocation of c-Jun into the nucleus to activate apoptosis-dependent genes. Future research will characterize the interaction between MEKK3 and caspase-cleaved Bcl-xL in mammalian cells and the possible signaling pathways that may be involved in etoposide-induced apoptosis.

1678 ROLE OF PROTEOLYSIS IN BCL-X_L DEPLETION DURING MK886-INDUCED APOPTOSIS IN FL5.12 CELLS.

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The caspase family of proteases is considered the major regulator of apoptotic death. However, several reports indicate the association of non-caspase serine proteases in apoptosis. Apoptosis induced by the 5-lipoxygenase activating protein (FLAP) inhibitor MK886 is accompanied by the rapid loss of the antiapoptotic bcl-2 and bcl-x_L proteins (Datta *et al., J. Biol. Chem.* **273**: 28163 [1998]). This loss could only partially be prevented by caspase inhibitors. In the current study, the participation of serine, cysteine or aspartic acid proteases in MK886-induced apoptosis and the loss of bcl-x_L protein in the murine pro-B lymphocytic (FL5.12) cell line was investigated. Phenylmethylsulfonyl fluoride (PMSF), pepstatin and leupeptin were capable of significantly inhibiting MK886-induced apoptosis. Disappearance of bcl-x_L from FL5.12 cells upon MK886 treatment was completely prevented by pretreatment with these inhibitors. Pretreatment with leupeptin, pepstatin or PMSF blocked about half the increase in caspase-3 activity spurred by MK886. None of the inhibitors were able to directly inhibit active caspase-3 activity suggesting they were preventing the activation of this caspase. The broad-spectrum caspase inhibitor, Boc-D-FMK, inhibited all markers of MK886-mediated apoptosis including caspase-3 activity in cell lysates *in vitro*. Together, these observations suggest the involvement of either serine cysteine or aspartic acid proteases, upstream of active caspase-3, in the loss of bcl-x_L protein and in the signaling pathway of MK886-induced apoptosis in FL5.12 cells. (Supported by HL51005 and Center Grant ES07784.)

1679 DIFFERENTIAL INVOLVEMENT OF CASPASES IN HYDROQUINONE-INDUCED APOPTOSIS IN HUMAN LEUKEMIC HL-60 AND JURKAT T CELLS.

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Hydroquinone (HQ), a major metabolite of benzene is known to induce apoptosis in hematopoietic cells. We examined the role of caspases in HQ induced apoptosis in MPO-rich HL-60 human promyelocytic leukemia and MPO-deficient Jurkat T cells. Treatment of HL-60 and Jurkat cells with HQ (75 μM and 50μM respectively) resulted in apoptosis as assessed by ultrastructural, flow cytometric and biochemical apoptotic changes, including phosphatidylserine (PS) exposure, activation of caspases-3 and -7, cleavage of poly(ADP-ribose)polymerase (PARP) and DNA. In HL-60 cells, pre-treatment with the caspase inhibitor Z-VAD.FMK blocked caspases-3 and -7 processing and cleavage of both PARP and DNA, but PS externalisation, nuclear morphology and cytoplasmic changes were relatively unaffected. Interestingly, pre-treatment with the caspase-3 specific inhibitor, Z-DEVD.FMK, did not block any apoptotic features in these cells. In marked contrast, in HQ-treated Jurkat cells, all ultrastructural, flow cytometric and biochemical changes associated with apoptosis were completely inhibited by pre-treatment with Z-VAD.FMK. Pre-treatment with Z-DEVD.FMK blocked caspases-3 and -7, cleavage of PARP and DNA, but PS exposure and ultrastructural nuclear changes were only partially blocked. We propose that HQ induces apoptosis via a caspase-independent pathway in HL-60 cells but requires caspase activation in Jurkat cells. The different mechanisms of apoptosis in HL-60 and Jurkat cells may reflect the MPO activity of these cells and the amount of reactive 1,4-benzoquinone that is generated. (Supported by HHS ES 09554 and EPA R825281010.)

1680 REGULATION OF HEPATOCYTE SURVIVAL: DIVERSE ROLES FOR MAP KINASE AND AKT (PKB) MEDIATED PATHWAYS.

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Perturbation of hepatocyte growth regulation is associated with a number of liver diseases such as fibrosis and cancer. These diseases are mediated by a network of growth factors and cytokines that regulate the induction of hepatocyte proliferation and apoptosis. In this study, we have investigated the role of signalling pathways activated by tumor necrosis factor α (TNFα) and epidermal growth factor (EGF) in the regulation of apoptosis induced by transforming growth factor β1 (TGFβ1) since this physiological factor is believed to regulate spontaneous apoptosis in the liver. We show that while both EGF and TNFα can suppress apoptosis in isolated rat hepatocytes, they act by engaging different protein kinase signalling pathways. Suppression of apoptosis by EGF is dependent on activation of PI3 kinase and the ERK MAP kinase pathways but not p38 MAP kinase. In contrast, TNFα does not require PI3 kinase and Akt (PKB) - mediated pathways but is dependent on ERK and p38 MAP kinases to suppress apoptosis. These data contribute to our understanding of the intracellular survival signals which play a role in normal liver homeostasis and in diverse pathological conditions.

1681 SALICYLATE ENHANCES NECROSIS AND TNFα-INDUCED APOPTOSIS MEDIATED BY THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) IN RAT HEPATOCYTES.

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Salicylate, the major metabolite of aspirin, lowers the threshold for onset of the MPT in isolated liver mitochondria (JPET 278:1000). At higher concentrations, salicylate causes MPT-mediated necrotic death of cultured hepatocytes (TAP 147:431). The MPT also mediates hepatocyte apoptosis after tumor necrosis factor-alpha (TNFα) and necrotic cell killing after oxidative stress and Ca^{2+} ionophore (Molec Cell Biol 18:6353; Biochem J 307:99; TAP 154:117). Accordingly, our aim was to determine whether non-toxic salicylate concentrations potentiate MPT-mediated cell killing by other agents. Cultured rat hepatocytes were exposed to Br-A23187 (Ca^{2+} ionophore, 0.5-5 μM) or *tert*-butylhydroperoxide (*t*-BuOOH, 5-50 μM), and cell killing was assessed by propidium iodide fluorometry. *t*-BuOOH and Br-A23187 both caused dose-dependent cell killing. After 1 μM Br-A23187, cell killing after 1 hr increased from 14.6% to 25.0, 41.6 and 55.3% in the presence of 0.1, 0.5, and

1 mM salicylate, respectively. Cell killing was prevented by cyclosporin A (1-10 μM), a specific MPT inhibitor, and salicylate by itself was not cytotoxic. Salicylate also potentiated the toxicity of 20 μM *t*-BuOOH from 30.9% to 46.4, 58.6 and 74.1% after 0.1, 0.5 and 1 mM salicylate. In hepatocytes infected with IκB adenovirus, TNFα apoptotic cell killing of 12.5, 35.2, 54.5 and 77.6% after 12, 16, 20 and 24h. Salicylate (1 mM) increased this cell killing to 38.5, 65.9, 77.2, and 83.2%, respectively. Salicylate also accelerated TNFα-induced caspase 3 activation from 105, 121 and 308% of control after 8, 12 and 16 hr to 169, 264 and 389%. In conclusion, salicylate potentiated both necrotic and apoptotic cytotoxicity mediated by the MPT. Thus, aspirin has the potential to enhance the hepatotoxicity of xenobiotics. Promotion of MPT-mediated apoptosis may also account for salicylate-induced potentiation of anticancer drug cytotoxicity and the protection of aspirin against colon, lung and breast cancer.

1682 POST-TRANSLATIONAL MODIFICATION OF P53 FOLLOWING TREATMENT WITH PSORALEN AND UV-A RADIATION (PUVA).

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The p53 protein participates in the regulation of several cellular processes, including cell cycle arrest, DNA repair, and apoptosis. p53 is regulated at the post-translational level and it appears to exist in a latent, inactive form that requires modification to become activated. Phosphorylation of p53 on specific residues can apparently modulate its activity. A number of studies have shown that cells treated with different DNA-damaging agents exhibit different patterns of phosphorylation. The generation of cleavage products may be another mechanism for regulating the activity of p53. Using cell-free systems, investigators have shown that treating p53 protein with single-stranded DNA, damaged DNA, or proteases results in the generation of cleavage products. In this study, we determined whether wild-type or mutant p53 exhibit different patterns of phosphorylation following treatment with PUVA. The aim of these studies was to elucidate the functional relevance of different phosphorylation patterns with respect to cell cycle arrest and/or apoptosis. Immortalized mouse keratinocytes (JB6) that have wild-type p53 and two mouse skin tumor cell lines containing mutant p53 were used for these studies. Following treatment, gel electrophoresis was performed and the blots were probed with several N- and C-terminus phosphoserine-specific antibodies. The results indicated that p53 was phosphorylated following PUVA treatment in JB6 cells while it was constitutively phosphorylated in the tumor cell lines. In addition to phosphorylation of p53, PUVA treatment of JB6 cells resulted in the generation of two cleavage fragments that were each missing the N-terminus. However, only one tumor cell line exhibited both fragments constitutively. Interestingly, treatment of JB6 cells with UV-C did not result in the generation of cleavage products. These results suggest that PUVA treatment causes phosphorylation and cleavage of p53 in JB6 keratinocytes. It is possible that these post-translational modifications in p53 play a role in PUVA-induced apoptosis.

1683 THE ROLE OF DNA MISMATCH REPAIR, P53 AND OTHER SIGNALING PATHWAYS IN DNA DAMAGE-INDUCED APOPTOSIS.

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All cells are unavoidably exposed to chemicals that can alkylate DNA to form genotoxic damage. Among the various DNA lesions formed, O6-alkylguanine lesions can be highly cytotoxic and we recently demonstrated that O6-methylguanine (O6MeG) and O6-chloroethylguanine (O6CEG) specifically initiate apoptosis. Here we show that the MutS-alpha branch of the DNA mismatch repair (MMR) pathway (but not the MutS-beta branch) is absolutely required for signaling the initiation of apoptosis in response to O6MeGs, and partially required for signaling apoptosis in response to O6CEGs. Further, O6MeG lesions signal the stabilization of the p53 tumor suppressor and such signaling is also MutS-alpha-dependent. Despite this, MutS-alpha-dependent apoptosis can be executed in a p53-independent manner. This experimental system will allow dissection of the signal transduction events that couple a specific type of DNA base lesion with the final outcome of apoptotic cell death. Currently, we are examining the role of p53-independent signaling pathways in this process.

1684 DELINEATION OF THE GLUCOCORTICOID-INDUCED APOPTOTIC PATHWAY IN RAT THYMOCYTES.

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In primary rat thymocytes, glucocorticoids induce a rapid apoptotic response, which is dependent upon gene expression and involves the activation of the caspase cascade. However, these conclusions were based largely on studies that analyzed DNA fragmentation, a late event in apoptosis. To determine whether upstream components of the glucocorticoid-induced apoptotic pathway are also dependent upon gene expression, we have now conducted a multiparametric analysis of cell viability, cell shrinkage, the loss of mitochondrial membrane potential, and externalization of phosphatidyl serine in the absence and presence of protein and RNA synthesis. Because caspases are activated during glucocorticoid induced apoptosis, we also analyzed the role of caspase activity in glucocorticoid-induced cell death. We show that the glucocorticoid-induced loss of viability, phosphatidyl serine externalization, and cell shrinkage in rat thymocytes are dependent upon both macromolecular synthesis and caspase activity. Interestingly, the loss of mitochondrial membrane potential was also dependent upon macromolecular synthesis in glucocorticoid-induced death, but was independent of caspase activity. We have also shown that glucocorticoids activate both caspase-9-like and caspase-8-like activity within thymocytes, the latter finding suggesting that glucocorticoid-induced apoptosis of rat thymocytes may also involve components of the Fas cell death pathway. In this regard, we show that glucocorticoids can impede Fas-mediated apoptosis, suggesting that glucocorticoids may act distal to the Fas receptor to induce thymocyte apoptosis.

1685 N-ACETYLSPHINGOSINE (C2-CERAMIDE) DISRUPTION OF LYSOSOMES AND CATHEPSIN B DISTRIBUTION.

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Exposure to N-acetylsphinogosine (C2-ceramide) triggers apoptosis in a variety of cell lines. The basis for this response is not known. The murine hepatoma cell line 1c1c7 undergoes apoptosis following exposure to C2-ceramide. Caspase-3 activation occurs within 12 hr of treatment, but does not reach its maximum (~500-fold increase) until 18-20 hr post treatment. DNA laddering is detectable within 16 hr of treatment and readily observed in an additional 8 hr. The fluorescent probe MTODT was used to analyze mitochondria respiration and membrane potential. MTODT staining was weakly diminished in some cells 6 hr post C2-ceramide exposure. Staining progressively, but slowly, diminished thereafter. Analyses with the fluorescent probes acridine orange and LysoTracker™ demonstrated coalescence of acidic organelles/lysosomes within 3 hr of treatment. Coalescence was complete by 6 hr. Thereafter, the fluorescent signals with these probes diminished with increasing treatment time. Analyses with an antibody to human pro- and processed cathepsin B revealed a punctate, peri-nuclear staining pattern in solvent-treated cultures. Within 3-6 hr of C2-ceramide treatment cathepsin B staining became diffuse and throughout the cell. *In situ* enzymatic analyses revealed a time-dependent progressive increase in cytoplasmic cathepsin B activity beginning 3 hr post C2-ceramide treatment. These studies raise the issue of whether C2-ceramide triggered apoptosis in 1c1c7 cells is initiated by released lysosomal enzymes. Such enzymes might facilitate the activation of procaspase 9 by damaging mitochondria and causing the release of cytochrome c. (This work was supported by grant ES09392.)

1686 1,2-DIACETYLBENZENE: A NEUROTOXIC & CHROMOGENIC AROMATIC HYDROCARBON.

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Isomers of diethyl benzene have been selected to initiate studies on the neurotoxic potential of aromatic hydrocarbons. Occupational exposure to 1,2-diethylbenzene (1,2-DEB) and 1,3-diethylbenzene (1,3-DEB) occurs in workers handling jet fuels, such as JP4 and JP8. 1,2-Diacetylbenzene (1,2-DAB), the putative metabolite of 1,2-DEB, reacted differentially with amino acids and proteins to form a blue chromogen that was not seen in comparable experiments with 1,3-DAB (Kim et al., this meeting). Male Sprague-Dawley rats

were treated intraperitoneally with 10 or 20 mg/kg/day 1,2-DAB 5 days/week for 30 and 9 days, respectively. Animals developed hindlimb weakness and blue discoloration of tissues, including brain, spinal cord and peripheral nerves. Lumbar spinal motor neurons and corresponding dorsal root ganglia exhibited pathological changes in the form of proximal axonal swellings filled with 10-nm neurofilaments. Sequestration of axonal organelles by adaxonal oligodendrocyte or Schwann cell cytoplasmic processes was observed in the central and peripheral nervous system, respectively. By contrast, rats treated comparably for 30 days with 1,3-DAB lacked tissue discoloration and showed no behavioral or pathological evidence of neurotoxicity. These data demonstrate a close relationship between the neurotoxic and chromogenic properties of diethyl benzene and suggest chromogenicity could serve as a biological marker of human exposure to the neurotoxic isomer. 1,2-DAB, but not 1,3-DAB, shares the molecular arrangement (gamma-diketone) and neuropathological property (induction of proximal neurofilamentous axonopathy) of aliphatic hydrocarbons (e.g., 3,4-dimethyl-2,5-hexanedione) that have high mammalian neurotoxic potency.

1687 DIACETYLBENZENE: AMINO ACID, PROTEIN AND ENZYME REACTIVITY.

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We have reported a relationship between the neurotoxic and chromogenic properties of the isomers of diacetylbenzene, the first of several chromogenic aromatic hydrocarbon compounds to be examined for neurotoxic properties (Kim et al., this meeting). 1,2-Diacetylbenzene (1,2-DAB), a gamma diketone-like neurotoxic isomer that blocks neurofilament transport in central and peripheral nerve fibers, forms a blue chromogen with certain amino acids *in vitro*. Of 20 amino acids (0.1 M) tested with 1,2-DAB (1 mM, pH 7.0, 37OC, 1 h), glycine and basic amino acids (lysine > histidine > asparagine) formed strong chromogenic reaction products. Proline, valine, tyrosine, and isoleucine were least reactive. 1,3-Diacetylbenzene (1,3-DAB), a non-chromogenic and non-neurotoxic isomer reacted modestly only with asparagine and glutamine. Protein reactivity of 1,2-DAB (but not of 1,3-DAB) was evident from the formation of a blue color with crystalline glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and, to a lesser extent, with lactate dehydrogenase (LDH). Incubation of these glycolytic enzymes with 125, 250, 500 or 1000 micromolar concentrations of 1,2-DAB produced, respectively, ~10%, ~20%, ~40% and ~60% inhibition of the enzyme activity of GAPDH but not of LDH, which was essentially unaffected. Similar concentrations of 1,3-DAB had no significant effect on the activity of GAPDH or LDH. These data demonstrate that 1,2-DAB, one of several aromatic hydrocarbons reported to have chromogenic properties, reacts differentially with amino acids and proteins. 1,2-DAB showed a time-dependent, concentration-dependent and selective inhibition of GAPDH enzyme activity. The failure of 1,2-DAB to react with glutathione suggests that inhibition of GAPDH activity is unrelated to thiol groups in the protein.

1688 ACUTE AND SUBCHRONIC NEUROTOXICOLOGICAL EVALUATION OF TETRAHYDROFURAN BY INHALATION IN RATS.

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The potential neurotoxicity of THF was evaluated in Crl:CD®BR male and female rats by acute or subchronic exposure followed by a battery of functional tests designed to assess nervous system dysfunction and neuropathology. Acute exposure concentrations were 0, 500, 2500, or 5000 ppm for 6 hours. Behavioral sedation, as indicated by diminished startle response to an unexpected auditory stimulus, occurred during exposure to 2500 and 5000 ppm. Immediately after removal from the inhalation chambers, rats in the 5000 ppm group exhibited lethargy, abnormal gait or mobility, slow or absent righting reflex, and splayed rear feet. A few animals (not statistically significant) exposed to 2500 ppm exhibited lethargy and splayed rear feet. Females exposed to 5000 ppm had reduced number and duration of movements after exposure on test day 1. No compound-related effects were observed during observations conducted on or after day 2 post-exposure. During the 14-week subchronic study, rats were exposed 6 hours per day, 5 days per week to 0, 500, 1500, or 3000 ppm. Neurobehavioral evaluations occurred on non-exposure days at approximately monthly intervals. Transient sedation, which appeared to be a daily reinstatement of acute sedation, was observed during exposure to concentrations of 1500 and 3000 ppm. There were no other compound-related effects on any neurobehavioral parameter or any morphological changes in nervous system tissues. The NOEL for both the acute and subchronic studies was 500 ppm in males and females. This project was sponsored by the THF Task Force.

1689 NEUROBEHAVIORAL ASSESSMENT OF HYDROCARBONS AND ITS APPLICATION TO THE DEVELOPMENT OF OCCUPATIONAL EXPOSURE LIMITS FOR HYDROCARBON SOLVENTS.

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A neurobehavioral testing program on aliphatic, cycloaliphatic, and aromatic hydrocarbons was conducted to develop a database on acute hydrocarbon solvent neurotoxicity. The data from this research program provide additional information for setting occupational exposure limits (OELs) for hydrocarbon solvents. Twelve hydrocarbons or hydrocarbon mixtures, with carbon chain lengths ranging from C5-C11, were tested for acute neurotoxicity. Male WAG/RijCrlBR rats were exposed by inhalation for 8 hr on 3 consecutive days and evaluated daily for effects on motor activity, functional observation measures, and learned performance of a visual discrimination task. C5 aliphatics (pentane, isopentane, cyclopentane) did not cause significant effects at exposures up to 20 g/m^3, and C7/C8 aliphatics (methylcyclohexane, n-octane, iso-octane) did not cause any significant effects at exposures up to 14 g/m^3. n-Decane caused some limited, but statistically significant neurobehavioral effects at 5 g/m^3, with no effects at 1.5 g/m^3. A commercial solvent containing a mixture of C9 aromatics caused significant neurobehavioral effects at 5 g/m^3 and reversible effects on learned performance at 1 g/m^3, with a NOAEL of 0.2 g/m^3. These data will be used with existing toxicological data on hydrocarbon solvents to recommend occupational exposure limits for the whole range of complex hydrocarbon solvents.

1690 INVESTIGATION OF THE EFFECTS OF REPEATED EXPOSURE TO JP-8 VAPOR ON THE COGNITIVE CAPACITY OF RATS.

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Forty-eight male Sprague-Dawley rats were exposed by whole body inhalation to JP-8 jet fuel vapor (1000 mg/m3 + 10%) or room air control conditions for 6 hr/day, 5 days/wk for 6 consecutive weeks (180 hr). No physiological or neurobehavioral deficits were observed, although jet fuel-exposed rats gained body weight at a lower rate (not significantly different) than controls. Following a 60-day recovery period, rats were trained successively on increasingly complex operant schedules of reinforcement from CRF to successive lever press, in which rats were required to learn a new response sequence to a 3-lever array during each operant session. The effects of repeated JP-8 vapor exposure are reported for cognitive performance capacity on tasks ranging from simple acquisition of lever-pressing behavior to development of complex cognitive strategies.

1691 PBPK MODELING OF PEAK EXPOSURES TO WHITE SPIRIT.

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Ocupational exposure limits are often 8-hour time-weighted averages (TWAs), but, in the workplace exposures fluctuate and TWAs may be exceeded for short periods of time. To assist in using animal data to set occupational exposure levels, we conducted toxicokinetic studies in rats and in human volunteers to develop a physiologically based pharmacokinetic (PBPK) model for two constituents of white spirit (WS), namely 1,2,4-trimethylbenzene (TMB) and n-decane (nDEC), representing the aromatic and aliphatic fractions. The model then compared the kinetic behavior of TMB and nDEC in blood and brain following different WS exposure scenarios, all equivalent to the 8-hour TWA value for WS (100 ppm). The modeling studies showed

that concentrations of these hydrocarbons may be higher in brain than in blood regardless of exposure scenario with a brain:blood ratio higher for nDEC than for TMB. External exposure scenarios involving short exposures above the TWA value are expected to produce concentrations in brain and blood that are higher than those from a constant exposure when total doses (i.e., ppm x hours) are equal. However, the expected increase is relatively less than the increase in external exposure levels. Finally, with respect to total dose, external exposures involving short exposures higher than the TWA value are not expected to significantly affect the area under the concentration vs. time curve (AUC) for the parent compound unless metabolism is saturated.

1692 METHYL *TERTIARY*-BUTYL ETHER (MTBE) AND RELATED COMPOUNDS INHIBIT BINDING AT A RECOGNITION SITE OF THE $GABA_A$ RECEPTOR IN MEMBRANES FROM RAT BRAIN.

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A major concern in the use of methyl *t*-butyl ether (MTBE) as a gasoline additive is the risk of neurological signs and symptoms after exposure. Since a variety of alcohols and ethers are known to modify aspects of the $GABA_A$ receptor function, we tested MTBE and related compounds for effects on binding of tritiated *t*-butyl-bicycloorthocarboxylate (^{3}H-TBOB), a ligand for the convulsant site of the complex. Whole forebrains of male albino rats were homogenized in 500 mM sodium chloride including 50 mM potassium phosphate buffer (pH 7.4), and the membrane fragments were frozen at -70°C and washed repeatedly by centrifugation and resuspension in newly added salt-buffer solution. Varying amounts of the compounds were incubated with ^{3}H-TBOB and resuspended membranes for 60 min at 25°C. Membrane-bound radioactivity was determined after filtering the membrane suspensions in a vacuum manifold and washing the filters twice. Data were corrected for non-specific binding in the presence of 10μM picrotoxin. All the ethers and alcohols tested were inhibitory of ^{3}H-TBOB binding at 30-300 mM concentrations. The most potent of the compounds tested was *t*-amyl alcohol (TAA), followed by ethyl *t*-butyl ether (ETBE), *t*-amyl methyl ether (TAME), *t*-butyl alcohol (TBA), and methyl *t*-butyl ether (MTBE), in descending rank order of potency. Generally, then, the potency of inhibition correlated roughly with the carbon chain length, and, for compounds with equal numbers of carbons, the inhibition of ^{3}H-TBOB was greater for the alcohols than for the ethers. In additional studies, MTBE was found to have primary effects on ^{3}H-TBOB receptor density (B_{max}) rather than affinity (K_d). (Support: NSF IBN-9809943.)

1693 BEHAVIORAL EFFECTS OF TRICHLOROETHYLENE (TCE): EXPOSURE VS. MODEL PREDICTIONS.

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The hazard from inhaled volatile organic compounds is presently assessed in terms of lifetime exposure to the average vapor concentration (C). This strategy yields rational predictions of risk if the product of C and the exposure duration (t) yields a constant effect. This assumption was tested by assessing the acute behavioral effects of inhaled TCE vapor in rats in terms of C, t and the concentration of TCE in the target organ (brain), $[TCE]_{Br}$. Adult male Long-Evans rats (n=11) were trained to perform a signal detection task in which a press on one lever produced food on trials with a signal (a 300-msec light flash); a press on a second lever produced food on trials lacking a signal. Proportions of correct detections of the signal [P(hit)], proportions of errors on non-signal trials [P(fa)] and response time (RT) were calculated at 20-min intervals during repeated daily 70-min exposures conducted in air containing 0, 400, 800, 1200, 1600, 2000, or 2400 ppm TCE. Each measure remained stable during tests in air. In TCE, P(hit) declined, and P(fa) and RT increased with increasing C and t; these effects depended more on C than t. The effects of TCE were larger during early exposures to TCE than during the later exposures. $[TCE]_{Br}$ at times of behavioral testing was estimated with a PBPK model for TCE using a fat volume and cardiac output appropriate for the tested rats. $[TCE]_{Br}$ accurately predicted the behavioral effects of TCE during both early and late exposures. Thus, behavioral effects of TCE depended more closely on the internal dose of TCE than on either its inhaled concentration (C) or cumulative inhaled dose (C × t product). (This abstract does not necessarily reflect U.S. EPA policy.)

1694 TIME COURSE OF TOLERANCE TO REPEATED INHALATION OF TRICHLOROETHYLENE (TCE) IN RATS.

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Previous work showed that tolerance to the organic solvent trichloroethylene (TCE) develops in rats performing a visual signal detection task (SDT) while inhaling TCE. The present experiment was designed to assess the persistence of this tolerance. In Phase 1, two groups of adult male Long-Evans rats with equivalent accuracy on the SDT inhaled TCE at 2000 ppm in daily 1-hour sessions for 2 weeks (Monday - Friday). One group performed the SDT during TCE exposure (Paired) while the other group performed the SDT prior to daily TCE exposure (Unpaired). The Paired group showed a decrease in accuracy and an increase in response time (RT) on Day 1; these effects abated during the two weeks of exposure, displaying the development of tolerance. In contrast, the Unpaired group maintained their pre-exposure baseline accuracy and RT. In Phase 2, all rats were challenged with SDT tests in 2000 ppm TCE on Days 1, 7, 14, 28, and 56 after termination of Phase 1. All rats performed the SDT in air only on the days between each challenge. The Unpaired rats showed reduced accuracy and increased RT during each challenge test. The RT of the Paired rats remained unaffected by TCE until Day 56, whereas their accuracy progressively deteriorated after Day 7. Thus the tolerance in the Paired group lasted from 7 to 28 days after induction, depending on the index measured. In contrast, the Unpaired group showed no beneficial effects from the pre-exposure to TCE received in Phase 1 on accuracy or RT during Phase 2. (This abstract does not necessarily reflect EPA policy.)

1695 ACUTE NEUROTOXIC EFFECTS OF INHALED TOLUENE ON PATTERN VISUAL EVOKED POTENTIALS AS A FUNCTION OF EXPOSURE AND ESTIMATED BLOOD AND BRAIN CONCENTRATION.

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The effects of acute inhalation exposure to the volatile organic compound trichloroethylene (TCE) are related to the target tissue concentration of TCE at the time of testing (Boyes et al., Toxicologist 48:291, 1999). The current studies examined exposure to another VOC, toluene, for comparison to the previous results with TCE. A physiologically-based pharmacokinetic (PBPK) model was developed for ad-lib feed (350-450 g) adult male Long-Evans rats. Rats were exposed by inhalation to 3,000 ppm toluene for 1.3 hr, or 4,000 ppm toluene for 1 hr. Both conditions gave constant C x t products of about 4,000 ppm-hrs. Pattern-elicited visual evoked potentials were recorded before and at the end of the exposure period. The pattern stimulus was a 0.16 cpd vertical sinusoidal grating with a contrast of either 60% or 0% ("noise" evaluation), that was modulated in an appearance/disappearance mode at 5 Hz. Exposure to toluene, like TCE, reduced the amplitude of the frequency-double spectral component (F2) without changing response amplitude at the primary stimulation rate (F1). The mean±SEM F2 amplitude was reduced from 11.2±1.6 to 3.8±0.4 uV at 3000 ppm, and from 13.2±2.3 to 4.7±0.7 uV at 4000 ppm. At the time of VEP testing, blood and brain concentrations of toluene were estimated to be approximately 68 and 152, or 81 and 182 mg/l, following exposure to 3,000 or 4,000 ppm, respectively. Toluene appears to reduce VEP F2 amplitude at roughly similar brain concentrations as did TCE. Further research at lower concentrations is planned to more fully examine this relationship. (This abstract does not necessarily reflect EPA policy.)

1696 ACUTE BEHAVIORAL EFFECTS OF n-DECANE, 1,2,4-TRIMETHYLBENZENE AND WHITE SPIRIT IN RATS IN RELATION TO BRAIN AND BLOOD LEVELS.

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Studies are being conducted to assist in setting occupational exposure limits for hydrocarbon solvents. The current series examined the acute behavioral effects of inhalation exposure to white spirit (WS: –100, 400, 800 ppm), a complex substance, and two WS constituents, 1,2,4-trimethylbenzene (TMB: –25, 250, 1000 ppm) and *n*-decane (nDEC: –85, 260, and 860 ppm). Rats were exposed 8 hours/day for 3 consecutive days in two experiments. Exp. I included functional observational battery (FOB) and motor activity (MA) assessments; Exp. II examined effects on two-choice visual discrimination performance (VisDisc). In separate experiments, brain and blood concentra-

tions were determined to validate toxicokinetic models for nDEC and TMB alone and as components of WS. FOB testing indicated changes in gait and body temperature for the high WS group and changes in forelimb grip strength in the high nDEC group. MA was reduced in a dose-responsive manner in WS- and TMB-exposed rats. All three substances produced dose-responsive psychomotor slowing in the visual discrimination test. Clearance rates of nDEC and TMB increased in WS- and TMB-exposed rats over the 3-day exposure period. In contrast, exposure to n-DEC did not affect its kinetics. It is hypothesized that these differences are due to induced metabolism by TMB and other WS consitutents but not n-DEC. The no-effect level (NOELs) for neurobehavioral effects were 0.6 g/m^3 (100 ppm) for WS, 1.25 g/m^3 (250 ppm) for TMB, and 1.5 g/m^3 (260 ppm) for nDEC.

1697 PBPK MODELING OF *n*-DECANE AND 1,2,4-TRIMETHYLBENZENE ALONE OR AS CONSTITUENTS OF WHITE SPIRIT.

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A physiologically-based pharmacokinetic (PBPK) model, was developed for white spirit (WS), n-decane (nDEC) and 1,2,4-trimethylbenzene (TMB) to facilitate the use of animal data in setting occupational exposure levels for hydrocarbon solvents. Blood and brain levels of nDEC and TMB, as representative of the aliphatic and aromatic WS constituents, were measured in rats. Parameters which described the saturable metabolism of nDEC and TMB (K_m and V_{max}) which were obtained by best simultaneous visual fits of the blood kinetics in the rat were 20 mg/kg/hr for V_{max} and 0.1 mg/l for K_m for nDEC and 3.5 mg/kg/hr and 0.25 mg/l for TMB. The simulated brain concentrations of these two compounds were consistent with measurements. The rat model was then allometrically scaled to humans. The predicted concentrations of TMB and nDEC in blood and expired air in humans were compared to those measured during and following a 4-hour exposure to WS at 100 ppm. For TMB, good agreement was found. The model tended to underestimate nDEC blood levels during WS exposure, but simulations agreed well with the data following the termination of exposure. Because the white spirit model, did not accurately simulate the rat blood and brain kinetics for nDEC and TMB, the maximum capacity of biotransformation parameter was increased. This suggests competition for metabolism of TMB and DEC by other WS constituents.

1698 SUPPRESSION SUBTRACTION HYBRIDIZATION IDENTIFIES GENES DIFFERENTIALLY EXPRESSED IN ETHYLENE GLYCOL MONOMETHYL ETHER (EGME)-INDUCED TESTICULAR APOPTOSIS.

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The solvent Ethylene Glycol Monomethyl Ether (EGME) produces the same testicular lesions in rodents and human testis cultures, which is characterized by apoptosis of pachytene spermatocytes. To identify gene changes early in the lesion and determine the possible involvement of cells other than the spermatocytes, we employed a suppression subtractive hybridization technique using whole testes from mice treated 8 hours previously with 500 mg/kg EGME, to generate two subtracted mouse testis cDNA libraries enriched for gene populations either upregulated or downregulated by EGME. A total of seventy clones were screened, and six of them were shown by Northern blotting to be differentially expressed in the EGME lesion. The three clones with increased expression after EGME were one identical to t-complex testis expressed gene 1 (tctex1), a gene encoding ribosomal protein S25 and a heretofore uncharacterized mouse testis EST 774, while three other genes suppressed by EGME were tctex2, alpha-2,6-sialyltransferase (STF) and another uncharacterized mouse testis EST 842. These clones contain multiple motifs for phosphorylation, glycosylation and myristoylation. Anti-sense RNA probes were generated, and in situ hybridization with these probes localized the expression changes of these six clones in multiple germ cell stages as well as other cell types: Sertoli cell (upregulated: S25, EST 774; downregulated: tctex2, EST 842), the interstitial cell (upregulated: tctex1; downregulated: STF). These data at the gene expression level are the first to demonstrate the early involvement in this lesion of cell types other than the dying spermatocytes, and yield new insights into the early responses to this testicular toxicant.

1699 LACK OF EFFECTS OF CYCLOSPORIN A ON SPERM MOTILITY AND TESTICULAR SPERMATID HEAD COUNTS IN WISTAR AND SPRAGUE-DAWLEY RATS.

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Cyclosporin A is an immunosuppressive drug derived from soil fungi that is often used in organ transplantation. Based on previous reports of adverse effects on male reproduction in Sprague Dawley rats, the present study was designed to use Cyclosporin A as a positive control compound for effects on sperm motility. Groups of 3-4 Wistar or Sprague Dawley rats were given daily s.c. doses of 0, 5, 10, or 20 mg/kg Cyclosporin A for 14 days. At termination, male reproductive organs were weighed, sperm motion parameters were analyzed using CASA, and testicular spermatid head counts were determined. In contrast to previous reports, 20 mg/kg Cyclosporin A produced significant body weight loss, morbidity and mortality in Wistar rats within 9 days of dosing, but had no consistent effect on sperm parameters or testis and epididymis weights in surviving animals. Doses of 5 and 10 mg/kg were tolerated with minimal effects on body weight in Wistar rats, and had no significant effects on testis and epididymis weights, sperm motility and velocity (VCL), or testicular spermatid head counts. Absolute and relative accessory sex organ weights were significantly reduced by 11% - 52% at all doses. In order to determine if the lack of effects on sperm parameters was due to strain differences, Sprague Dawley rats were given doses of 10 or 20 mg/kg Cyclosporin A. All animals survived, and body weights were significantly reduced (11%-24 %). As in Wistar rats, there were no significant effects on testis and epididymis weights, sperm motility and velocity, or testicular spermatid head counts. Accessory sex organs weights were reduced by 28% and 50% at 10 and 20 mg/kg, respectively; relative weights were reduced by 20% and 35%. These results suggest a strain difference between Wistar and Sprague Dawley rats in sensitivity to the general toxicity of Cyclosporin A, but no specific effect on testis or epididymis weights, or sperm parameters. However, the effects on accessory sex organs weight could suggest an adverse effect on androgen status.

1700 DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) CAUSES FORMATION OF GIANT MULTINUCLEATED GONOCYTES AND INHIBITS SERTOLI CELL PROLIFERATION BY SUPPRESSING CYCLIN D2 EXPRESSION IN THE NEONATAL RAT TESTIS.

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In this study, we explored the effects of di-(2-ethylhexyl) phthalate (DEHP) and the underlying mechanism in the neonatal rat testis. Three-day-old rats received a single oral dose of corn oil (control) or 20-500 mg/kg DEHP or 500 mg/kg of di-ethyl phthalate (DEP). The morphology of the testis was evaluated with light microscopy and proliferation of Sertoli cells was quantified by BrdU-labeling. Exposure to 200 or 500 mg/kg DEHP caused formation of giant gonocytes, each containing 2-4 apparently normal nuclei, while Sertoli cells appeared morphologically normal and there were no indications of cell death up to 48 hr after dosing. However, the percentage of BrdU-labeled Sertoli cells in pups 24 hr after treatment with 100, 200, or 500 mg/kg of DEHP was decreased to 20.83, 9.95% or 4.13%, respectively, compared to 27.03% of the control group ($p<0.05$). When serum FSH was measured by RIA, we found no significant difference between controls and pups treated with 200 or 500 mg/kg DEHP. Exposure to 20 mg/kg DEHP or 500 mg/kg of DEP had no effect on either morphology or Sertoli cell proliferation. To determine if DEHP reduces Sertoli cell proliferation by suppressing expression of cell cycle proteins, Western and Northern blotting were used to analyze and compare the relative protein and mRNA levels of cyclin D1, D2, D3 and p27kip1 in testes of control and DEHP-treated pups. Exposure to 200 mg/kg DEHP resulted in decreased cyclin D2 protein at 8 and 12 hr after dosing, while cyclin D1, D3 and p27kip1 in DEHP-treated rat testes remained unchanged. Moreover, the relative level of cyclin D2 mRNA was also decreased 12 hr after exposure to 200- or 500-mg/kg DEHP. Taken together with our previous findings *in vitro*, these data clearly indicate that exposure to relatively low levels of DEHP causes abnormal morphology in gonocytes and significantly decreases Sertoli cell proliferation in the intact testis. Furthermore, our findings also suggest that DEHP inhibits Sertoli cell proliferation by suppressing expression of cyclin D2 in an FSH-independent manner. (Supported by NIH grant ES09102 to JMO.)

1701 A GUANYLATE CYCLASE INHIBITOR ATTENUATES THE RELAXATION RESPONSE OF UTERINE SMOOTH MUSCLE TO HYDROGEN PEROXIDE.

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Hydrogen peroxide (H_2O_2) stimulates contractile activity of the pregnant rat uterus through the activation of prostaglandin synthesis. This stimulation of activity is often preceeded by a period of relaxation. Using standard muscle baths and myometrial cells, the present study evaluated the possible roles of nitric oxide and cGMP in H_2O_2-induced relaxation of spontaneous oscillatory uterine contractions and myometrial cells isolated from gestation day 10 rats. Exposure of cultured myometrial cells to 25, 50 or 100 μM H_2O_2 increased the release of nitric oxide (as measured by the spectrophotometric quantitation of nitrite using the Griess reagent) to concentrations 50, 120, and 170%, respectively, above solvent control levels. However, pretreatment of uterine strips with the endothelial and inducible nitric oxide synthase inhibitors 3mM Nω-nitro-$_L$-arginine methyl ester (L-NAME) or 500 μM aminoguanidine did not protect against relaxation elicited by 200 μM H_2O_2. Uterine strips pretreated with 5 μM 1*H*-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one (ODQ), a selective inhibitor of soluble guanylate cyclase, were partially protected against the inhibition of uterine contractility induced by 200 μM H_2O_2. It is proposed that hydrogen peroxide can directly activate guanylate cyclase, increasing the level of cGMP and contributing to relaxation of the uterine smooth muscle. Direct activation of guanylate cyclase by hydrogen peroxide may have important implications for the regulation and timing of uterine smooth muscle activity during pregnancy and labor under pathophysiological and normal conditions.

1702 INHIBITION OF TESTOSTERONE BIOSYNTHESIS BY MOLINATE SULFOXIDE IN LEYDIG CELL-ENRICHED CULTURES.

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Molinate (Ordram) is a thiocarbamate herbicide widely used in rice fields to restrict the growth of grassy weeds. Our laboratory and others have previously shown that administration of molinate to rats produces testicular injury characterized by Sertoli cell vacuolation, phagocytosis of spermatids and failed spermiation. Previous studies have also shown that molinate is metabolized *in vivo* via a sulfoxidation pathway, and that molinate sulfoxidation products are electrophilic, more toxicologically potent than the parent and capable of covalently inhibiting a serine esterase, hydrolase A, that possesses cholesterol ester hydrolysis activity. Because the *in vivo* effects on hydrolase A activity and spermatid retention may represent a respective cause and effect of decreased testosterone levels, we have tested the hypothesis that molinate and molinate sulfoxide are capable of decreasing testosterone biosynthesis *in vitro*. Leydig cell-enriched cultures were stimulated with human chorionic gonadotropin and treated for one hour with a DMSO vehicle and either molinate, molinate sulfoxide or ketoconazole, a known inhibitor of testosterone biosynthesis, respectively. After one hour of treatment the medium was changed and the cells were allowed to recover until the experiment was terminated 12 hours later. Although molinate and molinate sulfoxide both decreased testosterone production, only molinate sulfoxide significantly inhibited testosterone biosynthesis without diminishing cellular ATP. These data indicate that only molinate sulfoxide inhibited testosterone biosynthesis through a mechanism not associated with impairment of cell health. Molinate sulfoxide completely inhibited nonspecific esterase activity at much lower concentrations (< 0.5 μM) than that required to significantly decrease testosterone biosynthesis (50 μM), suggesting that nonspecific esterase inhibition may not be directly involved in the sulfoxidation-dependent decrease in testosterone production.

1703 LEYDING CELLS MEDIATE LEUPROLIDE STIMULATION OF SPERMATOGENESIS IN 2,5-HEXANEDIONE-INDUCED TESTICULAR ATROPHY.

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2,5-Hexanedione (2,5-HD), a reactive metabolite of the environmental toxicant n-hexane, produces a persistent testicular atrophy in rats when administered for 3.2 weeks (1% v/v) in drinking water. While the initial injury to seminiferous tubules by 2,5-HD has been attributed to a disruption of Sertoli cell microtuble transport, it has been hypothesized that the persistent nature is due to an increase in intratesticular testosterone (ITT) levels. Consistent with this hypothesis, we observed a 2.5 fold increase, relative to controls, in concentration of ITT in atrophic testes of Fisher rats (n=6) 30 weeks after treatment with 2,5-HD. Leuprolide, a GnRH agonist that produces a transient increase followed by a sustained suppression of ITT, reverses testicular atrophy induced by 2,5-HD. When 2,5-HD rats (n=6) were administered leuprolide (1.5 mg/animal, 3 injections, 24 days apart) ITT levels were suppressed below the limits of detection during leuprolide treatment. Twelve weeks after the last leuprolide injection ITT levels (280±53 ng/mL) had returned to control values (214±26 ng/mL), with a concomitant 10-fold increase in the percent of populated seminferous tubules. To further examine the role of ITT in the maintenance of testicular atrophy, an additional group of 2,5-HD treated rats (n=7), received a single dose (85 mg/kg i.p.) of the Leydig cell toxicant ethane dimethanesulphonate (EDS), followed by 3 doses of Leuprolide. EDS caused an ablation of testosterone-producing Leydig cells, which was sustained for the duration of leuprolide treatment. The combination of leuprolide and EDS also significantly depressed ITT levels (33±ng/ml). As with the group of animals which had received leuprolide alone, ITT levels (241±31 ng/mL)had returned to control values (214±26 ng/mL) 12 weeks after the last leuprolide injection, however the combination of EDS and leuprolide failed to stimulate a repopulation of seminiferous tubules. These results indicate that paracrine acting factoring or testosterone precursors by Leydig cells, are necessary for the stimulation of spermatogenesis by leuprolide.

1704 EARLY RESPONSE OF THE TESTIS TO AN ORAL EXPOSURE TO THE FUNGICIDE CARBENDAZIM IN THE RAT.

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The fungicide carbendazim, a microtubule poison, is a toxicant that causes serious testicular damage. It is known that in the rat all seminiferous tubules, except in Stages III-V, show sloughing of elongate spermatids, as early as 3 hr after an oral exposure. However, the mechanism that is responsible for sloughing remains unknown. To study the pathophysiology of sloughing, the earliest changes in the seminiferous epithelium after a single exposure of carbendazim were determined in the present study. Adult Sprague Dawley rats were treated orally with the agent (400 mg/kg) and the testes were processed for light microscopy 45, 60, 90, 120 and 150 min later. Little change was noted in the testes up to 90 min post-exposure. The earliest change observed was mainly in Stages VI and XIII at 120 min, which was characterized by narrow gaps between the layers of elongate and round spermatids. These gaps became evident in more stages at 150 min, indicating sloughing of spermatids. Immuno-histochemistry for tubulin and vimentin indicated that, in tubules with the gaps and/or sloughing of spermatids, some Sertoli cells showed a diffused staining pattern for tubulin and collapse of vimentin, respectively, but staining patterns of both cytoskeletal elements in other Sertoli cells appeared to be normal. These results indicate that the earliest change after a carbendazim exposure is sloughing of spermatids in stages VI and XIII that occurs sometime between 90 and 120 min. It is suggested that depolymerization of tubulin and collapse of vimentin are related to sloughing of germ cells, but further studies are needed to explain the biochemical mechanisms.

1705 MICROTUBULE DISRUPTION BY CARBENDAZIM IS REGULATED BY MICROTUBULE-ASSOCIATED PROTEINS.

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The fungicide carbendazim is known to disrupt microtubular structures in the testis and to cause testicular toxicity in rats. To investigate how carbendazim affects testis cytoskeletal proteins, tubulin and microtubule-associated proteins (MAPs) were isolated from rat testes and brains using three techniques. The effects of carbendazim on microtubule (MT) assembly were compared with the known MT disruptors colchicine and nocodazole. Carbendazim (100 μM) had no effect on the assembly of MTs from tubulin containing MAPs isolated with taxol, while colchicine (40 μM) and nocodazole (12.5 μM) strongly inhibited the assembly reaction. Similarly, formation of MTs from tubulin prepared with glycerol was strongly inhibited by colchicine and nocodazole but only weakly by carbendazim. However, in the presence of carbendazim these MTs were less stable and more prone to depolymerization. The assembly of MTs from MAP-free tubulin isolated with glutamate was inhibited by all three compounds but the inhibition by carbendazim was reversed by the inclusion of high molecular weight MAPs and not by unrelated protein (BSA). Inclusion of colchicine or nocodazole but not carbendazim during isolation of microtubular proteins resulted in the loss of tubulin indicating a different mechanism of action for carbendazim. The data suggest that carben-

dazim enhances the dynamic instability of MTs and that specific MAPs prevent this effect. Further studies are exploring the possibility that carbendazim affects the binding and/or hydrolysis of guanosine triphosphate.

1706 PERINATAL/JUVENILE EXPOSURE TO METHOXYCHLOR REDUCES SERTOLI CELL NUMBERS IN ADULT RATS.

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Perinatal and juvenile treatment of rats with methoxychlor (MXC) reduced testicular size and other reproductive indices in those animals as adults (Chapin et al., 1997). The objective was to determine if these males have fewer Sertoli cells which might explain these effects. Rat dams were gavaged with MXC at 0, 5, 50, or 150 mg/kg/day for the week before and after they gave birth. Resulting male pups (14-16/group) then were dosed directly from postnatal day 7 to 42. Testes were fixed in Bouin's and in OsO4, embedded in Epon and sectioned at 0.5 μm, stained with toluidine blue, and evaluated stereologically or cut at 20 μm to measure Sertoli cell nuclei with Nomarski optics. Single Sertoli cell nuclear volume was calculated using a sphere times a correction factor for the nonspherical shape of the Sertoli cell nuclei. Sertoli cell number equaled [volume density of the nucleus times the parenchymal weight (90% of testicular weight)] divided by the volume of a single Sertoli cell nucleus. Across dose groups, there were no changes in the nuclear volume density (control: 1.46±0.04%), the volume of a single nucleus (474±16 fl), and the number of Sertoli cells per g parenchyma (28.2±1.3 million). There were dose-related changes in the volume of nuclei per testis (23.4±1.2, 23.1±0.8, *19.3±0.8, or *16.3±0.6 μl) and the number of Sertoli cells per testis (50.3±3.0, 48.6±2.0, *41.2±1.8, or *32.7±1.3 million). The two highest dose groups were the ones with fewer testicular spermatids. Male rats exposed to MXC prior to puberty had reduced numbers of Sertoli cells and spermatids as adults. (Supported by NIEHS.)

1707 NINETY DAY INHALATION TOXICITY STUDY OF HCC-230FA IN RATS.

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The inhalation toxicity of 1,1,1,3,3,3-hexachloropropane (HCC-230fa) was evaluated by exposure of male and female rats to 0, 0.50, 2.5, or 25 ppm over a 90-day period. Body weights and food consumption were measured weekly. Clinical pathology was evaluated at 45 and 90 days. A morphological evaluation was conducted at 14 weeks or at the end of the 1-month or 3-month recovery periods (males only). In addition, sperm assessment and primary humoral immune response evaluations were conducted at 14 weeks or at the end of the 1-month recovery period. Body weights and food consumption in rats exposed to 25 ppm were lower than the corresponding controls. Male and female rats exposed to 25 ppm and sacrificed immediately after the exposure period had minimal statistically significant decreases in total leukocyte and lymphocyte counts. These leukocytic changes in the 25 ppm groups were considered to be marginally adverse. Pathologic examination revealed hepatocellular hypertrophy in male rats from the 25 ppm group sacrificed after the exposure period, and an increased incidence and/or severity in chronic progressive nephropathy in 0-day, 1-month recovery, and 3-month recovery males at 25 ppm. The hepatocellular hypertrophy was considered a non-adverse pharmacological adaptive response to HCC-230fa. Evaluation of sperm parameters at the end of the exposure period showed biologically significant decreases in percent motile sperm at 25 ppm (63.1% vs. 78.3% for controls). In addition, decreases in epididymal sperm number per cauda epididymis and percent normal sperm morphology were seen in rats at 25 ppm; however, these changes were not believed to be biologically significant. There were no compound-related effects on humoral immune response. The no-observed-effect level for repeated exposure to HCC-230fa in male and female rats was 2.5 ppm based on effects on body weight, food consumption, percent motile sperm, clinical pathology parameters, and histology.

1708 EFFECT OF 4-VINYLCYCLOHEXENE DIEPOXIDE DOSING ON EXPRESSION OF *bax* IN RAT AND MOUSE SMALL PREANTRAL FOLLICLES.

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The occupational chemical 4-vinylcyclohexene diepoxide (VCD) is known to destroy small ovarian follicles in rats and mice. In rats, this destruction occurs by accelerating the basal rate of atresia in the ovary, however, the exact mechanism is unknown. Concurrent VCD and 17β-estradiol (E_2) treatment protects rat primary follicles from VCD-induced toxicity. The pathway of this follicular rescue is unknown. Previously, repeated daily dosing with VCD was demonstrated by semi quantitative RT-PCR to increase mRNA encoding the cell death enhancer *bax* in rat small preantral follicles. In the present experiment, quantitative ribonuclease protection assay (RPA) was used to compare expression of mRNA encoding *bax* in rat and mouse small preantral follicles. F344 rats (28 days) were treated for 15 days with either vehicle control (sesame oil), VCD (80 mg/kg, i.p.), E_2 (0.1 mg/kg, s.c.), or VCD and E_2. Four hours after the final dose, ovaries were collected and small follicles were isolated. RNA was isolated from follicles less than 100μ (target population for VCD), hybridized to ^{32}P labeled *bax* and *18s* probes overnight, visualized by PAGE, and quantified. Compared with controls, the ratios of mRNA for *bax* were similar across treatments (expressed as treatment/control ratios; 2.27, VCD; 2.20, E_2; 2.08, VCD plus E_2). Although, all treatments increased expression of *bax*, it is yet to be determined whether VCD- and E_2-associated pathways converge. $B6C3F_1$ mice (28 days) were treated daily for 15 days with either vehicle control or VCD (80 mg/kg, i.p.). Unlike rats, in mice, there was no difference in *bax* mRNA levels in mouse small preantral follicles between treated and control animals (VCD/control=0.97). This supports that VCD may destroy small preantral follicles by different mechanisms in rats and mice. (ADCRC 9809, NIEHS Center Grant 06694, ES09246, ES08979.)

1709 MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP)-INDUCED GERM CELL APOPTOSIS PRECEDES ITS DETACHMENT FROM SERTOLI CELLS.

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Germ cell apoptosis occurs as a mechanism to limit the number of germ cells in the seminiferous epithelium. It has been proposed that Sertoli cells regulate germ cell apoptosis. Exposure of mice to the Sertoli cell toxicant MEHP results in a detachment of germ cells from the seminiferous epithelium and increases the numbers of apoptotic germ cells. The Fas receptor (Fas)/Fas ligand (FasL) signaling system has recently been demonstrated to be a key regulator of germ cell apoptosis. To investigate the relationship between germ cell detachment and apoptosis, we used Sertoli cell-germ cell co-cultures prepared from gld mice that express a dysfunctional form of FasL. Addition of MEHP (200 μM) to co-cultures prepared from wild-type (C57/b16) mice led to a progressive increase (at 6 & 12 h) in the numbers of detached germ cells. However, in co-cultures from gld mice, only a marginal increase in detached germ cells was observed at 6h after MEHP treatment, and no further increase was observed after 12h,. Caspase-8 and caspase-3 are Fas downstream signal effectors that participate in the early phases of apoptosis. Processing of the pro-forms of caspases to the active form is revealed by western blot analysis. An early (3 h) increase in the processing of caspase-8 and caspase-3 was observed in testis homogenates from 28-day-old rats exposed to a single oral dose of MEHP (2 g/kg). This corresponds to a time that precedes the morphological observation of germ cell detachment. Taken together, these observations indicate that the initiation of germ cell apoptosis precedes germ cell detachment and that the primary toxic mechanism of MEHP is not likely at the germ cell-Sertoli cell adhesion complex. (Supported by NIEHS grants ES09145 & ES07784.)

1710 DOES CIGARETTE SMOKE AFFECT MYOMETRIAL ACTIVITY DURING ESTRUS AND EARLY PREGNANCY *IN VIVO*?

M. M. Rubio. *University of California, Riverside, CA.* Sponsor: J. T. Arey.

To determine if cigarette smoke interferes with normal myometrial activity, we developed an experimental technique that employs video laparoscopy to monitor the changes in myometrial activity in nonpregnant and pregnant hamster *in vivo*. Using video laparoscopy, we are able to record myometrial activity before, during, and after a single 40-minute smoke exposure. Analysis of the videotape data revealed that during the cigarette smoke exposure period

(for both mainstream and sidestream smoke), the number of unidirectional peristaltic and longitudinal contractions decreased in estrus females and day 4 pregnant females. These inhalation experiments were followed up by additional *in vivo* experiments to determine if nicotine, a major component of cigarette smoke, was responsible for decreasing in the number of contractions seen during smoking. In the first follow-up experiment, nicotine was applied topically to the uterine horns. The nicotine solution concentrations ranged from 10 nM to 10 mM. Even at the highest concentration nicotine had no effect on the myometrial activity, suggesting that nicotine cannot exert an effect directly on the uterine horns. The next experiment delivered nicotine tartrate solution directly into the heart then throughout the body. Within 15-20 minutes after being injected directly into the bloodstream it caused a transient decrease in unidirectional peristaltic contractions in these nonpregnant females. T-tests comparing total number of unidirectional peristaltic contractions/5-minute intervals during the control and after the injection showed that the 40-45 minute and the 45-50 minute intervals were significantly different from the control intervals. A decrease or inhibition of uterine smooth muscle contractions could slow blastocyst movement in the uterus. If the blastocyst transport rate is delayed, then sites may be crowded, as seen previously in hamsters exposed to smoke. When taken together, these data could explain why women who smoke have decreased rates of fecundity.

1711 COMPARATIVE STUDY IN LABORATORY RATS TO VALIDATE SPERM QUALITY METHODS AND ENDPOINTS.

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The Naval Health Research Center, Detachment (Toxicology) performs toxicity studies in laboratory animals to characterize the risk of exposure to chemicals of Navy interest. Research was conducted at the Toxicology Detachment at WPAFB, OH in collaboration with Wright State University, Department of Biological Sciences for the validation of new bioassay methods for evaluating reproductive toxicity. The Hamilton Thorne sperm analyzer was used to evaluate sperm damage produced by exposure to a known testicular toxic agent, methoxyacetic acid and by inhalation exposure to JP-8 and JP-5 in laboratory rats. Sperm quality parameters were evaluated (sperm concentration, motility, and morphology) to provide evidence of sperm damage. The Hamilton Thorne sperm analyzer utilizes a DNA specific fluorescent stain (similar to flow cytometry) and digitized optical computer analysis to detect sperm cell damage. The computer assisted sperm analysis (CASA) is a more rapid, robust, predictive and sensitive method for characterizing reproductive toxicity. The results presented in this poster report validation information showing exposure to methoxyacetic acid causes reproductive toxicity and inhalation exposure to JP-8 and JP-5 had no significant effects. The CASA method detects early changes that result in reproductive deficits and these data will be used in a continuing program to characterize the toxicity of chemicals, and combinations of chemicals, of military interest to formulate permissible exposure limits.

1712 A SINGLE HEAT SHOCK OF JUVENILE MALE MICE CAUSES A LONG-TERM DECREASE IN FERTILITY AND REDUCES EMBRYO QUALITY.

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Testicular hyperthermia adversely affects spermatogenesis in a wide range of mammalian species. Indeed, mild testicular heating has been reported as a possible safe, effective and reversible contraceptive method for men. In adult mice, a single acute heat shock has been shown to reduce fertility for about 1 spermatogenic cycle. Scrotal heating of adult sires has also been shown to have direct effects on embryo growth in untreated female mice. However, there have been no reported studies examining the effects on fertility of testicular heating in juveniles. We examined the long-term reproductive consequences of a single, acute heat shock on juvenile male C57BL/6 mice. Heat shocks were given at 7, 14, 21, 28, 42 or 56 days after birth. The animals were sedated with ketamine and the posterior half of their body submerged in a 43°C waterbath for 20 min. The males were mated at 14 weeks of age. The dams were sacrificed at gestation day 14.5, and litters assessed for number and weights of embryos, resorptions and terata. Juvenile male mice exposed to a single heat shock at 14 days postnatal (dpn) have a long-term reduction in fertility as indicated by the total number of litters and embryos sired per male. Although the incidence was not significant, we also observed more terata in progeny of the heat-shocked males (3/14 litters contained one embryo with anophthalmia) than the control males (1/11 litters contained one embryo with microphthalmia). There was also a significant reduction in the weights of embryos sired by the heat shocked males compared to the controls. Similar results were not obtained from males exposed at time points other than 14dpn. Overall, these observations suggest that the developing testis has a window of susceptibility to heat shock in terms of long-term adverse effects on fertility, and that male-mediated developmental toxicity may be initiated by the heat-shock event. The 14dpn susceptibility window we have found in this juvenile model may provide a useful starting point for investigating the effects of environmental toxicants on spermatogenesis and the developing testis. (This is an abstract for a proposed presentation and does not necessarily reflect EPA policy.)

1713 ANTIANDROGENIC EFFECTS OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP) ON MALE REPRODUCTIVE AND SEXUALLY DIMORPHIC CNS DEVELOPMENT IN RATS.

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Research was conducted to determine effects of *in utero* and lactational DEHP exposure on male reproductive and sexually dimorphic CNS development. Sprague-Dawley rats were dosed with corn oil or DEHP (0, 375, 750, or 1500 mg/kg/day, po) from Gestation Day 3 through Postnatal Day (PND) 21. Dose-related effects on male offspring include reduced ano-genital distance, nipple retention, undescended testes, and delayed or incomplete preputial separation. Dose-related reductions in testis, epididymis, seminal vesicle, ventral prostate, anterior prostate, dorsolateral prostate, and glans penis weights were observed at PND 21, 63, and/or 105-112. Additional effects include unilateral or bilateral absence of the anterior prostate and seminal vesicles, partial or complete absence of the ventral prostate, dorsolateral prostate agenesis, testicular and epididymal malformations, and cleft phallus. No abnormalities were found in any of 8 control litters, but DEHP caused reproductive toxicity in 5 of 8 litters at 375 mg/kg/day, 8 of 8 litters at 750 mg/kg/day, and 5 of 5 litters at 1500 mg/kg/day. Masculine sexual behaviors were also examined. Seven of 8 control males displayed typical behaviors and ejaculated within the 45-min observation period (ejaculatory latencies averaged 14 min). In contrast, 3 of 7 low dose males never mounted, intromitted, or ejaculated; all 7 males at the middle dose mounted but 3 never intromitted and 4 didn't ejaculate; and neither rat at the high dose mounted, intromitted, or ejaculated. These results strongly suggest that sexual differentiation of the CNS was incomplete. In summary, *in utero* and lactational DEHP exposure profoundly alters male reproductive system development and appears to alter sexually dimorphic CNS development in rats. The LOAEL was the lowest dose tested (375 mg/kg/day), which prevented testis descent, reduced weights of several sex organs, caused permanent nipple retention, and demasculinized sexual behaviors. The pattern of effects demonstrates that DEHP (and/or its metabolites) is an antiandrogen. (Supported by NIH Grant ES06806.)

1714 *PARA*-NONYLPHENOL (NP) ADMINISTERED TO CD RATS IN A SOY- AND ALFALFA-FREE DIET AFFECTS TESTES AND FEMINIZES LIVER METABOLIC PATTERNS IN 50-DAY-OLD PUPS.

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NP is widely used in the manufacture of nonionic surfactants, lubricant additives, polymer stabilizers, and antioxidants. The weak estrogenic activity of NP has led to much recent research on its toxicity, and we have evaluated NP in a dose range-finding study to determine the doses of NP to be further tested in a multigeneration study. NP was fed in the diet at 0, 5, 25, 200, 500, 1000 and 2000 ppm to F_0 dams beginning on gestation day 7. Exposure of the F_1 pups continued via the dams milk until postnatal day (PND) 21 and then by feed until sacrifice on PND 50 (n=5 litters with 4 pups of each sex per litter). We previously reported the severe polycystic kidney disease and weight loss observed in both sexes of the 2000 ppm dose group (Latendresse *et al.*, Toxicologist **48**: 321, 1999). No other significant effects were observed in females of the lower dose groups. In the testes, retention of elongated spermatids was observed in both control and dosed groups, but the degree of retention appeared higher in the 25 ppm dose group and above. Depletion of elongated spermatids was observed only in dosed groups, with an apparent increase in severity at 500 ppm and above. In addition to the lesions noted above, cytoplasmic vacuolization of interstitial cells, an increase in severity of degeneration of pachytene and prepachytene spermatocytes, and minimal to

mild depletion of round spermatids were observed in testes from the 2000 ppm animals. The severe kidney toxicity and body weight loss seen at that dose make it unclear as to whether observed effects are primary or secondary. Hepatic microsomes from the 25, 500, and 2,000 ppm dose groups were also assayed for testosterone metabolism. No significant effects were observed in females. In males, a decreasing dose trend in formation of androstenedione and decreased formation of 2α- and 16α-hydroxytestosterone at 2000 ppm were observed. In addition, significant, dose-dependent increases in the formation of dihydrotestosterone (DHT) and DHT diols were observed, and 7α-hydroxytestosterone production was increased at 25 and 500 ppm. These effects suggest a feminization of hepatic metabolism by NP as well as effects on spermatogenesis at doses that are not overtly toxic.

1715 FUNCTIONAL STUDIES TO INVESTIGATE DYSTOCIA AND STILLBIRTHS OBSERVED DURING A REPRODUCTIVE BIOASSAY WITH AN EXPERIMENTAL CYANAMIDE (YRC 2894).

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During the conduct of a two-generation reproductive toxicity study with [3-[(6–chloro-3-pyridinyl]methyl]-2-thiazolidinylidene]-cyanamide (YRC 2894) an increase in stillbirths and evidence of dystocia were observed. In order to investigate the potential mechanisms by which YRC 2894 may have interfered with parturition a modified one-generation study was conducted. In this study male and female SD rats were continuously exposed to dietary levels of 0 or 1000 ppm YRC 2894 during premating, mating, and gestation phases. Specific functional and morphologic changes in the uterus and cervix were examined during the third week of pregnancy and also at parturition. Cervical evaluations included morphology (collagen content and organization), gross weight and water content, and extensibility. Uterine examinations included electrical activity (EMG), mechanical activity (intrauterine pressure), evaluated *in vivo*; uterine contraction, evaluated *in vitro*; and morphologic changes (histology and gross weight). Additionally, the potential effect of YRC 2894 on tissue innervation was investigated via enumeration of uterine alpha-adrenergic receptors. As determined following these studies, YRC 2894 did not affect the functional capabilities of the cervix or uterus, nor did it affect the uterine alpha adrenergic receptor population. Based on these results, the original observation of dystocia is not explained by YRC 2894 affecting the functional aspects of parturition.

1716 DISRUPTION OF OVARIAN CYCLES BY CHLOROTRIAZINES AND THEIR METABOLITES IN THE RAT.

R. L. Cooper, W. K. McElroy and T. E. Stoker. *USEPA, Research Triangle Park, NC.* Sponsor: R. J. Kavlock.

Previously, we reported that the chlorotriazine (CZ) herbicide atrazine (ATZ) disrupts the ovarian cycles in rats [i.e., induces repetitive pseudopregnancies (RPP) or prolonged diestrus (di)]. In the present study we examined the effect of ATZ, simazine (SIM) and cyanazine (Cy), as well as three CZ metabolites [2-amino-4-chloro-6-isopropylamino-S-triazine (IS), 2-amino-4-chloro-6-ethylamino-S-triazine (EA) and diaminochlorotriazine (DACT)]. Regularly cycling, 90-day old female Long-Evans hooded rats were gavaged with 75, 150 and 300 m/kg ATZ, SIM, IS, EA, or DACT or 18.8, 37.5 and 75 mg/kg CY for 21 days. Vaginal smears were monitored during treatment and for the ensuing 21 days. A dose-dependent disruption of ovarian cycles (i.e., RPP or di) was observed for all three parent compounds (ATZ, SIM & CY) and all doses altered cycling. IS, EA, and DACT also disrupted cycling at each dose tested in a dose-dependent manner. Compared to ATR and SIM, ovarian cycles were disrupted in a greater number of animals receiving the same dose of IS, EA and DACT. These data demonstrate that the CZ and their metabolites share a common endocrine disrupting ability. Such information should provide insight into the CZ structure responsible for the disruption of ovarian function. (This abstract does not reflect U.S. EPA policy.)

1717 THE EFFECT OF ATRAZINE (ATR) ON PUBERTY IN MALE WISTAR RATS.

T. E. Stoker, D. L. Guidici, W. K. McElroy, S. C. Laws and R. L. Cooper. *NHEERL USEPA, Research Triangle Park, NC.* Sponsor: R. J. Kavlock.

The chlorotriazine herbicide ATR is known to suppress serum prolactin (PRL) and luteinizing hormone and alter neurotransmitter concentration in female rats. Because puberty in mammalian species is a period of rapid interactive endocrine and morphological changes, we hypothesized that exposure of male rats to ATR may interfere with several of the endpoints described in the Endocrine Disrupters Screening and Testing advisory Committee prepubertal assay including preputial separation (PPS), body weight, testes, seminal vesicle, prostate and epididymal weights, serum testosterone (T), thyroxin (T4) and thyroid stimulating hormone (TSH). ATR (0, 50, 100 or 200 mg/kg) was administered by gavage to male rats from postnatal day (PND) 23 to 53. PPS was significantly delayed by all ATR doses. On PND 53 males were killed by decapitation. ATR (200 mg/kg) decreased ventral and lateral prostate weight but testes weight was not altered. ATR (200 mg/kg) significantly decreased serum T, while serum estradiol was significantly increased at this dose. Serum T3 and T4 were unaltered at any dose. As ATR suppresses serum PRL and LH in the adult rats, these data would indicate that puberty is delayed in the male rat by altering hypothalamic-pituitary maturation, although a direct effect on the testes can not be ruled out at this time. (This abstract does not reflect EPA policy.)

1718 THE EFFECT OF ATRAZINE ON PUBERTY IN FEMALE WISTAR RATS.

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Atrazine (ATR), a chlorotriazine herbicide, has been shown to decrease serum LH and prolactin in adult female rats. In this study, the effect of ATR on the onset of puberty was evaluated using the 20-day Pubertal Female Protocol recommended by Endocrine Disupters Screening and Testing Advisory Committee. Female Wistar rats were dosed from postnatal days (PND) 22-41 with ATR (0, 50, 100, 200 mg/kg). Vaginal opening (VO) was significantly delayed 3 or 4 days in rats dosed with 50 or 100 mg/kg ATR. VO did not occur in 9 of 16 rats at 200 mg/kg ATR during the 20-day dosing period. Body weight gain during the 20-day dosing period was unaltered at 50 and 100 mg/kg ATR, but was reduced by 8.5% in the 200 mg/kg group. Half the animals in each treatment group were killed on PND 41 and tissue weights (e.g., liver, kidney, adrenal, ovaries, uterus, pituitary) and serum were collected. Uterine weights (no fluid) were significantly reduced in the 100 and 200 mg/kg groups. Kidney and pituitary weights were also significantly reduced in the 200 mg/kg group. Estrous cyclicity was evaluated in the remaining rats from VO until 30 days after treatment was terminated. Irregular cycles were observed in ATR (50 and 100mg/kg) rats between VO and PND 41. However, ATR did not alter the number of regular cycles during the ensuing 30 days. Of the 8 rats in the 200 mg/kg group, VO occurred in only 3 rats by PND 41. However, once treatment was terminated, VO occurred in the remaining 5 rats within 3 days (PND 44). The estrous cycles of the rats in the 200 mg/kg group were initially irregular, but were similar to controls by the end of the 30 day post treatment period. In summary, these data demonstrate that atrazine can modify the onset of puberty in the female rat. (This abstract does not reflect U.S. EPA policy.)

1719 METALLOTHIONEIN ISOFORM 1 AND 2 GENE EXPRESSION IN THE HUMAN PROSTATE: DOWN REGULATION OF MT-1X IN ADVANCED PROSTATE CANCER.

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Metallothionein (MT) over expression has been associated with a wide variety of human tumors. However, a family of genes underlies the expression of these proteins. The goals of this study were to define the expression of MT genes and protein in normal prostate tissue and to provide evidence that expression of the MT isoforms is altered in prostate cancer. Immunohistochemistry was used to localize MT protein, RT-PCR to determine MT isoform-specific mRNAs, and immuno-blot analysis to determine MT protein levels in fresh and archival biopsy specimens. The localization of MT in the prostate was defined using E9 antibody. This antibody determines the co-expression of MT-1 and MT-2 proteins. Immunohistochemical analy-

sis demonstrated that MT protein was expressed in the normal prostate and this was supported by mRNA from the MT-1A, MT-1E, MT-1X and MT-2A genes. In advanced prostate cancer, MT protein was over expressed. MT-2A was expressed in all cases of advanced prostate cancer, whereas MT-1A and MT-1E expression were present in some, but not all cases of prostate cancer. No expression of the MT-1X gene was demonstrated in any of the cases of advanced prostate cancer. The expression of MT-1 and MT-2 isoform-specific mRNA varied among three commonly utilized prostate cancer cell lines providing further evidence that MT gene expression may be altered among individual prostate cancers.

1720 METALLOTHIONEIN ISOFORM 3 EXPRESSION IN THE HUMAN PROSTATE AND CANCER-DERIVED CELL LINES.

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Expression of metallothionein isoform 3 (MT-3) was initially reported to be confined to neural tissues. However, it was recently demonstrated that MT-3 is expressed in epithelial cells of human kidney. This motivated the current examination of MT-3 expression in human prostate. Selected epithelial and stromal cells of normal human prostate were shown to have low levels of MT-3 expression based on immunohistochemical localization using an MT-3 specific antibody. This protein was increased in prostatic intraepithelial neoplasia (PIN) lesions and further increased in a highly variable fashion in prostatic adenocarcinoma. Expression was also measured in cultures of three commonly utilized human prostate tumor cell lines, DU-145, LNCaP, and PC-3, using RT-PCR with MT-3 specific primers. MT-3 was detected in DU-145 and LNCaP cells at 35 cycles of cDNA amplification; however, for the PC-3 line, the level of expression was below the detection limit (40 cycles). Expression of the house keeping gene, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was detected in all three cell lines at 30 cycles. MT-3 mRNA was also detected in RNA isolated from normal prostate tissues using this technique. Authenticity of the MT-3 RT-PCR product from normal tissues and tumor cell lines was verified by FokI restriction enzyme analysis. These results show that MT-3 is expressed in the human prostate and indicate a possible dysregulation of its expression in prostate cancer.

1721 DIBROMOACETIC ACID (DBA)-INDUCED EFFECTS ON ESTROUS CYCLICITY AND FOLLICULAR STEROID SECRETION IN THE RAT.

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Chlorination of public water supplies generates a large number of treatment-related chemicals. A number of these disinfection by-products (DBPs) are reported to have adverse reproductive effects in rodent test populations. The haloacetic acid DBA has been found to alter testosterone levels in male rats and affect sperm quantity and quality. Since there are limited data in the female rat, the present study investigated the influence of DBA on estrous cyclicity and the effects of *in vitro* exposures on ovarian follicular steroid secretion. In exp. 1, DBA (0,10,30,90 or 270 mg/kg, in distilled H2O adj. to pH 6.5) was administered daily by gavage for 14d to regularly cycling Sprague-Dawley rats. Vaginal cytology was monitored for 6 wks over the pre-dosing, dosing, and post-dosing periods (2 wks each). Cyclicity was significantly affected at the 2 highest doses, with periods of persistent estrus most evident in the 270 mg/kg group during dosing/ post-dosing segments. At this dose, plasma concentrations (measured by capillary GC) averaged 130 μg/ml at a 1hr peak (T1/2=1.05 hr.). Values over a 5 hr period were used to select a concentration for Exp. 2. Immature females were given 10IU PMSG on d26 and killed 2d later. Preovulatory follicles from 8-10 rats were separated out and paired in teflon vials containing oxygenated Medium 199 (M199, pH 7.4) with 0.1% BSA, 10 mM HEPES and 50 μg/ml gentamicin sulfate. Follicles were exposed to 1 of 4 conditions: M199 only, DBA (50 μg/ml), hCG (100 IU), or DBA+hCG. Aliquots taken at 4, 8, 12 & 24 hrs were assayed for estradiol (E) and progesterone (P). Data showed that in non-stimulated follicles, DBA significantly elevated E secretion, while P release was unaffected. In contrast, DBA completely inhibited the hCG-stimulated increase in P, with no effect upon the hCG-induced rise in E. Results indicated that follicular steroid secretion could be altered by DBA exposure, although the effect was differentially influenced by hCG stimulation. Such alterations may also have had an impact on estrous cyclicity.

1722 TWO-GENERATION ORAL (DRINKING WATER) REPRODUCTIVE TOXICITY STUDY OF PHENOL IN RATS.

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This study was designed to assess the potential reproductive toxicity of Phenol in rats. Phenol was administered to 30 Sprague-Dawley rats/sex/group in the drinking water at concentrations of 0, 200, 1000 or 5000 ppm. Parental (P_1) animals were treated for 10 weeks prior to mating, during mating, gestation, lactation, and until sacrifice. The F_1 generation (P_1 offspring) was treated using a similar regimen, while the F_2 generation was not treated. After mating, 10 P_1 males/group were evaluated using standard clinical pathology parameters and an immunotoxicity screening plaque assay. No evidence of immunotoxicity, and only a minimal increase in BUN, were noted in the 5000 ppm group. Significant reductions in water and food consumption were observed in the 5000 ppm group in both generations; corollary reductions in body weights/body weight gain were also observed. Mating performance and fertility in both generations were similar to controls, and no adverse effects on vaginal cytology or male reproductive function were observed. Vaginal opening and preputial separation were delayed in the 5000 ppm group, and were considered to be secondary to the reduction in F_1 body weight. Litter survival of both generations was reduced in the 5000 ppm group. Absolute uterus and prostate weights were decreased in the F_1 generation at all dose levels; however, no underlying pathology was observed and there was no functional deficit in reproductive performance, therefore these findings were not considered to be adverse. Most of the adverse effects noted were presumed to be associated with flavor aversion to Phenol in the drinking water. Based on a comprehensive examination of all of the parameters, the no-observed-adverse-effect level (NOAEL) for reproductive toxicity of phenol administered in drinking water to rats is 1000-ppm. (This study was sponsored by the Chemical Manufacturers Association Phenol Panel.)

1723 DEVELOPMENTAL REPRODUCTIVE EFFECTS OF A MIXTURE OF DIOXINS, FURANS AND CO-PLANAR PCBS ON LONG EVANS RATS.

J. T. Hamm and L. S. Birnbaum. *USEPA/University North Carolina-Chapel Hill, Research Triangle Park, NC.*

TCDD causes a spectrum of adverse biological responses including alterations in reproductive development. However, within the environment and within animal tissues, TCDD is found as part of a complex mixture of dioxins and dioxin-like compounds. Therefore, the effect of a mixture of dioxins, furans and co-planar PCBs on reproductive development was examined. This mixture was based upon the relative mass ratio of the compounds as they are found in foodstuff and doses were calculated using the Toxic Equivalency Factor (TEF) scheme. Single oral doses of 0, 0.05, 0.2, 0.8 and 1.0 μg/kg toxic equivalency were administered on gestation day 15 (GD15). As with TCDD, our mixture delayed the time to puberty, from 39.8±0.3 to 41.9±0.5 in the male and from 31.3±0.2 to 33.7±1.1 in the female at the high dose. In contrast, the weights of the reproductive tract tissues were not affected at PND49 or 63. However, PND32 is a more sensitive time point for effects on the seminal vesicle and at this time tissue weight was decreased in a dose response fashion, from 40.4mg in controls to 26.4mg at the high dose. Comparison of ethoxyresorufin-*O*-deethylase (EROD) induction between the mixture and TCDD alone revealed relatively higher levels of induction during gestation but lower postnatal induction. Since effects on reproductive development may occur pre- or postnatally, results with EROD may help to explain the variable degree of responsiveness to the mixture observed between endpoints. (This abstract does not necessarily represent USEPA policy. JTH, EPA, CT 902908.)

1724 A SIMPLE TECHNIQUE FOR DETERMINING FAILURE OF FERTILISATION IN THE RAT.

L. M. Burns and F. W. Ross. *Quintiles, Ledbury, United Kingdom.* Sponsor: A. K. Palmer.

In the commonly used design of reproduction studies, assessment of whether a reduction in the numbers of implantations results from a failure in fertilisation or early death of the embryos is impossible. We report here on the successful use of a simple technique to identify if fertilisation has taken place. In a recent male fertility study reduced numbers of implantations were observed

in untreated females mated with males dosed for 10 weeks but pregnancy rate was unaffected. In the males the incidence of morphological sperm abnormalities was increased and cauda epididymis weights were reduced. There were no effects on homogenisation resistant spermatid counts nor were there any histopathological findings. From these results it was impossible to establish whether the reductions in implantations were due to failure of fertilisation or early post-fertilisation mortality and an additional study was designed to provide clarification. Males were mated 1:1 with untreated females to provide baseline assessments of fertility. On Day 4.5 after mating the females were killed and the ova were flushed from the uterus and examined for fertilisation and subsequent degree of development. Males shown to be fertile were selected to provide a group of 20 and were dosed at a level known to induce effects before being mated 1:1 with a second group of untreated females. Ova were again flushed from the uterine horns on Day 4.5 after mating and examined for fertilisation and degree of development. Results clearly indicated that the reduced numbers of implantations were a result of failure of fertilisation.

1725 EVALUATION OF A SOY- AND ALFALFA-FREE DIET FOR USE IN STUDIES OF ENDOCRINE-ACTIVE COMPOUNDS.

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Standard laboratory rodent chows contain hormonally active agents, including the isoflavones genistein and daidzein, that could modulate responses to agents under test for endocrine activities. In a series of studies of endocrine active agents, we are utilizing a soy- and alfalfa-free irradiated diet (Purina 5K96) that is formulated to be similar to NIH-31 and contains a low percentage of casein (<3%). Diet analysis indicated that NIH-31 contained approximately 24 ppm genistein and 22 ppm daidzein whereas these compounds were present at approximately 0.5 ppm each in 5K96. 50 CD rats of each sex were removed from NIH-31 pellets at weaning and placed on either NIH-31 or 5K96 meal (25 animals of each sex per diet). Body weight and food consumption data were collected for 6 months; males and females were bred at 10-12 weeks and data on the litters collected. 24 F_1 male pups were maintained on the same diet as their parents until PND 63 for evaluation of kidneys, mammary glands, and thyroid peroxidase (TPO). These endpoints were selected since diet composition could significantly alter kidney function, and stimulation of male mammary gland and inactivation of TPO had been previously observed at doses of genistein similar to those present in NIH-31. For the F_0 animals, food consumption was significantly lower in both sexes in the 5K96 groups throughout the experiment. Only the males in the 5K96 group showed a significantly lower body weight than NIH-31 males, with the difference first evident at about 11 weeks of age. No differences in fertility or litter parameters were observed between the two groups. Serum genistein and daidzein levels at sacrifice were 0.35-0.62 μM and 0.20-0.25 μM, respectively, in NIH-31-fed animals, with females slightly higher than males. In contrast, serum levels in animals fed 5K96 were <0.02 μM for both isoflavones. TPO activities were significantly lower in F_0 and F_1 NIH-31 animals, while thyroid hormones and TSH were unchanged or slightly elevated. Other endpoints remain to be evaluated, but the data above suggest that 1) 5K96 is a suitable diet for reproductive toxicity studies and 2) isoflavones in rodent chows are bioavailable and may affect responses to other endocrine active test agents.

1726 ONE GENERATION INHALATION REPRODUCTIVE TOXICITY STUDY OF HEXAMETHYLDISILOXANE (HMDS) IN RATS.

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This study was conducted to evaluate the potential adverse effects of whole body vapor inhalation exposure of F0 parents and F1 offsprings to HMDS. Groups of male and female Sprague-Dawley rats (24/sex) were exposed to either 0, 100, 1,000 or 5,000 ppm HMDS for 6 hours/day for at least 28 days prior to mating, during mating, and through gestation day (GD) 20. Exposure was suspended from GD 21 through postnatal day (PND) 4 and resumed on PND 5 until weaning. Clinical observations, body weights and food consumption were recorded at appropriate intervals. All F0 dams were allowed to deliver and rear their pups to weaning; were necropsied on PND 21; selected organs weighed and designated tissues from the control and 5,000 ppm groups examined microscopically. Spermatogenic evaluations were performed on all F0 males. F1 offsprings (60/sex/group) were selected for HMDS exposure from PND 22 to PND 27, necropsied on PND 28 and selected organs were weighed. All F0 animals survived to the scheduled necropsy. A slight reduction in body weight gains was observed in the 5,000 ppm group males and females during the first 3 weeks of the study. No other exposure-related findings were noted for any of the F0 endpoints evaluated. F1 pup survival in the 5,000 ppm group was slightly, but not statistically significantly decreased from birth to PND 4 when compared to controls. No exposure-related findings were noted for any of the other F1 endpoints evaluated. (Supported in part by the Silicone Environmental Health and Safety Council.)

1727 FERTILITY, DEVELOPMENTAL AND REPRODUCTIVE EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF LEPTIN IN MICE.

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Leptin is a protein hormone, produced by adipose tissue, involved in the regulation of body fat. Recombinant leptin administered to normal animals results in rapid loss of body weight primarily due to body fat reduction. These studies were conducted in mice to evaluate the fertility, developmental and reproductive toxicity of leptin. To assess the fertility and early embryonic development doses of 1, 10, and 30 mg/kg/day were administered to male mice 28 days prior to mating until sacrifice and to female mice 14 days prior to mating until Gestation Day (GD) 6. Equivalent doses were given to female mice GD 6 through 15 to evaluate embryo-fetal development. To assess the pre- and postnatal development, including maternal function 3, 10, and 30 mg/kg/day were administered to female mice on GD 6 through Lactation Day (LD) 21. Reproductive parameters and spermatogenic endpoints were unaffected by r-metHuLeptin administration. Mean body weights were reduced in a dose-related manner. Food consumption was reduced for males. Intrauterine growth and survival were unaffected by the test article and there were no treatment related fetal malformations or variations. Postnatal pup survival was reduced from birth to postnatal day (PND) 0, PND 0-1 and birth to PND 4. Mean body weights were reduced and developmental landmarks were delayed for the F_1 pups. Mean body weight for the F_1 adults were decreased. There were no adverse effects on F_1 reproductive performance or on the F_2 fetuses. The NOEL for reproductive and developmental toxicity was 30 mg/kg/day and the NOAEL for neonatal toxicity was less than 3 mg/kg/day.

1728 REPRODUCTIVE EFFECTS OF JP-8 JET FUEL ON MALE AND FEMALE SPRAGUE-DAWLEY RATS AFTER EXPOSURE BY GAVAGE.

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The jet engine fuel designated JP-8 is the standardized fuel for the U.S. military, which has resulted in exposure to both male and female personnel. Limited information is available on the reproductive effects of JP-8 jet fuel in humans and animals. In this report, data are presented from two reproductive studies. In the first study male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naive females to assess fertility and semen parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30% lower than control weights. There were no significant changes for pregnancy rate, gestation length or semen parameters as compared to control values. In the second study female rats were dosed with neat JP-8 (0, 325, 750, 1500 mg/kg) daily by gavage for a total of 21 weeks (90-days plus mating with naïve males, gestation and lactation) in an effort to assess fertility and reproductive endpoints. There were no statistically significant changes from control values for gestation length, pregnancy rate, percent live pups at birth and numbers of pups per litter. Pregnancy rates were decreased among dose groups but not significantly or in a dose-dependent manner. There was a trend for decreased pup weight with increasing dose from postnatal days 4-21 with the 1500 mg/kg pups significantly lower in weight than controls at 21 days. Recovery occurred by 90 days. Based on the results of both studies, the no observable adverse effect level for JP-8 reproductive effects is 750 mg/kg with 1500 mg/kg as a lowest observable adverse effect level.

1729 REPRODUCTIVE EFFECTS OF AZIDOTHYMIDINE IN CD-1 MICE WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.

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Azidothymidine (AZT) was evaluated for potential reproductive toxicity using the multigenerational Continuous Breeding protocol. With the increasing prophylactic use of AZT come concerns about the function of the offspring of treated parents. AZT was administered twice daily via oral gavage at dose levels of 0, 50, 100, and 200 mg/kg/day to adult male and female mice (N=20) during the F_0 generation only to test for effects of prenatal/lactational exposure on fertility, gestation, and development of the subsequent generation. F_1 animals were not dosed. Mating pairs were allowed to produce three litters (F_{1a}, F_{1b}, and F_{1c}). On PND 74±10, F_{1c} animals were assigned to mating pairs and allowed to produce three litters (F_{2a}, F_{2b}, and F_{2c}). Endpoints evaluated included body weight, food consumption, clinical signs, number and weight of pups, anogenital distance, sperm analysis, vaginal cytology, organ weights, and gross and microscopic pathology. In the F_0 generation, the Pregnancy Index in the high-dose was 65% and 60% for the 2nd and 3rd litters, respectively, compared to 100% in controls. Days to Litter was increased (4-11%) in all treated groups. There was a dose-related decrease (35-72%) in the Number of Pups/Litter in all treated litters from F_0 mice. In addition, there was a trend toward decreased Percent Males in all treated groups. F_0 sperm analysis revealed decreases in sperm motility, velocity, linearity, density and spermatid head counts at all dose levels. There were no differences in estrous cycles for the F_0 generation. In the F_1 matings, there were no effects on Pregnancy Index or Number of Pups/Litter, except for 23% fewer Pups/Litter in the F_{2c} litter at 200 mg/kg. Sperm and vaginal cytology for the F_1 generation were unchanged. Under conditions of this study, AZT had a significant reproductive effect at 50, 100, and 200 mg/kg/day in the F_0 generation when these mice were dosed, but had little reproductive effect in the F_1 generation when the F_1 pups were not dosed.

1730 EVALUATION OF DECAMETHYLCYCLOPENTASILOXANE (D5) IN A 2-GENERATION INHALATION REPRODUCTIVE TOXICITY STUDY IN RATS.

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Sprague-Dawley rats (30/sex/group; 45 days old at study start) were exposed to Decamethylcyclopentasiloxane (D5) by whole-body vapor inhalation (30, 70, or 160 ppm) 6 hr/day for 70 consecutive days before mating. A concurrent control group (30/sex) was exposed to filtered air. Exposure continued during mating and gestation through Gestation Day (GD) 20. Maternal exposure was interrupted from GD 21 through Postnatal Day (PND) 4, resumed on PND 5, and continued until weaning on PND 21. Starting on PND 22, F1 weanlings were exposed to D5 for at least 70 consecutive days before mating and continuing through mating, gestation, and lactation as described for the F0 generation. The F2 pups were not directly exposed to D5. Clinical observations, body weights and feed consumption were recorded at appropriate intervals. Parental rats were necropsied after weaning. Unselected F1 and F2 weanlings were necropsied on PND 21 or 28. There were no effects in any generation on appearance, behavior, clinical signs, body weight, body weight gain, or feed consumption. Estrous cyclicity, time between pairing and mating, mating and fertility indices, duration of gestation, and parturition in the F0 and F1 were unaffected by D5 exposure. Litter size, sex ratio, pup weights and pup viability were also not affected. Anogenital distance, vaginal patency, and balanopreputial separation were unchanged. Spermatogenic endpoints and primordial follicle and corpora lutea counts were unchanged. There were no effects on organ weights. Other than minimal alveolar histiocytosis in all exposed groups, there were no noteworthy microscopic findings. The NOAEL (no-observed-adverse-effect level) for reproductive toxicity was equal to or greater than 160 ppm. (Supported in part by the Silicones Environmental, Health and Safety Council.)

1731 EFFECT OF MANGANESE ON REPRODUCTIVE PERFORMANCE OF SD RATS.

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Manganese (Mn) is a ubiquitous constituent in the environment occurring in air, water, and soil. While Mn is beneficial at low intake levels, inhalation or oral exposure to high levels can cause adverse effects. Effects of manganese acetate treatment on the reproductive performance of SD rats were evaluated. Male and female Sprague-Dawley rats were dosed by oral gavage with 76, 153, 306 and 1838 mgMn/kg for 63 days. At the end of the dosing period, one half the animals were sacrificed. Significant decreases in ovarian weights were observed in all treatment groups. This effect was more pronounced in rats treated with 153 mgMn/kg. Insignificant increases in testicular weight were observed. The second half of animals were sacrificed after two weeks following mating, during which time Mn was continuously administered. Control groups (0 mgMn/kg) had an 80% pregnancy rate whereas 25, 0 100 and 24% pregnancy rates for 76,153, 306 and 1838mgMn/kg, respectively were observed. Mean litter size were reduced in the treated groups compared to the controls. These results suggest that manganese administration adversely affected the fertility index of rats while the animals were orally dosed with manganese during the two week mating period.

1732 A SEGMENT I REPRODUCTION STUDY OF LY353381 HYDROCHLORIDE ADMINISTERED ORALLY TO MALE CD RATS.

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LY353381 hydrochloride is a selective estrogen receptor modulator (SERM) which acts as a potent estrogen antagonist in uterine and mammary tissue while acting as an estrogen agonist to maintain bone density and lower serum cholesterol. The Segment I reproduction study was designed to evaluate the reproductive toxicity of LY353381 in adult male CD rats. LY353381 was administered by oral gavage in daily doses of 0, 0.3, 3, or 30 mg/kg to 20 male rats/group for 10 weeks prior to cohabitation, throughout cohabitation, and until termination. Males were cohabited with untreated females (1:1) for up to 2 weeks. Female rats were euthanized on Postmating Day 20 for assessment of reproductive parameters, and fetuses were collected for examination. Necropsies were conducted on the males after a total of 13 weeks of treatment. Epididymal and testicular sperm concentrations and epididymal sperm motion characteristics were evaluated. Testis and epididymis were collected, weighed, and preserved for histological examination. All rats survived until scheduled termination with no compound-related clinical signs of toxicity. Body weight (~20%), body weight gain (42% to 52%), and food consumption (9% to 31%) were decreased in a non-dose-responsive manner in males of all LY353381-treatment groups when compared to controls. The mating index was decreased for males of the 30-mg/kg group (6 of 20 failed to mate); however, there were no decreases in time to mating or the fertility index for males that mated. The decreased mating index at the 30-mg/kg group was likely an effect of LY353381 on libido. Epididymal sperm concentration parameters, epididymal sperm motion parameters, and spermatid numbers per testis were not adversely affected. In addition, there were no significant differences in absolute epididymal and testicular weights and no compound-related gross or microscopic changes in these tissues. Nineteen, 20, 18, and 12 females in the 0-, 0.3-, 3-, and 30-mg/kg groups, respectively, were pregnant. No important effects were seen in maternal reproductive parameters or in fetal viability, weight, gender, or external morphology. Based on upon the decreased mating index at 30-mg/kg, the no-observed-effect level for reproductive toxicity in male CD rats was 3 mg/kg.

1733 A PRELIMINARY STUDY ON THE REPRODUCTIVE CAPABILITY OF RATS TREATED WITH ZINC CHLORIDE.

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The effects of zinc chloride (Zn) on the reproductive performance of Sprague-Dawley rats was evaluated. Both male and female rats (45-50 days old) were administered 0.0 (control), 7.50 (low), 15.0 (mid) and 30.0 (high) mg/kg/day of Zn via oral gavage for 77 days prior to mating. At the end of premating dosing, males and females were paired as follows: 0.0:0.0, 7.5:7.5, 15.0:15.0 and 30.0:30.0 mg/kg/day. Dosing was continued for both sexes throughout mating (21 days). All males were euthanized at the conclusion of mating. Dosing was continued in females throughout gestation (21 days) and lactation (21 days). Evaluation of reproductive performance included sex ratios, fertility index, viability index, weaning index, litter size, andbody weights of pups on days 0, 4, 7, 14, 21 of lactation. There was no effects on fertility, weaning indices and sex ratios. However, pup viability indices on days 0 and 4 at the high dose were significantly lower than the control. Also, the body weights of pups in high dose group on days 14 and 21 were significantly lower than the control. These results suggest that Zn produced mild effects on reproductive perfor-

mance of rats. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU398948-07.)

1734 EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) IN A 2-GENERATION REPRODUCTIVE TOXICITY STUDY IN RATS.

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Sprague-Dawley rats (30/sex/group) were exposed to 0, 70, 300, 500 or 700 ppm D4 vapor by whole-body inhalation 6 hr/day for at least 70 consecutive days prior to mating, during mating and gestation through gestation day (GD) 20. Exposure was suspended from GD 21 through postnatal day (PND) 4 and resumed on PND 5 until weaning on PND 21 (F_0 generation) or until euthanasia (F_1 generation). Starting on PND 22, F_1 weanlings were exposed to D4 for at least 70 consecutive days before mating and continuing as described for the F_0 generation. The F_2 pups were not directly exposed to D4. Clinical observations, body weights and feed consumption were recorded at appropriate intervals. F_0 animals were mated once to produce the F_1 generation; F_1 parental animals were mated twice to produce two F_2 litters. Also, the F_1 males were mated with unexposed, nulliparous females. Extended parturition and/or dystocia were observed in 2 and 3 F_0 females in the 500 and 700 ppm groups, respectively, and in 1 F_1 dam in each of the 300, 500 and 700 ppm groups. The cause of death for 2 F_0 700 ppm group dams and 1 F_1 500 ppm group female was considered to be dystocia. Reductions in the mean number of pups born and mean live litter size were observed in the 300, 500 and 700 ppm groups in the F_0 generation and all D4-exposed groups in the F_1 generation; the reductions were statistically significant in the 500 and 700 ppm groups for both the F_0 and F_1 generations. No adverse effects were observed at any exposure level on anogenital distance, vaginal patency and preputial separation. Prolonged estrous cycles, delayed ovulation progressing to an anovulatory state, and decreased mating and fertility indices were observed in all F_1 groups exposed to D4. No adverse effects were seen on reproductive parameters on male spermatogenic endpoints, on microscopic evaluation of male reproductive tissue, or when the D4-exposed F_1 males were mated with the unexposed, nulliparous females, demonstrating that the reproductive toxicity observed was due to D4 exposure to the females. (Supported in part by the Silicones Environmental Health and Safety Council.)

1735 A VALIDATION STUDY OF DOG SPERM MOTILITY ASSESSMENT WITH A COMPUTER-ASSISTED SPERM ANALYZER.

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Male reproductive assessments in dogs have been subjected to problems associated with substantial variability in the methodology for the assessment of motility of dog spermatozoa between laboratories. The Computer Assisted Sperm Analyzer (CASA) system has provided a rapid, reliable and accurate assessment of rat spermatozoa motility and was considered appropriate to measure consistently the motility of dog spermatozoa.The purpose of this study was to validate the technique for evaluating motility of semen samples from male Beagle dogs with the use of the CASA.Semen samples were collected from 10 untreated Beagle dogs (approximately 15 months of age) by manual stimulation on 7 occasions. Samples collected on the last 4 occasions were used for the validation. Computer assisted motility assessments were performed, following semen dispersion in medium that was adjusted to facilitate optimum motility. Comparison of analyses over time (reanalysis of sample following 30 minutes, 1 hour and 2 hours at room temperature), reanalysis of stored disk images, assessments on non-motile samples and comparison to on screen manual assessments were performed.The differences between the CASA results and the manually assessed values were within the acceptance criteria of ±10% for the relevant samples analysed. Reanalysis of digitally stored images with the same settings were 100% accurate when compared to the results obtained by the original analysis. It was concluded that the use of a computer-assisted sperm analyzer gave consistent, reliable results of parameters such as overall motility, progressive motility and motion parameters (straight-line velocity, curvilinear velocity and path velocity) for spermatozoa collected from Beagle dogs.

1736 IMPAIRED SEMEN QUALITY IN MEXICAN AGRICULTURAL WORKERS EXPOSED TO ORGANOPHOSPHOROUS PESTICIDES.

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Organophosphorous pesticides (OP) are used worldwide. Human beings are exposed through environmental contamination and occupational exposure. It has been suggested that these chemicals disrupt human germinal cell homeostasis. Disruption is a common cause of poor reproductive outcomes, therefore, is of major public health significance. The objective of this study was to evaluate semen quality before and during pesticide spraying period between a non-occupationally exposed group (less exposed) and agricultural/sprayer workers (highly exposed). The study was conducted in an agricultural community where OP are the most widely used and sold pesticides. We constructed an exposure index based on OP use. Semen quality was assessed according to WHO methodology in 240 semen samples. In addition, 25 semen slides (13 from the highly exposed group and 12 from the less exposed group) were chosen for aneuploidy scoring by the three color chromosome fluorescence in situ hybridization (FISH) method for chromosomes X, Y and 18. Bivariate and multivariate analyses were performed. The results obtained after adjustment for several confounders disclosed that volume, total sperm count, total motility, motility grade A, viability, dead forms and mobile live forms were affected significantly in pesticide sprayers when compared with a non-occupationally exposed group, in both sampling periods. The parameters most affected were motility and viability. The crude proportion of all aneuploidy combined was 0.68% and 0.67% for sperm from highly exposed and less exposed men, respectively. However, these values were twice as high as those reported in similar studies. The most frequent aneuploidy observed was the lack of one sexual chromosome. Therefore, our results indicate that exposure to OP significantly impair semen quality.

1737 DIISOPROPYLFLUOROPHOSPHATE (DFP) ANTAGONISM BY RECOMBINANT ORGANOPHOSPHORUS ACID ANHYDROLASE (OPAA) ENCAPSULATED WITHIN STEALTH LIPOSOMES (SL).

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Stealth liposomes containing recombinant OP hydrolyzing enzyme (OPAA) were employed as a carrier model to antagonize the lethal effects of DFP. DFP was employed as a model OP, as it serves as a good substrate for OPAA as well as some chemical warfare agents, e.g., soman and sarin. The properties of this carrier system and the antidotal protection was studied alone and in a combination with 2-PAM and/or atropine. The carrier liposomes were made by mechanical dispersion method, and the hydrolysis of DFP was determined by measuring the increase of fluoride ion concentration using fluoride sensitive electrode. Recombinant OPAA enzyme originated from Alteromonas Strain J6D has a broad substrate specificity for OP compounds, especially for DFP, soman and sarin. The OPAA encapsulated within (SL) could rapidly hydrolyze DFP. The rate of DFP hydrolysis was directly proportional to the amount of (SL)* added to the reaction mixture. (SL) with no enzyme did not hydrolyze DFP. Rate of hydrolysis was first order to fluoride and diisopropylfluorophosphate. These studies suggest that the antidotal protection of the classic OP antidotes 2-PAM and/or atropine can be strikingly enhanced when they are used in a combination with the OPAA encapsulated (SL)*.

1738 METABOLITES OF HEXAMETHYLDISILOXANE AND DECAMETHYLCYCLOPENTASILOXANE IN FISCHER 344 RAT URINE.

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Hexamethyldisiloxane (MM or HMDS) and decamethylcylclopentasiloxane (D_5) are examples of a linear and a cyclic siloxane respectively. These volatile low molecular weight siloxanes are of significant commercial importance. They find use as ingredients in a wide range of cosmetic and personal care product formulations. These applications stem from a variety of favorable

physical characteristics such as adequate evaporation rate, low surface tension, lack of odor, a high degree of compatibility with other ingredients in formulations, solubility characteristics etc. Typical examples of applications include moisturizing creams, lotions, bath oils, colognes, shaving products and perfumes. They are also used as cleaners, lubricants and penetrating oils. To aid in the pharmacokinetic investigations, major metabolites of MM and D_5 were identified in urine collected from rats following exposure to [^{14}C]-MM and [^{14}C]-D_5. The structural assignments were based on GC-MS analysis of the tetrahydrofuran extract of urine containing the metabolites. The metabolites in the extract were first protected with trimethylsilyl groups prior to GC-MS analysis using bis(trimethylsiloxytriflouro- acetamide. The structures were also confirmed by comparisons with synthetic ^{14}C-labeled metabolite standards. The following are among the major metabolites identified in the case of MM: $Me_2Si(OH)_2$; $HOMe_2SiCH_2OH$; $HOCH_2Me_2SiOSiMe_2CH_2OH$; $HOMe_2SiOSiMe_2CH_2OH$; $HOCH_2Me_2SiOSiMe_3$; Me_3SiOH. The metabolites of D_5 are as follows: $Me_2Si(OH)_2$; $MeSi(OH)_3$; $MeSi(OH)_2OSi(OH)_3$; $MeSi(OH)_2O$- $Si(OH)_2Me$, $MeSi(OH)_2OSi(OH)Me_2$; $Me_2Si(OH)OSi(OH)Me_2$; $Me_2Si(OH)OSiMe_2OSi(OH)Me_2$; nonamethylcyclopentasiloxanol and hydroxymethylnonamethylcyclopentasiloxane. The presence of certain metabolites clearly established some demethylation at the silicon-methyl bonds. Although the initial oxidation step in the metabolic path way may be similar, at the end, the cyclic D_5 yielded, following ring opening, metabolites that are structurally different from that obtained for MM except for the commonly present $Me_2Si(OH)_2$.

1739 [13C8]LABELED STYRENE: TOXICOKINETICS AND URINARY METABOLITES IN MAN.

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Styrene is used in the manufacture of plastics and polymers. Styrene is metabolized to styrene oxide (SO) in rodents and humans. SO is hydrolyzed and metabolized to products (e.g. mandelic acid, MA; phenylglyoxylic acid, PGA) that have been used as markers of exposure in humans. Formation of SO-mercapturic acids (rodents and humans) and metabolites derived from phenylacetaldehyde (PAA, rodents) have also been described. The purpose of this study was to obtain quantitative information on the metabolism of styrene in humans for comparison with rodent data. Four healthy male volunteers were exposed to 50 ppm [13C8]styrene for 2 hr. Blood, expired air, and urine were time-course collected and analyzed by GC, HPLC, and NMR. Subjects exhaled 1-2% of the dose as styrene. Peak ~2 hr blood levels of SO were 3-13 nM and peak styrene blood levels were 7-9 μM. Peak urinary concentrations of MA (~22% of uptake) were reached ~2 hr following exposure termination and peak concentrations of PGA (~6% of uptake) were reached between 4 and 6 hr. MA and PGA were detected in urine up to 23 hr and 42 hr, respectively, following exposure. Hippuric acid (0.11 mM) and phenylacetic acid (0.09 mM) were quantitated in urine collected at 4 hr following exposure initiation. Based on evaluation of urinary metabolites, >95% of styrene metabolism in humans occurs via hydrolysis to SO while <5% occurs via conversion to PAA. These observations are similar to a low level of conversion to PAA (3-5%) in rats and in contrast to a significantly greater conversion (~12-20%) in mice (rodents were exposed to 125-500 ppm styrene for 6 hr). (This study was funded by the Styrene Information and Research Center.)

1740 METABOLISM AND DISPOSITION OF BISPHENOL A IN FEMALE RATS.

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Bisphenol A (BPA), which is used to manufacture polycarbonates, is weakly estrogenic in *in vitro* and some *in vivo* test systems. The effects of BPA on rodent offspring have been investigated following administration to pregnant animals during late gestation and continuing during lactation. The objectives of this study were to compare the metabolism and excretion of ^{14}C BPA in female Fischer 344 (F-344) and Sprague Dawley (SD) rats, to evaluate the transfer of BPA and metabolites from lactating dams to pups, and to assess the estrogenic activity of BPA metabolites *in vitro*. Adult female SD and F-344 rats were administered ^{14}C BPA (100 mg/kg) by gavage. Over 6 days, F-344 rats excreted more radioactivity in urine and less in feces than SD rats. Both strains had similar urine radioactivity profiles, with F-344 rats excreting more free BPA. The major urinary metabolite was characterized by ^{1}H and ^{13}C NMR spectroscopy as BPA glucuronide. In lactating SD rats administered ^{14}C BPA (100 mg/kg), the majority of the dose was recovered in the intestines at 1, 8, and 26 h after gavage. Only a small fraction of the dose was found in milk, with 0.95±0.66, 0.63±0.13, and 0.26±0.10 μg equivalents/ml (mean±SD) in the milk collected at 1, 8, and 26 h after dosing, respectively. Radioactivity in pup carcasses ranged from 44-78μg equivalents/kg. BPA glucuronide was the major metabolite in milk and plasma. In an *in vitro* test system for activity with the estrogen receptors α and β, BPA glucuronide (in contrast to BPA) did not show any appreciable activity at concentrations of up to 0.03 mM. These results indicate that metabolism of BPA via glucuronidation is a detoxication reaction.

1741 BENZENE METABOLISM BY ISOLATED PERFUSED RABBIT LUNG.

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Benzene is an occupational hazard and environmental pollutant associated with acute myelogenous leukemia in humans. There is limited evidence suggesting that lung cancer may result from benzene exposure. Pharmacokinetic models take into account benzene metabolism by liver but not lung, although recent analysis suggests they underestimate metabolite formation and that extrahepatic tissues may be important. *In vitro* studies have shown that lung is capable of metabolizing benzene. To further evaluate the capacity of lung to metabolize benzene in an *in vitro* preparation simulating *in vivo* metabolism, the isolated perfused rabbit lung was used. Lungs from male New Zealand White rabbits were placed in an artificial thorax and perfused via the vasculature with 55 μM ^{14}C-benzene for 30 min. One ml samples were taken at predetermined time intervals, and metabolites were separated by HPLC and quantified by liquid scintillation counting. Lung samples were also analyzed following the perfusion. They were solubilized, and metabolites were determined by HPLC and liquid scintillation counting. Analysis of the perfusate samples showed the decrease of benzene was 1st-order. Phenol in the perfusate increased in a non-linear manner for the first 20 minutes and then decreased. Phenylsulfate was detected in a few samples but not consistently. Tissue samples contained hydroquinone, phenol, and an unidentified metabolite. The results demonstrate that lung has the capability of metabolizing benzene in an *in vivo* simulation and should be included in pharmacokinetic models. (Supported by EPA Grant R826191.)

1742 BIOCHEMICAL EVIDENCE FOR INVOLVEMENT OF TYR381 AND TYR465 IN THE CATALYTIC MECHANISM OF MOUSE SOLUBLE EPOXIDE HYDROLASE.

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Mammalian soluble epoxide hydrolase (sEH) plays an important role in the detoxification of xenobiotic epoxides as well as in the biosynthesis of inflammation mediators. In order to improve our understanding of the catalytic mechanism, we produced mutant mouse sEH based on the X-ray diffraction structure of the corresponding enzyme-inhibitor complex, the potent dialkylurea N-cyclohexyl-N'-(3-phenyl)propyl urea (CPU) being the inhibitor (M. Argiriadi et al. Proc. Natl. Acad. Sci. USA, 96, 10637-10642, 1999). The site directed mutagenesis was performed in a baculovirus expression system. The mutant enzymes, Y381F and Y465F reduced 98 and 97% of the activity of hydrolysis of trans-diphenylpropene oxide, respectively, compared to that of the wild-type enzyme. The Y465F-Y381F double mutant lost the activity completely, indicating that both tyrosines are required for the activity. Inhibition by potent urea inhibitors were effected in both Y381F and Y465F mutants. Particularly Y381F increased the IC50 by 8-10 fold. To investigate the roles of Y381 and Y465 in the catalytic mechanism, kinetic experiments were carried out with a series of para-substituted chalcone oxides. These biochemical and kinetic results suggest that the two tyrosine residues in the active site have important functions in the inhibition by urea and in the catalytic mechanism.

1743 CHARACTERIZATION OF COUMARIN 3,4-EPOXIDATION IN B6C3F1 MOUSE, F-344 RAT AND HUMAN LUNG MICROSOMES.

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Coumarin is a mouse lung and rat liver toxicant. Acute coumarin administration (200 mg/kg) causes the selective necrosis of mouse lung Clara cells. However, equimolar dosages of this chemical do not cause rat lung injury, indicating that coumarin-mediated lung injury is species-specific. The goal of the current study was to relate species differences in susceptibility to lung toxicity with levels of pulmonary coumarin bioactivation. Coumarin-mediated toxicity results from the formation of coumarin 3,4-epoxide and its product *o*-hydroxyphenylacetaldehyde (*o*-HPA). Coumarin bioactivation was quantitated via the formation of *o*-HPA in whole lung microsomes. Clear species differences were observed in the rate of pulmonary microsomal *o*-HPA formation, with *o*-HPA production in mice being 20-fold higher than in rats. *o*-HPA formation was biphasic in pooled mouse lung microsomes. The apparent K_m and V_{max} values were 134 μM and 1.21 nmol/min/mg, and 753 μM and 2.58 nmol/min/mg for the high and low affinity enzymes, respectively. Consistent with the lack of Clara cell toxicity observed in rat lung *in vivo*, the single enzyme involved in *o*-HPA formation in F344 rat lung produced low levels of *o*-HPA, exhibiting a K_m and V_{max} of 183 μM and 0.218 nmol/min/mg, respectively. Furthermore, *o*-HPA formation was not detected in 10 individual human lung microsomal samples, indicating that humans are unlikely to be susceptible to coumarin-mediated pneumotoxicity.

1744 INHIBITION OF CYTOCHROME P450 2E1 ACTIVITY BY NICOTINE, COTININE, AND AQUEOUS CIGARETTE TAR EXTRACT *IN VITRO*.

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Tobacco-specific N-nitrosamines, N-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are both activated by cytochrome P450 (CYP) 2E1 in rat *in vivo*. Previous reports indicate that nicotine and its main metabolite, cotinine, reduce the mutagenicity of both NNK and NDMA in *Salmonella typhimurium*. To study the mechanism for this inhibition, we examined the effect of nicotine, cotinine, and aqueous cigarette tar extract (ACTE) on CYP 2E1 activity, as assessed by hydroxylation of *p*-nitrophenol, in CYP 2E1-expressing microsomes. Both nicotine and cotinine inhibited 2E1 hydroxylase over a range of pNP concentrations. At 0.12 mM *p*NP, IC_{50} for inhibition of *p*NP hydroxylase was 3.93 and 64.37 mM for nicotine and cotinine, respectively. At 0.24 mM *p*NP, IC_{50} values were 3.87 and 37.71 mM, respectively. Similarly, ACTE inhibited 2E1 activity over a range of pNP concentrations, and the extent of inhibition was dependant on ACTE concentration. These data show that nicotine, cotinine, and ATCE inhibit CYP 2E1 activity in this system, and that nicotine is a more potent inhibitor than cotinine. Thus, CYP 2E1 inhibition is a possible mechanism for the observed inhibition of NNK and NDMA mutagenicity by these compounds *in vitro*.

1745 TROGLITAZONE TOXICITY IN PRIMARY CULTURES OF HUMAN HEPATOCYTES.

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Treatment of primary human hepatocytes cultured in serum-free media for 2 hr with 10, 20, 25, 35 and 50 μM of troglitazone resulted in concentration-dependent decreases in total protein synthesis, with 90% inhibition observed at 50 μM. Decreases at 10 and 20 μM were reversible by 24 hr. However, protein synthesis did not recover at concentrations >25 μM. This change was associated with decreased inducible enzymatic and immunochemical levels of CYP3A. Troglitazone at 50 μM was lethal to the cells and associated with 70% decrease in MTT reduction by 24 hr. Addition of 5 mM acetaminophen or 2 mM phenobarbital 30 min. before and concomitant with 10 μM troglitazone, resulted in 70% and 60% decreases in protein synthesis, respectively. The effect on protein synthesis was associated with 3.6- and 1.4-fold decreases in formation of the troglitazone-sulfate metabolite by acetaminophen and phenobarbital, respectively. Troglitazone-quinone formation was increased 2-fold with acetaminophen and unaffected by phenobarbital. Exposure of human hepatocytes to a combination of 10 μM troglitazone and an inhibitor of sulfation (pentachlorophenol) resulted in a 90% inhibition in protein synthesis. Analysis of total cellular glutathione after treatment of hepatocytes with 50 μM troglitazone showed only a 25% decrease by 2 hr. These data suggest that inhibition of troglitazone metabolism by drugs competing for a common metabolic pathway may affect hepatotoxicity from troglitazone.

1746 THE HYPEROXIDATION OF L- DOPA BY CHLORPROMAZINE IN A LIPOXYGENASE CATALYZED REACTION.

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This study tests the effect of chlorpromazine (CPZ) on the lipoxygenase-catalyzed oxidation of L-Dopa in the presence of hydrogen peroxide. Soybean lipoxygenase (SLO)-catalyzed oxidation of L-Dopa was evaluated in the presence and absence of CPZ. The oxidation of L-Dopa to dopaminochrome was found to be proportional to the concentration of SLO, L-Dopa, CPZ, hydrogen peroxide, and linear with time. In the presence of 1.0 mM CPZ and 20 μg of SLO, the optimal conditions required to observe the maximal stimulation of the rate of L-Dopa oxidation at room temperature, included pH 4.0, 1.0 mM L-Dopa, 0.4 mM hydrogen peroxide. Under these assay conditions; CPZ radical was found to stimulate L-Dopa oxidation by ~25 fold. This stimulatory response was markedly suppressed by the addition of the classical inhibitors of lipoxygenase, nordihyroguaiaretic acid (NDGA) and gossypol. These results explain the ineffectiveness of CPZ-Dopa combination therapy for Parkinson's disease.

1747 REGENERATION OF QUERCETIN AND OTHER FLAVONOIDS BY NAD(P)H:QUINONE OXIDOREDUCTASE 1.

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Flavonoids are a class of naturally occurring compounds which are found in vegetables and fruits. Epidemiological studies suggest that dietary intake of flavonoids may reduce the incidence of heart disease and certain types of cancer. Quercetin in both aglycone and glycosylated forms is one of the most common flavonoids found in the diet and recent experiments suggest that quercetin has both antioxidant and prooxidant properties. Oxidation of quercetin has been proposed to generate a transient quinone metabolite. The purpose of this study was to examine whether any metabolites generated during quercetin oxidation could serve as substrates for NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) and undergo two-electron reduction to regenerate quercetin. HPLC analysis was used to examine quercetin oxidation reactions in the presence and absence of purified rhNQO1. Enzymatic oxidation (tyrosinase, myeloperoxidase), superoxide-dependent oxidation (xanthine/xanthine oxidase) and copper-stimulated, pH-dependent (pH 8) oxidation of quercetin resulted in generation of a metabolite which could be reduced by purified rhNQO1. In the absence of NQO1, quercetin (50μM) was substantially depleted (60-70%) following oxidation. In the presence of NQO1, however, only 5-10% of quercetin was depleted. In addition, the presence of NQO1 increased oxygen consumption 2-fold during copper-stimulated, pH-dependent oxidation of quercetin. The NQO1-dependent regeneration of quercetin and the increase in oxygen consumption was NAD(P)H-dependent and dicumarol-inhibitable. Similar results were observed with NQO1 and other flavonoids. These data suggest that NQO1 may be able to regenerate quercetin and other flavonoids following oxidation and individuals who are homozygous for the NQO1*2 polymorphism and lack NQO1 activity may have a decreased capacity to regenerate these compounds. (Supported by CA51210 and ES 09554.)

1748 ETHANOL METABOLISM IN HUMAN FETAL BRAIN CATALYZED BY A NOVEL OXIDASE ENZYME.

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Ethanol is a well-known teratogen that often causes a pattern of morphologi-

cal and neurological abnormalities in the fetus referred to as fetal alcohol syndrome. Mechanisms have been proposed to explain alcohol's deleterious effects, several involving the enzyme-catalyzed oxidation of ethanol to its highly reactive metabolite acetaldehyde. In prenatal human brain (gestational days 90-115), the contribution of several enzymes to acetaldehyde production was evaluated using high performance liquid chromatography with fluorescence detection. The addition of specific inhibitors and cofactors/cosubstrates did not affect the activity substantially suggesting that an enzyme system other than alcohol dehydrogenase, CYP2E1, or catalase may be involved during early development. Heat inactivation and substrate saturation confirmed this was an enzymatic process while additional experiments determined the activity was localized in the cytosolic fraction, dependent on molecular oxygen, and inhibited weakly by sodium azide. Collectively, these results indicate that an alcohol oxidase might be largely responsible for catalyzing the conversion of ethanol to acetaldehyde in human fetal brain. Alcohol oxidases have not been reported in mammals but have been identified in lower species, such as yeast and bacteria. Further characterization of the enzyme revealed a two-component system consisting of a large protein (molecular weight>100,000) and smaller molecule (molecular weight<1,000) essential for activity. The specific activity in human fetal brain was approximately 20% of that measured in adult rat liver. The significant level of ethanol-oxidizing activity observed in prenatal brain suggests that the quantities of reactive metabolite generated may be of extreme importance and this novel enzyme could play a major role in eliciting neurotoxic effects during human development. (Supported by ES-04041 and ES-07032.)

1749 FETAL N-ACETYLTRANSFERASES: A POTENTIAL ROLE IN 4-AMINOBIPHENYL PRENATAL GENOTOXICITY.

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Exposure to aromatic amines such as 4-aminobiphenyl (4ABP) results in hemoglobin or DNA adducts in adults, neonates or fetuses. Biotransformation of 4ABP is required for production of DNA-reactive species. One pathway involves N-hydroxylation followed by O-acetylation, which is catalyzed by N-acetyltransferases (NAT). The contributions of maternal and fetal NAT to the genotoxicity of 4ABP were investigated in C57Bl/6 mice. NAT activity in maternal liver and conceptal tissue at gestational days (GD) 10, 15, and 18 was evaluated by measuring the formation of 4-acetylaminobiphenyl (4AABP) by S9 fractions. NAT activities were 0.2±0.03 nmol 4AABP/min/mg protein at GD 10, 0.26±0 at GD 15 and 0.36±0.04 at GD18 compared to 1.55±0.43 for maternal liver. There was a statistically significant (p=0.05) increase in 4ABP-NAT activity from GD10 to GD15 and from GD 15 to 18. The formation of 4ABP-DNA adducts was assessed in maternal liver and fetal tissue at GD15 and GD18. Pregnant animals were killed 24h after a single oral dose of 120 mg 4ABP/kg or corn oil. Tissue was frozen, histological sections prepared and adducts detected by immunofluorescence. Little or no fluorescence was observed with corn oil. For animals exposed to 4ABP, the relative fluorescent intensities were similar at GD15 and GD18, 23.4±1.4 and 24.5±3.3 respectively. At GD15, the value for maternal liver was 35.6±3.6 and 39.6±4.0 at GD18. The presence of 4ABP-DNA adducts is further evidence that maternal exposure to 4ABP can damage fetal DNA. Although it is not clear whether the DNA-reactive products are formed *in utero*, the present data demonstrate that conceptal tissue has the ability to acetylate 4ABP. Consequently, fetal biotransformation may contribute to the prenatal genotoxicity of 4ABP. (Supported by Arizona Disease Control Research Commission and NIEHS, ESO6694 (CAM) and ESO8846 (MAP).)

1750 NON-LYTIC EXPRESSION OF XENOBIOTIC-METABOLIZING ENZYMES IN INSECT CELLS.

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Insect cells are widely used for heterologous expression due to high yield and capability of producing functionally active proteins. However, the currently used insect cell expression systems are cell-lytic, which creates uncertainties when cell injuries are used as experimental end points. We used a non-lytic system and established insect cell lines which expressed several xenobiotic-metabolizing enzymes (e.g., carboxylesterases and cytochrome P450s). cDNAs encoding these enzymes were inserted into the pIZ/V5-His vector, which were then used to transfect Sf9 insect cells. The transfected cells underwent selection against Zeocin-containing media for stable integration in the genome. Western immunoblotting analyses showed that specific antibody to each enzyme detected a single protein only in the Sf9 cells expressing the corresponding enzyme which co-migrated with the native protein in human liver microsomes. Two cell lines expressing human carboxylesterases (HCE-1 and HCE-2) were further characterized for the correlation of the expression levels with plating density and requirement for selective media. Cells at all plating densities (30-80%) exhibited little difference in the expression levels of the recombinant protein. However, over-confluence caused slightly lower expression levels. Cells cultured in Zeocin-containing media expressed higher levels of the recombinant protein but exhibited a lower proliferation rate. The recombinant carboxylesterases were enzymatically active and both HCE-1 and HCE-2 rapidly hydrolyzed *para*-nitrophenylacetate. HCE-1 was more sensitive to the inhibition by phenylmethylsulfonyl fluoride (PMSF), whereas HCE-2 was more sensitive to the inhibition by sodium fluoride SF). Finally, carboxylesterase-expressing cells are more resistant (20-30 times) to either PMSF or SF than the control Sf9 cells. (Supported by a PHS grant ES07965.)

1751 DOSE-DEPENDENT EFFECT OF TRI-*O*-TOLYLPHOSPHATE ON HEPATIC AND PANCREATIC FATTY ACID ETHYL ESTER AND METHYL ESTER SYNTHESIZING ACTIVITIES.

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Background: Fatty acid ethyl ester synthase (FAEES) is an enzyme that catalyzes the conjugation of alcohols with fatty acids. The formation and accumulation of these lipophilic conjugates in target organs may result in toxicity. Previous studies from this laboratory have shown that FAEES purified from rat liver is structurally and functionally different from that of pancreas. **Objective:** In this study the functional relationship of hepatic and pancreatic FAEES is further characterized *in vivo*, using tri-o-tolylphosphate (TOTP; a β esterase inhibitor) in a dose-dependent manner. **Methods:** Five groups of rats received i.p. injection of 10, 25, 50, or 100 mg/kg TOTP in corn oil, or vehicle alone. The animals were sacrificed 18 hours later, and the liver and pancreas were excised. FAEES activity was analyzed by measuring the ester formation following incubation of [1-^{14}C] oleic acid with ethanol or methanol in the presence of post-nuclear fraction of liver or pancreas at 37°C for 2 hours, while esterase activity was determined using p-nitrophenyl acetate (PNPA) as a substrate. **Results:** The percent inhibition of FAEES activity was found to be 61, 65, 78 and 87% in the liver (at 10, 25, 50, and 100 mg/kg TOTP respectively). Using methanol as the substrate, the percent inhibition was 74, 72, 88, and 93%, respectively. No inhibition was found in the pancreas of rats treated with TOTP as compared to the controls. The PNPA assay gave similar results with the percent inhibition in the liver being 73, 77, 88 and 93%, respectively. In the liver, maximal inhibition of fatty acid methyl ester or ethyl ester synthesizing activities was at the 100 mg/kg dose. Western Blot analysis demonstrated that the pancreatic FAEES does not cross-react with the FAEES antibody that recognizes both liver and plasma FAEES. **Conclusions:** From these studies we conclude that hepatic ethyl ester and methyl ester synthesizing activities could be expressed by the same enzyme, inhibited by TOTP, and are functionally and structurally different from that found in the pancreas.

1752 PURIFICATION AND CHARACTERIZATION OF RAT PANCREATIC FATTY ACID ETHYL ESTER SYNTHASE AND ITS RELATIONSHIP TO FATTY ACID METHYL ESTERS AND ANILIDE SYNTHESIZING ACTIVITIES.

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Fatty acid ethyl esters (FAEEs), formed by a fatty acid ethyl ester synthase (FAEES)-catalyzed reaction of ethanol with fatty acids, have been implicated in pathogenesis of diseases of extrahepatic organs during alcohol abuse. Among all the organs and tissues, pancreas possesses the highest FAEES activity. In previous studies, we demonstrated that FAEES, purified from rat liver microsomes, is identical to carboxylesterase (pI 6.1) and structurally and functionally different than that of pancreas. In the present studies, synthesizing activities towards FAEEs, fatty acid methyl esters (FAMEs) and fatty acid anilides (FAAs) from rat pancreas were purified and their interrelationship was evaluated with cholesterol esterase (ChE). p-Nitrophenyl acetate (PNPA) hydrolyzing activity (a measure of esterase activity) was also determined. The synthesizing activity for FAEEs, FAMEs and FAAs as well as hydrolyzing activities for PNPA were co-purified at each step of purification by ammonium sulfate saturation, and Q Sepharose XL and heparin-agarose column chromatography. The FAEE, FAME and FAA synthesizing activity of heparin-agarose purified protein was found to be 3380, 5800 and 670 nmol/hr/mg protein, respectively, while PNPA hydrolyzing activity was 35 micromol/min/mg protein. The purified protein showed a molecular weight of ~68kD by SDS-

PAGE under reduced denaturing conditions and cross-reacted with antibodies to rat pancreatic ChE. Based on molecular weight and cross-reactivity with

antibodies to rat pancreatic ChE, the purified protein appears to be ChE, which catalyzes the formation of FAEEs as well as FAMEs and FAAs.

1753 CLONING, MUTAGENESIS AND EXPRESSION OF A MAJOR RAT CARBOXYLESTERASE REST1 IN THE BACULOVIRUS SYSTEM.

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Liver microsomal carboxylesterases (EC 3.1.1.1) hydrolyze xenobiotics containing ester, thioester or amide groups and thus play an important role in drug metabolism and detoxification of many chemicals in the environment. They also are common activators of prodrugs and are proposed as therapeutics agents. The baculovirus expression of these enzymes allows the study of the metabolism of drugs and the efficient production of antibodies towards these proteins. In this study, a major rat liver carboxylesterase, rEST1, was cloned from a double-stranded library using PCR. The 1.7 kb insert was subcloned to a baculovirus vector, pFASTBac. The rEST1 carboxylesterase was expressed successfully in the High5 derived cells from Trichoplusia ni egg cell. Carboxylesterases are located in the luminal site of the endoplasmic reticulum and attached to a specific receptor through an HXEL motif located at the C-terminal. A mutation in the last amino acid of the rEST1 carboxylesterase, from leucine to arginine, perturbed the trafficking of the enzyme. The mutant form of rEST1 is secreted to the media and thus purification is achieved easily through one step classical or affinity purification. In conclusion, the expression of carboxylesterase in the baculovirus system combined with the single mutation of the last amino acid which makes the carboxylesterase rEST1 secretory results in inexpensive, high yield and homogenate enzyme.

1754 ORAL MUCOSAL METABOLISM OF VINYL ACETATE.

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Statistically significant increases in tumor incidences in several sites within the oral cavity of the rat and mouse have been observed following chronic oral exposure to vinyl acetate (VA). Since carboxylesterase-dependent hydrolysis of VA to acetic acid and acetaldehyde has been implicated in the nasal inhalation carcinogenesis of this ester, the potential for oral mucosa of the rat and mice to hydrolyze VA was examined. Homogenates were prepared by scraping the mucosa from four regions of the oral cavity: dorsal interior (all tissues interior to the teeth), dorsal tongue surface, ventral interior (sublingual area and lower interior tissues) and exterior (all tissues exterior to the teeth). The oral cavity was rinsed once with saline prior to dissection to determine if oral secretions possessed metabolic capacity. Aliquots of the homogenates or rinse fluid were incubated for 30 minutes with varying concentrations of vinyl acetate (0.05-10 mM), and the production of acetaldehyde was quantitated by HPLC. All tissue regions possessed VA hydrolysis activity. In both species the hydrolysis activity was greatest in the dorsal interior region (Vmax of 90 and 6 nmol/min in the rat and mouse, respectively, Km values of 0.5 and 0.9 mM). Activity in the other oral regions was 2-15 fold lower. Activity was observed in the rinse fluid, but was 30-50 fold less than the dorsal interior region. Local hydrolysis of VA may play a role in the oral toxicity of ingested VA.

1755 DEVELOPMENTAL EXPRESSION AND CHARACTERIZATION OF HEPATIC MICROSOMAL AND SERUM A-ESTERASES IN THE RAT.

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A-esterases (AEST) are primarily involved in the metabolism of oxidized lipids and detoxification of the oxons of organophosphorus (OP) insecticides. AEST have been found in two isozyme forms in several species. Previous studies have shown that differences in the pattern of AEST activity for direct and indirect methodologies (high and low substrate concentrations, respectively) may be due to different isozymes operating in the assays. This study investigated the developmental expression of hepatic (microsomal) and serum AEST isozymes in male and female rats at postnatal days (PND) 1, 3, 12, 21, 33, and 80, using native polyacrylamide gel electrophoresis (PAGE), with B-naphthylacetate (BAC) as the substrate (esterase stain). Equivalent amounts of tissue (for each age) were added to the gels. Hepatic AEST activity was present at all ages in both sexes studied, with two high molecular weight (MW) proteins (250 kDa and 200 kDa; isozymes 1 and 2, respectively) stained for activity. At PND 1 and 3, isozyme 1 was faintly detected. Both isozymes stained with increasing intensity at PND 12, 21, 33, and 80. Serum activity toward BAC was also present at all ages and in both sexes studied. As in the liver, two high MW proteins were identified (200 kDa and 137-200 kDa; isozymes 1 and 2, respectively). At PND1 and 3, isozyme 2 was only faintly detected. At PND12, 21, 33, and 80, both isozymes stained for activity. To further characterize the biochemical aspects of AEST and determine the relative contribution of each AEST isozyme to the hydrolysis of OP oxons, liver AEST were purified through the application of detergent solubilized microsomes to a stirred ultrafiltration cell, followed by excision from a native PAGE gel and application to an anion exchange chromatography column. By observing the rate of development of AEST isozymes and determining their role in the detoxification of OP insecticides, the reasons for age-related differences in OP toxicity may be discerned further. (Supported by NIH RO1 ES04394 and F31 Eso5752.)

1756 INVOLVEMENT OF THE TYROSINE CATABOLIC PATHWAY IN THE INHIBITION OF DCA METABOLISM.

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Dichloroacetate (DCA) has been shown both clinically and in animal models to inhibit its own metabolism. The mechanism of this inhibition remains to be elucidated. The enzyme responsible for the dechlorination of DCA to glyoxylate is GST Zeta (GSTz), also known as maleylacetoacetate isomerase, an enzyme in the tyrosine catabolic pathway responsible for the cis/trans isomerization of maleylacetoacetate (MAA) and its decarboxylation product, maleylacetone (MA), to fumarylacetoacetate (FAA) and fumarylacetone, respectively. MAA is a known alkylating agent, and it is theorized that inhibition of MAA and/or MA metabolism by DCA could lead to alkylation of GSTz, rendering it inactive. In vitro pre-incubation of DCA (0.5 mM) with dialyzed rat cytosol caused a direct inhibition of GSTz activity which was not seen in dialyzed human hepatic cytosol. Incubation of rat and human hepatic cytosol with 0.2 mM DCA in the presence of MA (0.05-0.4 mM) led to a concentration dependent inhibition of DCA metabolism. Administration of DCA to male Sprague Dawley rats, 4 mg/kg x 5 days-1000 mg/kg for one or five days, led to a dose dependent inhibition of *in vitro* GSTz activity in hepatic cytosol and up to a 30 fold increase in urinary excretion of MA in rats dosed with greater than 50 mg/kg. Using a polyclonal antibody to human GSTz, western blots of hepatic cytosol from these animals revealed a dose dependent decrease in GSTz expression. These data taken together suggest more than one mechanism may be operational in DCA's inhibition of its own metabolism. (Supported by U.S. Public Health Service ES 07375.)

1757 *IN VITRO* METABOLISM AND GLUCURONIDATION OF THE HOP FLAVONOID XANTHOHUMOL.

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Xanthohumol (XN) is the major prenylated flavonoid of hops (*Humulus lupulus*) and has been detected in beer. Recent studies have suggested it may have potential cancer chemopreventive activity, but little is known about its metabolism. We investigated the glucuronidation and conversion of XN to various metabolites by rat and human liver microsomes. Three major polar metabolites were found with hepatic microsomes from either untreated rats or phenobarbital pretreated rats as detected by HPLC analysis. Isosafrole- and β-naphthoflavone-pretreated rat liver microsomes formed an additional major nonpolar metabolite. Human liver microsomes produced the same three metabolites formed by untreated rat liver microsomes. *In vitro* glucuronidation of XN using rat liver microsomes and human liver microsomes produced two monoglucuronide conjugates. As determined by LC-MS and ^{1}H-NMR analysis, two of the major polar microsomal metabolites of XN were tentatively identified as 5''-(2-hydroxyisopropyl)-[2'',3'':3',4'] dihydrofurano-2',4-dihydroxy-6'-methoxychalcone and a derivative of XN with a hydroxyl addition on the B ring. Additionally, the nonpolar metabolite appears to be dehydrocycloxanthohumol. By the same analytical methods, the monoglucuronide conjugation was shown to occur at either the 4- or 4'- position of

XN. The biological activity of these metabolites and their involvement in cancer chemoprevention remains to be established. (Supported by the Hop Research Council and NIEHS grant ES00210.)

1758 INDOLE-3-CARBINOL, A GLUCOSINOLATE BREAKDOWN PRODUCT OF CRUCIFEROUS VEGETABLES, INDUCES QUINONE REDUCTASE GENE EXPRESSION THROUGH TWO GENE REGULATORY ELEMENTS, ARE AND XRE.

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Epidemiological studies show that cruciferous vegetables play a role in protection against cancers. These protective effects are thought to be associated with plant secondary metabolites, termed glucosinolates. Indole-3-carbinol (I3C), the breakdown product of the glucosinolate glucobrassicin, is known to increase phase I and phase II detoxification enzymes. We and others previously showed that I3C increased quinone reductase (QR), Glutathione-S-Transferases (GSTs), and CYP 1A activities and mRNA levels *in vivo*. In this study, we confirm the finding of others that both I3C and I3C acid condensates bind to the cytosolic aryl hydrocarbon receptor (AhR) to activate the xenobiotic response element (XRE) for QR gene expression. To measure the QR gene expression, we performed transient transfection of HepG2 human hepatoma cells with a reporter gene construct. 12-fold and 1.7-fold activation of the XRE over control were observed with 50 μM I3C or I3C acid condensates treatments, respectively. Here we show also for the first time that I3C acid condensates and I3C both caused dose-dependent activation of the antioxidant response element (ARE) in a dose range of 10 μM-200 μM. Both I3C and I3C acid condensates showed significant activation of the ARE at 25 μM and greater concentrations. The data suggest that QR is upregulated by I3C via both the ARE and the XRE.

1759 EFFECTS OF CHRONIC DIETARY INDOLE-3-CARBINOL (I3C) EXPOSURE ON BLOOD CHEMISTRY AND DRUG METABOLISM IN FISCHER 344 RATS.

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I3C is a naturally occurring component of the human diet and is found in high concentrations in cruciferous vegetables. I3C is also marketed as a dietary supplement and has been proposed as a chemopreventive agent for women against breast cancer. A sub-chronic exposure of I3C is known to induce several phase I and phase II drug metabolizing enzymes while inhibiting the flavin-containing monooxygenase (FMO). Less is known about the effects of long term dietary I3C exposure to rats. Fischer 344 rats were fed diets containing 0, 1000 or 2500 ppm I3C for 28 weeks. A small animal chemistry panel was performed, and serum testosterone levels determined. The total cytochrome 450 (CYP) content of the liver was also investigated. Alkaline phosphatase, creatine kinase, alanine aminotransferase and testosterone levels were significantly decreased in the rat serum while globulin levels were increased. As seen in sub-chronic studies, the liver total P450 content was significantly induced. The alterations observed in this study following long-term dietary I3C administration raises concerns relative to potential adverse effects on hormone levels and drug/xenobiotic metabolism. (Supported by PHS grant ES07060.)

1760 SELECTIVE INDUCTION OF TRIIODOTHYRONINE (T_3) GLUCURONIDATION BY PREGNENOLONE-16α-CARBONITRILE (PCN) IN RATS.

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It has been postulated that UDP-glucuronosyltransferase inducers decrease circulating thyroid hormone concentrations by increasing the biliary excretion of thyroid hormones. The inducers 3-methylcholanthrene (3-MC) and Aroclor 1254 (PCB) are extremely effective at reducing serum thyroid hormones, whereas pregnenolone-16α-carbonitrile (PCN) is less effective. However, only PCN treatment results in a marked increase in serum levels of thyroid stimulating hormone (TSH), whereas 3-MC and PCB cause little to no increase in TSH. Sustained TSH elevation is considered the primary stimulus for thyroid tumor development in rats, yet the mechanism by which enzyme induction leads to TSH elevation is not fully understood. Whereas PCN, 3-MC, and PCB all increase microsomal glucuronidation of T_4, only PCN causes an increase in microsomal glucuronidation of T_3. The purpose of this study was to determine whether PCN, which increases serum TSH, causes an increase in the glucuronidation and biliary excretion of T_3 *in vivo*. Male rats were fed control diet or diet containing either PCN (1000 ppm), 3-MC (250 ppm), or PCB (100 ppm) for seven days. Animals were then given [^{125}I]-T_3 *iv*, and bile was collected for two hours. Radiolabeled metabolites in bile were analyzed by reverse-phase HPLC with γ-detection. The biliary excretion of total radioactivity was increased 1.7 fold by PCN, but not by 3-MC or PCB. Of the T_3 excreted into bile, approximately 75% was recovered as T_3 glucuronide, with remaining amounts represented as T_3 sulfate, T_2 sulfate, T_3, and T_2. Biliary excretion of T_3 glucuronide was increased 1.5 fold by PCN, while neither 3-MC nor PCB altered T_3 glucuronide excretion. These findings indicate that PCN induces the glucuronidation and biliary excretion of T_3 *in vivo*, and suggest that increased elimination of T_3 may be the mechanism responsible for the increases in serum TSH caused by PCN. (Supported by NIH grants ES-08156 and ES-07079.)

1761 EFFECT OF PERINATAL EXPOSURES OF RATS TO THE PCB MIXTURE AROCLOR® 1254 ON HEPATIC MICROSOMAL METABOLISM OF ESTRADIOL AND THE PRO-ESTROGEN METHOXYCHLOR.

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Polychlorinated biphenyls (PCB's) are well known inducers of hepatic cytochrome P450. The influence of gestational and lactational exposures of rats to the PCB mixture Aroclor 1254 was determined for hepatic microsomal metabolism of 17β-estradiol (E2) and the proestrogenic organochlorine insecticide methoxychlor (MXC). Dams were administered 4 mg/kg-day of Aroclor 1254 in safflower oil p.o. from gestational day 6 to post-natal day (PND) 22 (weaning). Pups were sampled on PND 15, 23, or 31. There was little E2 metabolism in microsomes of controls of either sex at PND 15 or 23, but there was greater metabolism in the PCB-treated animals, with 2-hydroxyestradiol (2-OH-E2) the major metabolite. There was more 2-OH-E2 production in the control microsomes at PND 31 than from the younger ages, and several additional metabolites were observed in the microsomes from PCB-treated animals. Enhanced hepatic metabolism of E2 may have been responsible for lower serum E2 levels observed in PCB-treated prepubertal females. There was greater MXC disappearance and a higher level of appearance of the estrogenic metabolite hydroxyphenyl- trichloroethane (HPTE) as well as other MXC metabolites in hepatic microsomes from the PCB-treated animals compared to the controls in both sexes. In high dose *in vivo* treatments with MXC, the enhanced uterine weights and depressed testicular weights induced by MXC were attenuated in the animals also treated with PCBs. It appears that MXC is metabolized to non-estrogenic metabolites more quickly in PCB-treated animals than in controls. Therefore, PCB exposure is capable of altering the hepatic metabolism of both endogenous estrogens and the pro-estrogen MXC which may influence the response of the developing reproductive system to endogenous or exogenous estrogens. (Supported by EPA R825296.)

1762 NOVEL SUBSTRATE AND INHIBITOR PROPERTIES OF CATFISH INTESTINAL PHENOL SULFOTRANSFERASE (SULT) WITH HYDROXYLATED BENZO[A]PYRENES.

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Hydroxylated xenobiotics ingested in the diet, including phenolic and dihydrodiol metabolites of benzo(a)pyrene (BaP) may be sulfated in the intestine, and this may influence their absorption and toxicity. Mammalian SULT enzyme activity can be inhibited by environmental compounds, including pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP), as well as bioflavonoid natural products. Cytosol from intestinal mucosa of the channel catfish was incubated with 3- or 9-hydroxyBaP, BaP-7,8-dihydrodiol (BaP-7,8D) or 2-naphthol as substrates and PAPS as cosubstrate. The phenolic metabolites were readily sulfated, whereas BaP-7,8-D was a very poor substrate. Incubation of intestinal cytosol with 35S-PAPS and increasing concentrations (1 to 50 μM) of BaP-7,8-D revealed dose-dependent sulfation of unknown compounds in the reaction mixture. Preliminary results showed a comparable stimulation of the sulfation of 3-OH-BaP when low concentrations (0.05 to 5 μM) of BaP-7,8-D was added to the reaction mixture, suggesting separate regulatory and catalytic sites of the SULT enzyme. Neither PCP nor DCNP, 0.1 to 10 μM, inhibited sulfation of 3-OHBaP or 2-naphthol as substrates, whereas quercetin inhibited sulfation of both substrates in a

dose-dependent manner. The IC50 of quercetin for 3-OHBaP was 0.5 μM and for 2-naphthol was 5 μM. These results show the potential for intestinal sulfation of phenolic, but not dihydrodiol metabolites of BaP and suggest the catfish SULT differs from mammalian phenol SULT, which is effectively inhibited by PCP, DCNP and BaP-7,8-D. (Supported by ES 05781.)

1763 DIFFERENTIAL BENZO[A]PYRENE METABOLISM AND EXCRETION IN TWO RELATED FISH.

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Differential susceptibility of polycyclic aromatic hydrocarbon (PAH)-mediated liver cancer exists in two related species of Ictaluridae catfish. The hypotheses to explain this difference are that the relatively insensitive channel catfish 1) do not produce the PAH metabolites associated with DNA binding, 2) they more quickly eliminate PAHs due to greater phase II enzyme activities, and/or 3) they more rapidly repair PAH-DNA adducts than the more sensitive brown bullhead. Livers and bile were collected from each species 6, 24, 72, and 168 hr after a single 10 mg/kg i.p. BaP exposure. Cytosolic 1-chloro-2,4-dinitrobenzene-glutathione-S-transferase (GST) and cis-stilbene oxide-microsomal epoxide hydrolase activities were not significantly different in BaP-treated and control animals. Furthermore, enzyme activities were not significantly different across the time course or between species. HPLC-APCI-MS of bile and bile enzymatically digested to detect glucuronyl transferase (GT), GST, and sulfotransferase (ST) conjugated metabolites indicated that channel catfish more quickly eliminated total biliary metabolites compared to brown bullhead. GT and GST conjugates predominated; ST conjugates were minimal. BaP-diones accounted for the majority of metabolites in both species. Formation of BaP-7,8-dihydrodiol and slower metabolite excretion in brown bullhead samples may be linked to the increased PAH susceptibility in this species.

1764 3-METHYLCHOLANTHRENE (3MC) ALTERS BIOTRANS FORMATION BUT NOT THE BIOAVAILABILITY OF BENZO[A] PYRENE -7,8- DIHYDRODIOL (7,8D) IN AN *IN-SITU* INTESTINAL CATFISH PREPARATION.

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Catfish gavage fed semipurified diet with or without 3-MC (10 mg/kg diet) for 10 days were utilized for in situ isolated perfused intestinal preparations. A micelle solution containing either 20 or 40mM [3H]7,8D was administered in the lumen. After a 60 minute perfusion, mucosa, postinfusate and perfusion blood were utilized for assessment of bioavailability and intestinal biotransformation of [3H]7,8D. [3H]7,8D molar equivalents (Meq) were readily available from the intestinal lumen to the intestinal mucosa and the systemic circulation. The mucosa demonstrated dose responsive increases in [3H]7,8D Meq from the 20 to 40mM treatments, however, no significant differences were evident with 3-MC pretreatment from respective controls for both [3H]7,8D concentrations. [3H]7,8D Meq concentrations were consistently greater in the mucosa as compared to the postinfusate and greater for the postinfusate as compared to the blood. The blood demonstrated similar [3H]7,8D Meq concentrations for control and 3-MC treatments at the 40 mM dosage and marginally lower values for the 3-MC treatment at the 20mM [3H]7,8D dose. Postinfusate demonstrated dose related changes in [3H]7,8D Meq concentrations, but no significant changes were evident with 3-MC pretreatment. HPLC analysis demonstrated that [3H] 7,8D, was the primary constituent found in all biological media in all treatments with triols, tetrols and glutathione and glucuronide conjugates also evident. 3MC treated animals exhibited increased amounts of triols, tetrols and glutathione conjugates. These results indicate that dietary 7,8D was readily absorbed and biotransformed by the intestine with variations in metabolite profile evident with 3-MC pretreatment. (NIH ES05781.)

1765 GENDER SPECIFIC ALTERATION OF GLUCURONIDATION IN BROOK TROUT DURING THE ANNUAL REPRODUCTIVE CYCLE.

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There is limited information on the effect of physiological and biochemical changes associated with the annual reproductive cycle on hepatic phase II biotransformation rates in fish. The purpose of this study was to provide insight regarding metabolic deactivation of xenobiotics and to provide data for the prediction of altered hepatic biotransformation rates and pathways during the reproductive cycle. *In vitro* phase II biotransformation of phenol was assessed using hepatic microsomes isolated from three male and three female brook trout (*Salvelinus fontinalis*) every four weeks from June through December. Glucuronidation of phenol over a range of substrate concentrations (1 to 60 mM) was quantified for the determination of Michaelis-Menten constants (k_m, V_{max}) by HPLC with UV DAD. Initially there were no significant differences in glucuronide rate of formation (pmol/min/mg protein) or total capacity (pmol/min/liver) between immature adult females and males. At the peak of maturation, the maximum rate of glucuronide formation was 4-fold less in females, however, the total capacity was approximately equal between the sexes, due to the increased liver size in the females. The peak alteration in metabolic parameters coincided with increases in the hepatic and gonadal somatic indices and with changes in plasma hormone concentrations. These data provide valuable insight for resolving uncertainties which are central to fish predictive toxicity namely, the role of biotransformation and maturation in chemical disposition.

1766 SEX-LINKED CHANGES IN PHASE I BIOTRANSFORMATION OF PHENOL IN BROOK TROUT OVER AN ANNUAL REPRODUCTIVE CYCLE.

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The microsomal metabolism of phenol (11°C) over an annual reproductive cycle from June to December has been studied using fall spawning adult brook trout (*Salvelinus fontinalis*). Hepatic microsomes were isolated from three male and three female fish each month. Incubations were optimized for time, cofactor concentration, pH, and microsomal protein concentration. Formation of phase I ring-hydroxylation metabolites, i.e., hydroquinone and catechol, was quantified by HPLC with dual channel electrochemical detection. Sample preparation and chromatographic conditions were optimized to achieve the separation and sensitivity required for the analysis of these labile products. Metabolism of phenol over a range of substrate concentrations (1 to 150 mM) was quantified for calculation of Michaelis-Menten constants (K_m and V_{max}) for each month. Results indicate a nearly equal production of hydroquinone and catechol among males and females in late June. At the peak of maturity (October) there was an approximate ten fold greater production of ring-hydroxylation metabolites noted in females in comparison with males on a total liver basis. The increased production correlated with increased liver size and ovarian development in females. These experiments indicate the importance of quantifying changes in metabolic capacity, in response to large seasonal changes in physiology and biochemistry noted during sexual maturation and spawning.

1767 EVALUATION OF LIVE PRECISION-CUT TISSUE SLICES USING MULTIPLE INDICATORS OF CELLULAR INJURY VIA A CONFOCAL MICROSCOPIC TECHNIQUE.

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Previous studies have shown that cytotoxic effects of toxicants can be monitored in live precision-cut liver slices using confocal microscopic techniques. Initial studies used the live/dead probes Syto-10 and Dead Red to examine cytotoxic effects. These fluorescent probes were again used in conjunction with the intracellular calcium probes Fluo-4 and Oregon Green BAPTA-1, to examine the sequence of toxic effects produced by the toxicant vinylidene chloride (VDC). At multiple timepoints from 2 to 8 hr, toxicant exposed slices from male adult SD rats were removed and stained with various fluorescent probes. Living and dead cells were stained with Syto-10 and Dead Red. Slices were exposed to 2.5μl dye/500 μl media for 15 min in the dark at room temperature, followed by two washes. The Fluo-4 and Oregon Green BAPTA-1 dyes were used to visualize intracellular calcium changes. For each calcium probe, slices were exposed to 5μl dye/500μl media for 20 min. After staining, slices were placed in a 35 mm tissue culture plate (modified to contain a glass coverslip base) and were held in place with aluminum meshing. Slices were viewed using a Leica TCS confocal microscope containing an IRBE inverted microscope and Kr/Ar laser. Simultaneous visualization of the stains was obtained by excitation with the 488/568 laser lines accompanied by FITC and TRITC emission bandpass filters. VDC lead to a selective progressive dam-

age of the slices. Slices displayed little or no dead cells at 2 hr. Dead cells began to appear at 3 hr around blood vessels and progressively spread to encompass the entire slice by 7 hr. Slices stained with calcium probes displayed a similar pattern. Calcium was present in both control and dosed slices; however, dosed slices showed increased levels around the blood vessels. By 7 hr, calcium levels were increased throughout the slices. Control slices showed no cellular damage at timepoints up to even 8 hr. These results indicate that multiple indicators of cell viability can be monitored by evaluation of live tissue slices using confocal microscopy. (Procter & Gamble Exploratory Research Grant).

1768 OPTIMIZATION AND CHARACTERIZATION OF PRECISION-CUT PROSTATE SLICES AS A TOOL FOR TOXICOLOGICAL STUDIES.

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Precision-cut prostate slices offer a valuable new model for studying the prostate. This human slice system has advantages since it maintains stromal-epithelial relationships. In our initial studies, human prostate slices from the peripheral and transitional zones were maintained up to 72 hr. However, due to the androgen dependence for cell-cell interactions in prostate tissue, unsupplemented media was unable to maintain optimal prostate function. Thus, slices from peripheral and transitional zones of the prostate were incubated for 24, 48, and 72 hr in serum free keratinocyte basal medium supplemented with bovine pituitary extract, bovine insulin, hydrocortisone, bovine transferrin, and human epidermal growth factor. Androgen supplemented media contain either 30 nM 5α-androstan-17β-ol-3-one (A) or 30 nM cyclodextrin-encapsulated testosterone (T). Media was collected at 24, 48 and 72 hr and assayed for lactate dehydrogenase (LDH) and prostate specific antigen (PSA). These two viability markers are measures of cellular damage (LDH) and maintenance of secretory function (PSA). Slices were also collected and formalin fixed for histological evaluation. Androgen supplemented media slightly enhanced PSA secretion and reduced LDH leakage in transitional and peripheral slices. Of the two forms of testosterone, A showed the most effect. These results show that the supplementation of testosterone, in particular the 5α-androstan-17β-ol-3-one form, increases the viability and maintains cellular function. (Arizona DCRC Grant I-345.)

1769 PRECISION-CUT TISSUE SLICES FROM TRANSGENIC MICE AS AN *IN VITRO* TOXICOLOGY SYSTEM.

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Using transgenic animals with specific promoter regions encoding for biomarkers coupled to reporter genes as the source of tissue allows for an easily detectable signal for toxic insult, which can readily be measured by *in vitro* methods. This approach combines the power of the transgenic animals with the practicality of *in vitro* systems to investigate the biological impact of xenobiotics. When key regulatory sequences are "tagged," a wide array of mechanistic studies can be performed with specific detection of when and where the gene is expressed in the normal tissue architecture and, more importantly, its role in a sequence of toxic events. Two transgenic mouse lines, one of which couples a 2X tetradecanoyl phorbol acetate (TPA) repeat element (TRE) to luciferase, and another which couples cytochrome P450 1A1 to beta-galactosidase are used to determine the feasibility of this approach. Precision-cut kidney and liver slices from both transgenic lines remain viable as determined by slice ion content and enzyme release. Kidney and liver slices from the 2X TRE transgenic mice demonstrate induction of luciferase (up to 2.5 fold) when treated with TPA. The studies with the P450/beta-galactosidase transgenic mice exhibit an increase in beta galactosidase activity (1.5 fold induction in liver slices) when treated with beta-napthoflavone for 24 hr. These data indicate that precision-cut tissue slices from transgenic mice offer a novel *in vitro* method for reporter detection while maintaining normal cell homogeneity.

1770 A THEORETICAL MODEL FOR SIMULATING THE OUTCOME OF MECHANISM-BASED *IN VITRO* TOXICITY TESTING STRATEGIES.

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In order to investigate the fundamental principles that control the optimum selection of alternative test methods for the evaluation of chemical hazards, it is necessary to have a model system to explore possible alternative testing strategies. In general, our lack of detailed knowledge of the mechanisms of toxicity (including not only the early events in the interaction of chemicals with biological systems, but the sequence of subsequent events that lead to measurable toxicity) limit the development of quantitative dynamic models of the toxicological process for individual chemicals. We report here the development of a theoretical model that includes various potential mechanisms of toxicity. The "mechanisms" incorporated into the model are qualitatively similar to known mechanisms of action. The model is exercised to simulate the responses that would be observed using alternative batteries of *in vitro* endpoint measurements for a collection of test chemicals. The data generated are used to evaluate the relative effectiveness ("validity") of several testing strategies based on *in vitro* toxicity tests. (This research was supported by the Air Force Office of Scientific Research.)

1771 A NOVEL *IN VITRO* SYSTEM FOR EXPOSURES OF CELL CULTURES TO VOLATILE CHEMICALS.

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A major concern when conducting *in vitro* testing of volatile chemicals is loss of the chemical during the course of experimental studies. Such losses, due to volatility, affect dosimetry. Different engineering solutions have been developed, previously, to address this issue. However, the limitations of those systems make them impractical for many research applications. We have designed a novel system for exposing microwell plate cultures of primary rat hepatocytes to volatile chemicals. Our system allows for static or flow-through dosing methodologies, with ports for continuous sampling of both the headspace and media. Each chamber can hold multiple microwell plates or petri dishes and fits easily into a standard laboratory incubator. We have evaluated the efficacy of the chamber by comparing the responses of cells in the chamber to those cultured in a standard incubator, without chemical dosing and with dosing of low-volatility chemicals, e.g. hydrogen peroxide. The results show no difference between the treatments, as indicated by the responses of standard viability markers (MTT and LDH). Our current experiments have focused on halogenated chemicals. In one series of experiments, cell cultures were dosed with carbon tetrachloride in the chamber. When compared to cells not exposed to the chemical, cells receiving doses of 0.55, 1.75 and 3.11mM carbon tetrachloride exhibited catalase activity decreased to 66%, 25%, and 8%, lipid peroxidation products were elevated 205%, 294%, and 382%, and cellular viability was 95%, 90%, and 15%, respectively.

1772 THE DEVELOPMENT OF SIMPLE *IN VITRO* CYTOXICITY SCREENING MODELS FOR DRUG DEVELOPMENT.

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The goal of this study is to design two to three *in vitro* cytotoxicity screening models to support drug development and discovery. Cytotoxicity screening models are useful in assessing the toxicity in early stages of drug discovery, as well as investigating the toxicity of degradants, impurities (byproducts, intermediates), and metabolites in the later stages of drug development. Three cell lines (MDCK, HepG2, and Caco-2) representing kidney, liver, and colon, respectively, were used to evaluate the cytotoxic and apoptotic effects of different toxicants. The assays were performed in a 96-well plate format with each concentration of test article incubated in triplicate for 24 hours. Solvent (DMSO)-treated wells were used as the control at a final concentration of 1%. The three cell lines showed differences in sensitivity to known target organ toxicants as measured by colormetric (MTT) and fluorometric endpoints (apoptosis). The rank order for cytotoxicity in MDCK cells was cisplatin > 5-fluorouracil > acetaminophen. Cisplatin showed dose-dependent cytotoxicity, which induced significant cytotoxic effects at 5 mM. The rank order for cytotoxicity in HepG2 cells was bromobenzene > tamoxifen > alfatoxin B1. Less than 1% of bromobenzene and 50- 100 micro-M of tamoxifen induced substantial cytotoxicity within 24 hours. The rank order for cytotoxicity in Caco-2 cells was cisplatin > 5-fluorouracil > etoposide. Besides cisplatin at 5 mM, 5-fluorouracil and etoposide revealed minimal toxicity in Caco-2 cells. These established cells lines and endpoints provide for a rapid and simple method to screen a large number of compounds with a minimum amount of test material (<25 mg).

1773 *IN VITRO* CYTOTOXICITY TESTING WITH CULTURED HUMAN LUNG AND DERMAL CELLS.

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An extensive *in vitro* study using human cultured cells was conducted to test the basal cytotoxicity theory. This theory suggests that most chemical injury, at least *in vitro*, is a manifestation of one or more insults to the basic cellular structures and functions common to mammalian cells. This accounts for the similarity of results in multilaboratory studies. Human lung epithelial carcinoma (A549) cells, and human skin fibroblasts (WS1 and Detroit551) were studied in culture to evaluate their potential to screen for cytotoxicity. Confluent monolayers were incubated in the absence or presence of increasing concentrations of test chemicals for 24-hours, and fluorescent-labelled probes were used to assess toxicity. Inhibitory concentrations were extrapolated from concentration-effect curves after linear regression analysis. Ten chemicals, including arsenic, mercury, copper, sodium flouride, nicotine, caffeine, thioridazine, paraquat, amitriptyline, and chloroquin, were tested with each cell line using calcein-AM and Sytox. The data suggest that fluorescent probes are sensitive indicators of toxicity and contribute to understanding the mechanisms for each chemical. In addition, A549 cells revealed some significant differences from skin fibroblasts in their response to toxic insult. In combination with previously published reports, our study suggests that a basal cytotoxic phenomenon may only explain the similarity of results between skin fibroblasts and other finite human cell lines. (Supported by NIH, NIGMS Grant GM08153.)

1774 OXYGEN BIOSENSOR SYSTEMS: A HOMOGENEOUS FLUORESCENCE TECHNOLOGY FOR TOXICITY ASSAYS.

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BD ViaSante's Oxygen BioSensor System* represents a platform technology adaptable to a large number of cellular and subcellular applications in the Drug Discovery and Development arena. This technology allows researchers to fluorescently and unobtrusively monitor the level of dissolved oxygen in an assay medium, and may be adapted to any number of automation-friendly platforms, including 96- and 384-well FALCON® brand microtiter plates. Because they allow for continuous real-time read-out without the need for additional reagents, Oxygen BioSensor Systems are ideally suited for kinetic monitoring of cells in toxicity assays, proliferation assays, or any other assay where researchers would like to know the relative viability of the cells in the assay. Because the Oxygen BioSensor is entrapped within an inert polymeric matrix, the determination of oxygen concentration in no way perturbs the cell culture, this makes these systems an ideal platform for assay multiplexing. To demonstrate the simplicity and effectiveness of this platform technology, a series of standard toxicity screens were performed using a range of toxic agents and cell lines. In all cases, the signal from the Oxygen BioSensor System was easily detected, and correlated directly to the number of cells in the assay. In a side-by-side comparison with standard MTT assays for cellular toxicity, the Oxygen BioSensor System gave the same IC50 values as MTT, but without the need for additional reagents, handling steps, or incubation periods. Moreover, the fact that Oxygen BioSensor Systems could be read repeatedly, facilitated the generation of kinetic toxicology profiles. These profiles also correlated to the known modes of action of the drugs studied. Hence, with minimal additional effort, and no additional reagents, the BD ViaSante Oxygen Sensor System upgraded a standard toxicity screen into a more predictive and informative assay for cellular toxicity.

1774A EVALUATION OF THE EFFECT OF 2,3-DIMERCAPTO-PROPANE-1-SULFONATE (DMPS) AGAINST MERCURY CYTOTOXICITY IN PRIMARY LIVER AND KIDNEY CELL CULTURES.

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The effect of 2,3-Dimercaptopropane-1-sulphonate (DMPS) against the mercury cytotoxicity was studied in primary rat liver and kidney cells cultures. Hepatocytes and kidney cells were pretreated with DMPS (0.2mM) and 30 minutes later were exposed to $HgCl_2$ (60μM) for 12 and 24 h. Cytotoxicity was evaluated by neutral red (NR) uptake and mitochondrial metabolic activity (methylthiazoltetrazolium (MTT) test). A significant decrease of 96% on MTT reduction and of 75% on NR uptake was observed after 12 h exposure to 60μM $HgCl_2$. On the other hand in the DMPS pretreated cells and then exposed to $HgCl_2$ (12 h) a decrease of 14% on MTT reduction and of 20% on NR uptake was observed in the hepatocytes and 20% (MTT) and 25% (NR) in the kidney cells. DMPS (60 μM) did not produce cytotoxicity. **Conclusion:** DMPS produced a significant protective effect against the *in vitro* cytotoxicity of mercury in primary liver and kidney cell cultures.

1775 RELATIVE TOXICITY OF KETOCONAZOLE (KETO) AND CHLORAMPHENICOL (CAP) IN RAT AND HUMAN HEPATOMA CELL LINES: APPLICATIONS FOR *IN VITRO* TOXICITY SCREENING.

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Toxicity and mechanistic data, incorporated early in the drug discovery process, should allow compounds to be prioritized in a manner that would maximize efficacy while reducing toxicity. *In vitro* toxicity screening allows compounds within a therapeutic class to be prioritized for subsequent development based on the concentration that produces a half-maximal toxic response (TC50). Immortalized cell lines are routinely used for *in vitro* screening. However, the potential for cell lines from different species to respond differently when exposed to the same drug (e.g.TC50 values) must be defined. Therefore, the primary objective of the present study was to compare the toxicity of KETO and CAP in rat (H4Ile) and human (HepG2) hepatoma cell lines by monitoring cell viability via MTT reduction and ATP levels. Both cell types were seeded into 96-well plates at a density of 50,000 cells/mL in minimum essential medium with 10% serum in a final volume of 200 μl. Following a 48 hr growth period, media was removed and the cells were dosed with either KETO (0 to 300 μM) or CAP (0 to 20 mM) in media (0.5% DMSO). After 24 hrs, media was removed and MTT and ATP assays performed. KETO produced a dose-related decrease in MTT and ATP in H4Ile cells with TC50 values of 50 μM and 70 μM respectively. In HepG2 cells, exposure to KETO produced TC50 values for MTT and ATP of approximately 70 μM. Exposure to CAP produced a dose-related decrease in MTT and ATP with a TC50 of 2 mM in H4Ile cells. In HepG2 cells, CAP yielded TC50 values of 4 mM for MTT and 10 mM for ATP. In conclusion, both cell lines responded in a similar manner to CAP and KETO with H4Ile cells being slightly more sensitive than HepG2 cells. Ongoing studies of mitochondrial dysfunction as a common biological pathway of CAP and KETO toxicities are being performed in isolated hepatic mitochondria to elucidate and compare the subcellular responses of both cell lines to these agents.

1776 THE CELISA ASSAY CAN BE USED IN PLACE OF THE HEMATOPOIETIC COLONY ASSAY TO SCREEN FOR ERYTHROTOXICITY.

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The CELISA™ assay can be used in place of the hematopoietic colony assay to screen for erythrotoxicity. Erythrotoxicity is currently assayed through use of the clonogenic "colony assay". The extremely low throughput of the colony assay precludes its use in high throughput toxicity testing. A high-throughput CELISA assay of erythropoiesis based on the quantification of differentiation-dependant synthesis of hemoglobin has been developed. Either human hematopoietic progenitors or committed erythroid precursors can be utilized to initiate the assay; the time required for completion of the assay with these two cell types is 10 and 5 days respectively. The erythroid progenitors do not express classical markers of erythroid differentiation but rapidly begin to synthesize hemoglobin and express cell-surface glycophorin A after they are placed in culture. The CELISA assay was used to examine the effect of a known inhibitor of hemoglobin biosynthsis, succinylacetone, on hemoglobin accumulation in erythroid progenitors. Inhibition of hemoglobin biosynthesis by succinylacetone was 86%, a value nearly identical to that reported previously. After the required cell culture period, the CELISA assay requires only 90 minutes for quantification, as opposed to the lengthy process of manually counting colonys required by the colony assay. The CELISA assay is a 96-well-based suspension culture system available for use by researchers seeking a more high-throughput protocol for toxicity screening. These results indicate that the CELISA assay system can substitute for the clonogenic colony assay for screening potential erythrotoxic agents.

1777 PREVALIDATION OF THE EPIDERM PHOTOTOXICITY TEST (ED-PT).

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The EpiDerm™ phototoxicity test was chosen for prevalidation within ECVAM's tiered prevalidation procedure. In phase 1, ZEBET drafted an SOP and project plan for conducting the study. ZEBET's task in phase I was to adapt the existing Skin2 methodology to the specific needs of the epidermal model EpiDerm. UVA sensitivity experiments revealed 6J/cm2 as the highest non-cytotoxic UVA dose and exposure to test materials for 3, 6, and 21 hours before UVA irradiation revealed 21 hours to be the optimum exposure time. Established phototoxins were used to evaluate the method and were correctly identified. In phase II, taking into account the patch technique used *in vivo*, the three participating laboratories amended this technique and evaluated its usefulness for the EpiDerm test. Results revealed that the patch technique was useful for chemicals applied in oil but not in water. Since reproducibility of the data within, and between, the laboratories was excellent, it was decided to proceed to phase III. In phase III, ZEBET drafted the final SOP, and BIBRA selected ten chemicals for the blind trial. Each of the chemicals was tested twice independently in each laboratory and the data were submitted for biometrical analysis, which confirmed the expected predictivity and robustness of the test; only one positive chemical (tetracycline) was not detected as a phototoxin and none of the negative chemicals was over predicted as a phototoxin. We conclude that the ED-PT 1) meets the criteria previously determined for the Skin2 PT, 2) allows use of complex matrixes, 3) is an adjunct test to the 3T3 NRU PT, 4) is ready to undergo formal validation.

1778 CORROSITEX®, AN *IN VITRO* DERMAL CORROSIVITY TEST FOR PREDICTING THE *IN VIVO* CORROSIVITY POTENTIAL OF CHEMICALS/COMPOUNDS: ANALYSIS OF PERFORMANCE.

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The performance of Corrositex®, an *in vitro* dermal corrosivity testing method for predicting *in vivo* dermal corrosivity, was analyzed. Corrositex® data from several sources for 163 test materials were compared against data generated using the rabbit skin corrosivity test. The overall accuracy of Corrositex® was 79% (128/163) for all test materials; 75% (27/36) for inorganic and organic acids plus acid mixtures; 93% (13/14) for acid derivatives; 80% (20/25) for amines; 83% (29/35) for inorganic and organic bases plus base mixtures; and 81% (69/85) for organic and inorganic acids and bases. Corrositex® data were also compared to results obtained using pH extremes as an indicator of the corrosivity potential of chemicals. Current US Environmental Protection Agency regulations (40 CFR 158.690) specify that primary dermal irritation testing requirements can be waived for chemicals that have pH values less than 2 or greater than 11.5; such materials are labeled as toxicity category 1 because of the potential corrosive effect. A detailed comparison of data for these pH extremes and Corrositex® showed that both pH and Corrositex® are highly predictive of the corrosivity of chemicals in the pH extreme ranges. However, Corrositex® was more accurate than pH for predicting corrosivity potential. (Supported by NIEHS Contract N01-ES-85424 to ILS, Inc. Sponsored by the SOT Regulatory and Safety Subspecialty Committee.)

1779 RESULTS OF AN ICCVAM-SPONSORED PEER REVIEW PANEL EVALUATION OF CORROSITEX®.

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Corrositex®, an *in vitro* dermal corrosivity test method, was recently evaluated by an independent Peer Review Panel (Panel) convened by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The method was evaluated as to its usefulness as an alternative test method for predicting the *in vivo* corrosivity potential of chemicals and compounds. The Panel assessed inter- and intra-laboratory performance and the testing results for 163 chemicals tested with Corrositex® and for which there was *in vivo* data. In a January 21, 1999 public meeting in Bethesda, MD, the Panel concluded that, in specific testing circumstances such as that required by the US Department of Transportation, Corrositex® is useful as a stand-alone assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives. In other testing circumstances and for other chemical/product classes, the Panel concluded that Corrositex® may be used as part of a tiered testing strategy, where positive responses require no further testing and negative responses must be followed by dermal irritation testing. The Panel recommended that in either testing strategy, an investigator may conclude that confirmation testing is necessary based on consideration of supplemental information (e.g., pH, structure-activity relationships). The use of Corrositex® as part of a tiered testing approach reduces and refines animal use by eliminating the need to further test substances that are determined to be corrosive. Substances that are negative in Corrositex® or that do not qualify for testing are also unlikely to be corrosive. Corrositex®, when used as a stand-alone assay for certain chemical classes, replaces animal use for corrosivity testing. (NICEATM supported by NIEHS Contract N01-ES-85424 to ILS, Inc. Sponsored by the SOT Regulatory and Safety Subspecialty Committee.)

1780 AN *IN VITRO* MODEL TO STUDY THE PROTECTIVE EFFECT OF BISMUTH AGAINST CISPLATIN INDUCED NEPHROTOXICITY.

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Renal impairment due to tubular damage is an important, dose-limiting side effect of the cytostatic agent cisplatin. Animal and clinical studies provided evidence that bismuth salts are able to reduce cisplatin nephrotoxicity without compromising its anti-tumour activity. We developed an *in vitro* model to elucidate the mechanism of this protective effect using NRK-52E cells, a rat kidney cell line of tubular origin, grown to near-confluence on glass slides. The cells were pre-treated with 100 μM BiCl3 and 400 μM citrate in serum free medium for 12 to 48 hours. Subsequently, the cells were incubated in serum free medium for 6 to 24 hours before being treated with 50 μM cisplatin for 41 hours. Normal, necrotic and apoptotic cells could be discerned after staining with Hoechst 33258, a DNA-specific dye. Cisplatin treatment caused part of the cells to become apoptotic as detected by the fragmentation of the nuclei. Bismuth pre-treatment significantly lowered the percentage of apoptotic cells when compared to controls which had been pre-treated with citrate only. The largest reduction found was from 77% ±15 (SD) to 41% ±9 apoptotic cells. Conclusion: This *in vitro* model can serve to investigate the mechanism of the protective effect of bismuth against cisplatin induced nephrotoxicity in order to prevent or reduce this side effect or enabling the use of higher cisplatin doses.

1781 NEUROPROTECTIVE ROLE OF MELATONIN IN METHAMPHETAMINE-INDUCED DOPAMINERGIC NEUROTOXICITY.

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Methamphetamine (METH)-induced dopaminergic neurotoxicity is thought to be associated with the formation of free radicals. Since evidence suggests that melatonin may act as a free radical scavenger and antioxidant, the present study was undertaken to investigate the effect of melatonin on METH-induced dopaminergic neurotoxicity, both *in vitro* and *in vivo*. PC12 cell cultures were exposed to METH (250-1000 μM) in the presence or absence of melatonin (500 μM). METH caused a dose dependent depletion of dopamine (DA), and its metabolites DOPAC and HVA in PC12 cells. Treatment with melatonin did not protect against the depletion of DA or its metabolites. For *in vivo* studies, adult male Swiss-Webster mice were injected with METH (5 mg/kg x 3) and humanely killed after 72 hr. METH administration caused 45-57% depletion in the content of striatal DA and its metabolites, DOPAC and

HVA. The administration of melatonin (10 mg/kg) before each of the three injections of METH (on day 1), and thereafter for two additional days, afforded full protection against METH-induced depletion of DA and its metabolites DOPAC and HVA. Body temperature was also recorded during METH treatment. Melatonin significantly diminished METH-induced hyperthermia. Since it is postulated that METH-induced hyperthermia is related to its neurotoxic effect, the protective effect of melatonin observed *in vivo* but not *in vitro* may be due primarily to diminishing METH-induced hyperthermia.

1782 USE OF CELL AND ORGANOTYPIC CULTURES TO DEFINE MECHANISMS UNDERLYING THE MARKED TOXICITY OF THE TERATOGENIC RETINOID, TTNPB.

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The aromatic retinoid, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylenyl)-1-propenyl] benzoic acid (TTNPB) is 1000-fold more teratogenic than all trans-retinoic acid (tRA) in several species. The remarkable potency of TTNPB is due, in part, to binding to nuclear receptors (RARs) in the nanomolar range, reduced affinities for the cytosolic binding proteins and slow rate of metabolism (Toxicol. Appl. Pharmacol. 142:319-327, 1997). The present work investigates whether differences in transcriptional activity of the ligand receptor complex and/or the long half life of TTNPB could account for the great toxicity of TTNPB. The ability of TTNPB and tRA to activate the RARs was determined using a reporter construct driven by the tRA response element that was transfected into JEG3 cells. After 24 hours, tRA activated the RARs with greater efficacy and with EC50s that range from 30-90% greater potency than those of TTNPB. However, after 72 hours, TTNPB was more efficacious in activating RARs, and EC50s were 7- to 63-fold more potent than those for tRA. Mouse limb bud cell cultures, a well characterized model for retinoid teratogenesis, were used to examine the role of metabolic stability and teratogenesis. We examined whether inhibiting the metabolism of tRA in the limb bud assay markedly increases its potency in relation to that of TTNPB. Liarozole, a 4-hydroxylase inhibitor of tRA was used at concentrations that did not affect chondrogenesis. In the absence of liarozole, tRA was 465-fold less potent than TTNPB. In the presence of liarozole, the potency of tRA was only 12-fold less than tRA despite the fact that inhibition of metabolism was less than 2-fold. Thus, inhibiting metabolism of tRA by a small amount may have marked effects on the toxicity of this compound in the mouse limb bud cell assay. This data strongly suggests that the long half life of TTNPB may be the most significant factor in understanding the toxicity of TTNPB.

1783 REACTIVE OXYGEN SPECIES FORMATION IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE.

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Experimental models of Huntington's disease (HD) can provide information on biochemical and molecular aspects leading to brain damage. Three models of HD are well-known so far: a) Quinolinic acid (QUIN), a tryptophan metabolite, induces overactivation of NMDA subtype of glutamate receptors and enhances oxidative stress by lipid peroxidation; b) intrastriatal administration of kainic acid (KA) produces neuronal death involving non-NMDA and NMDA receptors, and calcium influx via voltage-gated Ca^{2+} channels, also activating toxic pathways and generating free radicals; and c) 3-nitropropionic acid (3-NP) acts at mitochondrial level producing a depletion of ATP synthesis and membrane depolarization, overactivation of voltage-gated NMDAr and excitoxicity. In this study we compared the effects of QUIN, KA and 3-NP on the formation of reactive oxygen species (ROS) in rat brain synaptosomes. Synaptosomes were incubated with QUIN, KA and 3-NP (25-2000 μM) during 60 min at 37°C. ROS technique used 2'7'-dichlorofluorescein-diacetate (DCFH-DA) to detect cerebral ROS. These toxins produced a concentration-dependent increase of ROS formation in synaptosomes. 3-NP resulted more potent than QUIN and KA to produce ROS at all concentrations tested (3-NP>QUIN>KA). Results indicate involvement of ROS in the pattern of toxicity elicited by these toxins as well as differential potencies to produce oxidative damage. (CONACyT Grant J28612-M.)

1784 EVALUATION OF THE EFFECT OF RODENT CARCINOGENS ON THE EXPRESSION OF BIOTRANSFORMATION ENZYMES IN HUMAN HEPATOCYTES.

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The objective of this study was to develop a feasible, reproducible and simple human hepatocytes culture system for evaluating xenobiotics as hepatic enzyme inducers, which could be used for routine toxicology. Human hepatocytes cultured with Matrigel in a serum-free medium containing low concentrations of insulin (1 μM) and hydrocortisone (0.1 μM), responded to prototypical liver CYP450 enzyme inducers (phenobarbital, PB; dexamethasone, DEX; rifampicin, RIF; 3-methyl-cholanthrene (3MC); and clofibrate; CLO) and rodent carcinogens (methapyrilene, MET; tamoxifen, TAM; cyproterone, CY; isoniazid, INZ, and 2-acetyl aminofluorene, 2AAF) with enhanced levels of expression of inducible forms of CYP450. The mRNA levels of human CYP450 isoforms (CYP1A, CYP2B, CYP3A, CYP4A) were measured by RT-PCR and fluorogenic DNA probe technology. Hepatocytes were found to respond to chemical CYP450 inducers with marked increases in enzyme expression at the mRNA levels and in a concentration related fashion. CYP3A was found to be induced by RIF, DEX, TAM, CY, MET, and PB; CYP1A was induced by 3MC, MET, TAM, INZ; CYP2B was induced by PB, MET, DEX, RIF and TAM. And CYP4A was found to be induced by CLO and MET. The results from this study indicate that human hepatocytes cultured with diluted Matrigel and coupled with enzyme mRNA expression analysis by RT-PCR have proven to be a valuable and important *in vitro* toxicological approach to assess the chemical-induced changes in expression of liver CYP450 enzymes in humans.

1785 DRUG METABOLIZING ENZYME ACTIVITIES IN THE HUMAN COLORECTAL CARCINOMA CELL LINES CACO2 AND HT29.

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Drug metabolizing enzyme activities were characterized in $CaCo_2$ and HT29, two frequently used human colorectal carcinoma cell lines, in order to give an overview of the metabolic potency of the cells and to use them as cellular model system in toxicological *in vitro* studies. The influence of the status of confluency of the cell monolayer on enzyme activities was studied by performing measurements after 2, 7, 14 and 21 days of culture. Scanning electron microscopic investigations demonstrated a differentiation during prolonged culture detectable by an increase of microvilli of the apical membrane and development of domes in $CaCo_2$ cultures. Cytochrome P 450 1A1/2-associated EROD activity was detectable only in induced $CaCo_2$ cells but not in HT29. In both cell lines no cytochrome P 450 3A4-associated activity was observed in the BEROD-assay. Expression of mRNA of the two isoforms of prostaglandin H synthase (PHS) was evaluated by RT-PCR. $CaCo_2$ cells exhibit only the PHS 2 isoform whereas HT29 exhibits both isoforms, PHS 1 and 2. PHS-mediated production of prostaglandin E2 was 2-fold higher in $CaCo_2$ cells compared to HT29 cells. N-acetyltransferase-1 could be detected in both cell lines and increased during the observed culture period. Total glutathion S transferase activity was constant in both cell lines. For UDP-glucuronyl-transferase a decline of activity was observed during the culture period. Only low activity of amino- and phenol-sulfotransferase was observed. Our results show that both cell lines maintain several enzyme activities required for the biotransformation of xenobiotics mostly independent from the confluency of the monolayer. In further studies the suitability of the cell lines for detection of genotoxic effects in the single cell gel electrophoresis assay (comet assay) should be investigated.

1786 PROCEDURE TO ESTIMATE THE PHAGOCYTIC ACTIVITY OF NAÏVE AND PARTICLE-EXPOSED MACROPHAGES USING LATEX MICROSPHERES.

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Lack of clear guidelines makes the comparison of phagocytic activity of macrophages difficult and inconsistent, as the results are subject to interlaboratory variations. This study was conducted to standardize the procedure to estimate the phagocytic activities of naïve and particle-exposed pulmonary alveolar macrophage (PAM). The procedure was adapted from Cohen et al.

(1997) and generated case-by-case pictures of PAM in various stages of phagocytosis. Young male F344 rats (BW: 200-250g) were used in all experiments (n=3-5/group). Naïve PAM were obtained from non-treated lungs and particle-exposed PAM were from lungs instilled with vanadium pentoxide (V_2O_5: 0, 0.63, or 6.3 mgV/kg). The bronchoalveolar lavage (~7 ml/wash) was done at 3 days following the instillation. Cell pellets from all six washes were combined to make the cell suspension that was diluted to 5.56×10^5 PAM/ml RPMI buffer and incubated at 37° for up to 150 min with serum-opsonized polystyrene latex microspheres ([bead:cell]=[100:1]). Slides were made by a cytospin, fixed in methanol, and stained for light microscopic examination. Xylene washing was effective in removing superficially attached beads on the slides. Incubation for at least 90 min was required to obtain the maximum estimates for the phagocytic index (PI:1-2 beads) and the phagocytic capacity (PC:≥3 beads), which were 40±6% and 21±5% for naïve PAM. Guidelines employed were: 1) the beads and the cells shall be in the same plane, 2) beads attached to cells or in the process of phagocytosis are not counted unless at least 2/3 engulfed by the cytoplasm of the cells, and 3) ingested beads exhibit a characteristic dark ring around, whereas merely attached ones are not. There was no difference in PI or PC among naïve or saline-instilled controls and the low V_2O_5 dose group. With the high dose group, PI increased to 56±9% while PC stayed the same (23±6%). V_2O_5-exposed lungs also contained significant numbers of PMN especially at high dose. Both PI and PC for PMN (PI=23±6%; PC=5±4%) of the high dose group were substantially lower than those of PAM.

1787 ESTABLISHMENT AND CHARACTERIZATION OF THE FOLLICULAR THYROID CARCINOMA CELL LINE ML-1 — A TOOL FOR TOXICOLOGICAL STUDIES.

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Chemicals may interfere with the production of thyroid hormones. Therefore, the aim of this study was to establish a human hormone-producing thyroid carcinoma cell line to obtain a permanent tool for toxicological investigations.The human cell line ML-1 was derived from a dedifferentiated follicular thyroid carcinoma recidive, which progressed despite of one preceding surgery followed by two radioiodine therapies. More than 90% of the cells of this line produce thyroglobulin, chondroitin sulfate and vimentin antigens, but only about 70% of them show cytokeratin filaments and a negative surface charge density like human erythrocytes. The cell line forms tumors with follicular structures, when transplanted to nude mice. More important, cells of this line are able to take up iodine and/or glucose *in vitro* as well as *in vivo* and to secrete thyroglobulin, chondroitin sulfate and fibronectin into the interstitial space. As measured by radioimmunoassay and ELIZA, even triiodothyronine is released constitutively into culture supernatants. Due to these unique features, the ML-1 cell line, so far the first human cell line derived from a follicular thyroid carcinoma, appears to be a very suitable test model for toxicological, pharmacological and cell biological studies.

1788 THE UROTSA CELL LINE AS A MODEL OF HUMAN UROTHELIUM.

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The only available human urothelial cell line for use in toxicology-based research studies is the UROtsa cell line obtained from normal human urothelium by immortalization using an SV40 large T-antigen construct. These cells are immortal, proliferate on a simple growth medium (RPMI with 10% fcs), form monolayers and have an epithelial morphology, but do not possess morphological features expected for bladder urothelium. Rather, they have features characteristic of undifferentiated basal cells. The goal of the present study was to determine if growth of UROtsa cells on serum-free medium could restore urothelial morphology. The serum-free medium consisted of a 1:1 mixture of Dulbecco's modified Eagles' medium (DME) and Ham's F-12 medium supplemented with selenium (5ng/ml), insulin (5μg/ml), triiodothyronine (4pg/ml), hydrocortizone (36.4ng/ml) and epidermal growth factor (10ng/ml). Under these conditions, the cultures became multilayered in selected areas and demonstrated a stratified organization of undifferentiated basal cells and apically located umbrella-like cells. The cultures remained viable and were able to be subcultured. When grown on filter inserts, cell multilayers were obtained that were morphologically similar to human urothelium. Ultrastructural analysis showed that tight junctions were present in the apical-most cell layers and freeze fracture analysis revealed tight junction sealing strands, gap junctions and desmosomes. The similarity of the UROtsa cells to human urothelium suggests they will be a valuable tool for the study of human bladder toxicology.

1789 ALTERNATIVE METHODS FOR ASSESSING CELL PROLIFERATION AND PROTEOGLYCAN PRODUCTION IN MICROMASS CULTURE.

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Micromass cultures of chick limb bud mesenchyme differentiate into cartilage provided cells are plated at densities greater than confluence. Cell proliferation and production of proteoglycans, predictable manifestations of chondrogenesis, are commonly measured variables in micromass cultures. Our objective was to evaluate alternative procedures for quantifying each of these variables. Cultures were seeded with 30μl aliquots (20E+06 cells/ml) and incubated for 24, 48, 72, 96 or 120 hr., or seeded with 10μl, 20μl or 30μl (20E+06 cells/ml) and incubated for 0 to 120 hr. Cell proliferation assessed by image analysis or optical density of crystal violet (Cv) stained cultures was compared with DNA, extracted from plated cells. Alcian green (Ag) extracted with 4M guanidine and propanol provided the standard against which optical density or image analysis of Ag stained cultures was compared. Stained and fixed unstained cultures were quantified by image analysis under controlled conditions for illumination and at known magnification. Variables evaluated included measured gray scale value and area as well as their calculated product. The image analysis product for unstained cultures correlates (R^2>0.960) both with the number of cell seeded as well as extracted DNA. Alcian green staining of fixed cultures measured spectrophotometrically with a plate reader correlated highly (R^2=0.989) with the optical density of extractable Ag. *In summary*: 1) Image analysis of unstained cultures provides a means to monitor cultures during the course an experiment. In addition in Ag stained cultures, by selecting the range of gray scale levels included in a given sample, a highly reproducible measurement of the portions of a culture dominated by Ag staining is obtained. 2) Plate read optical density of Ag stained cultures and fluorometric determination of extracted DNA provide quantitatively rigorous assessments of proteoglycan production and cell proliferation, respectively. (Supported in part by Hatch Funds and the US Air Force Office of Scientific Research.)

1790 DONOR VARIATION IN CHEMICAL ALLERGEN INDUCED IL-1β mRNA EXPRESSION BY CULTURED HUMAN BLOOD-DERIVED DENDRITIC CELLS.

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The development of *in vitro* methods for the identification of potential contact allergens based upon analysis of Langerhans cell (LC) function has been constrained by the fact that these cells represent only a minority population in the epidermis. However, methods have been developed recently that allow the expansion in culture of LC-like dendritic cells (DC) from human blood. We have shown previously that treatment with the contact allergen 2,4-dinitrofluorobenzene (DNFB) is associated with increased interleukin (IL-) 1β mRNA expression by DC derived from a proportion (3 of 8) donors. The differential ability of DNFB to influence IL-1β expression was donor dependent, with stable responder and nonresponder phenotypes being identified. We have therefore examined alterations in cytokine mRNA expression by human blood derived DC following exposure to a second contact allergen, paraphenylenediamine (PPD). Peripheral blood derived DC were expanded in culture for five days in the presence of IL-4 and granulocyte/macrophage colony-stimulating factor and then exposed to DNFB or PPD, or to vehicle alone, for 30 minutes at 37°C. Cytokine mRNA expression was analyzed by semi-quantitative reverse transcriptase polymerase chain reaction. All DC expressed IL-1β mRNA constitutively. Exposure to either DNFB or PPD resulted in up-regulation of mRNA for IL-1β in DC derived from two donors. DC derived from a third donor displayed a nonresponder phenotype to both allergens. These data demonstrate that the potent contact allergens DNFB and PPD upregulate IL-1β mRNA expression by human blood-derived DC, but that such responses are donor dependent.

1791 CHARACTERIZATION OF HUMAN DENDRITIC CELL CULTURES AND THEIR ROLE IN THE ANTIGEN PRESENTATION OF PENICILLIN, A PROTOTYPIC DRUG ALLERGEN.

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To develop a model *in vitro* system with the capacity to identify potential drug allergens, we studied the actions of penicillin on dendritic cell (DC) cultures and their influence on Th1/Th2 lymphocyte development. DCs were cultured from both human cord blood CD34+ haemopoietic progenitor cells (HPC) and peripheral blood monocytes. HPC-derived DC activated by LPS and CD40 ligation showed increased levels of the co-stimulatory surface molecules, CD54, CD80, CD86, CD40, MHC II and the cytokines, IL-12, IL-6 and IL-1β. Activated DC increased stimulation of allogeneic T cells which was inhibited by IL-10 or by neutralising IL-6 antibody. Cytokine treated HPC-derived DC were cultured with allogeneic CD4+CD45RA+ T cells. T cells stimulated with IL-4 treated DC failed to express IFNγ. IL-4 but not IL-6 treatment inhibited IL-12 expression by LPS-stimulated DC. IL-6 treated DC induced IL-4 and inhibited IFNγ expression within an allogeneic MLR. DCs were treated with penicillin (0, 0.5, 1.0 mg/ml) for 24 hours during their *in vitro* generation, then used to stimulate peripheral blood mononuclear cells in an allogeneic MLR. In cultures from atopic volunteers we found a dose-dependent increase in both IL-4 and IFNγ mRNA whereas from non-atopics we found a low stimulation of IL-4 and a dose- dependent decrease in IFNγ mRNA. There were also differences in DCs from atopics and non-atopics in the degree of inhibition of expression of CD40, CD80 and CD86 following treatment with penicillin. (Supported by EU BIOTECHNOLOGY contract CT96-0246.)

1792 EFFECTS OF AIRBORNE PARTICULATES ON THE RELEASE OF IL-1 BETA AND TNF ALPHA AND ON THE EXPRESSION OF HLA-DR BY A HUMAN MONOCYTIC CELL LINE (THP-1).

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The mechanisms by which exposure to environmental particulates may contribute to the occurrence of allergic diseases have not been elucidated. The aim of the present study was to investigate *in vitro* whether environmentally relevant particles may influence the immunological processing of allergens. The effects of particles of three different sources, namely carbon black, diesel exhaust particles (SRM1650, NIST) and urban particulate matter (SRM1648, NIST) were studied using the human monocytic THP-1 cell line, maturated to the macrophage phenotype with PMA (phorbol myristate acetate). The studied endpoints were the expression of HLA-DR molecules, assessed by fluorometry, and the release of Il-1b and TNFa, measured by RT-PCR. The cells were exposed for 48 hours with particles (0.16 - 1.6 - 16 - 160 - 1600 ng/ml) with or without IFNg (100U/ml). At least 3 independent incubations were performed. IFNg alone was able to significantly increase the expression of HLA-DR, Il-1b and TNFa by a factor 2 compared to basal values for non-exposed cells, while exposure to the particles alone did not change basal values. Particles in combination with IFNg were able to significantly increase the expression of HLA-DR by a factor 3 to 4 compared to IFNg exposure alone. The expression of Il-1b and TNFa were also raised when compared to IFNg alone. The increase in the release of inflammatory mediators and in HLA-DR expression caused by co-exposure of IFNg and particles in a cell type considered to be involved in antigen presentation is compatible with the hypothesis that particulates exert an "adjuvant" activity for environmental sensitisers.

1793 *IN VITRO* ASSESSMENT OF THE PULMONARY RESPONSE TO LOW SOLUBILITY PARTICLES: A SPECIES COMPARISON.

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At present, the mechanisms underlying the development of particle-induced rat lung tumors observed at doses that cause marked, persistant inflammation are not completely understood. This response seems to be unique to the rat and has not been observed in mice or hamsters. *In vitro* assay systems can be utilized to identify and evaluate mediators orchestrating complex signaling mechanisms involved in regulating the inflammatory process. By evaluating individual cell types, mechanistic data can be obtained and compared to *in vivo* findings. Briefly, pulmonary macrophages, neutrophils and epithelial cells were isolated from rats, mice and hamsters and exposed to varying doses of carbon black and silica for various times. Samples were evaluated for oxidant and anti-oxidant production, pro- and anti-inflammatory cytokines, and cytotoxicity. Activation of signal transduction pathways was also evaluated. A dose and time related effect was observed for all parameters measured in rat inflammatory and epithelial cells. Hamsters and mice were less susceptible to the cytotoxic and inflammatory effects of these particles. This data correlates with the *in vivo* data previously generated.

1794 TOXICITY OF ORGANIC CHEMICALS ASSOCIATED WITH URBAN AIRBORNE PATICULATE MATTER IN PUERTO RICO USING BRONCHIAL EPITHELIAL CELLS.

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Health issues with respect to airborne particulate matter arise as a consequence of urban development, population growth, and industrialization. Epidemiological studies throughout the world point at airborne particulate matter as one of the principal causes of respiratory illnesses, cardiovascular disease mortality, acute bronchitis, and asthma attacks. Although organic constituents of particulate matter have been suggested to contribute to the overall toxicity in urban areas, few work have been conducted evidencing this fact. We have evaluated the cytotoxicity of four organic compounds (Metylmorpholine, Ethylmorpholine, Diethyl Phthalate and Tributyl phosphate) using Normal Human Bronchiotracheal Epithelial Cells (NHBEC) with the Neutral Red Uptake bioassay (NR50). These compounds or family related compounds were identified in particles less than 10 μm (PM10) from an urban site in Puerto Rico. They were extracted using Soxhlet extraction and analyzed by GC/MS. The NR50's using NHBEC were determined as 1500 μg/mL (metylmorpholine), 1425 μg/mL (ethylmorpholine), 42 μg/mL (diethyl phthalate) and 5 μg/mL (tributyl phosphate). A combination of these chemicals at different proportions showed higher toxicity when combined at concentrations lower than their NR50's. A mixture of the two morpholines at higher ethylmorpholine concentration (but lower than their NR50's), showed higher toxicity levels when compared to the opposite concentration for the same morpholines. Other mixtures with the same two compounds at lower concentrations did not exhibit significant cytotoxicity. The mixture containing the NR50 for both compounds showed the highest toxic response. Four compound mixtures containing diethyl phthalate concentration above its NR50, but less toxic concentrations of the other three compounds exhibited a non-toxic effect suggesting a masking effect on this compound. Tributyl phosphate was found to exert a higher cytotoxic effect than any of the other three compounds evaluated.

1795 COMPARISON OF THE INTERACTIONS OF BISPHENOL A AND ITS METABOLITE BISPHENOL A GLUCURONIDE WITH ESTROGEN RECEPTORS α AND β.

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Bisphenol A (BPA) and its major metabolite BPA glucuronide (BPA-G) were assessed for their ability to compete with [^{3}H]17β-estradiol (E2) for binding to estrogen receptor (ER) α and β, and for inducing and antagonizing ERα- and β-mediated reporter gene activity *in vitro*. BPA competed with [^{3}H]E2 for binding to mouse uterine cytosol ER, a bacterially expressed glutathione-S-transferase (GST)-ER fusion protein consisting of the human ER α D, E and F domains linked to GST (GST-hERαdef) and a commercially available full length recombinant hERβ. The IC_{50} values for E2 were similar among receptor preparations (mouse uterine cytosol ER, GSThERαdef and hERβ were $3.0\pm0.2 \times 10^{-9}$ M, $2.2\pm1.4 \times 10^{-9}$ M and $5.5\pm1.9 \times 10^{-9}$ M, respectively). In contrast, BPA exhibited greater affinity for hERβ ($3.3\pm1.7 \times 10^{-7}$ M) than for GST-hERαdef ($2.7\pm1.4 \times 10^{-6}$ M) and for mouse uterine cytosol ER ($2.6\pm1.1 \times 10^{-5}$ M). BPA-G did not competitively displace [^{3}H]E2 from any of the ER preparations. In MCF-7 cells transiently transfected with Gal4-hERαdef (human) or Gal4-mERβdef (mouse) chimeric receptors and a Gal4-regulated luciferase reporter gene, 17m5-G-Luc, BPA fully induced luciferase reporter gene activity with comparable EC_{50} values ($7.1\pm2.9 \times 10^{-7}$ M and $3.9\pm1.2 \times 10^{-7}$ M, respectively). No significant response was seen for BPA-G. Cotreatment studies showed that concentrations of (1-30 ∝M) BPA and BPA glucuronide did not result in any significant changes in the level E2 (10 nM) induced luciferase activity in MCF-7 transiently transfected with either Gal4-hERαdef or Gal4-mERβdef. These results demonstrate that BPA can interact with ERα and β, and can induce ERα- and β-mediated gene expression *in vitro*. In contrast, BPA-G did not exhibit any *in vitro* estrogenic activity. (This research was supported by the Bisphenol A Global Industry Group.)

1796 DIFFERENTIAL ESTROGEN RECEPTOR BINDING OF ESTROGENIC SUBSTANCES: A COMPARISON ACROSS SPECIES.

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This study investigated the potential species-specific estrogenic receptor (ER) activity of several natural and synthetic chemicals for bacterially expressed fusion proteins using a semi high throughput competitive binding assay. The fusion proteins consisting of the D, E and F domains of human (α), mouse (α), chicken, green anole and rainbow trout ER linked to the glutathione-S-transferase (GST) protein were prepared and partially purified. Saturation ligand-binding analysis of GST-hERαdef (human), GST-mERαdef (mouse), GST-cERdef (chicken), GST-aERdef (green anole) and GST-rtERdef (rainbow trout) fusion proteins revealed a single high affinity binding component for E2 with dissociation constants (K_d) ranging from of 0.3 to 0.9 nM. Although, the ERs from the different species exhibited similar binding preferences and binding affinities for many of the compounds examined, several differences in absolute and relative binding affinities were observed. Of the 38 chemicals examined, 4-hydroxytamoxifen was found to bind with greatest affinity for the ERs of all 5 species. The ranking of the potency of the xenobiotics and phytoestrogens varied among species with no predictable patterns and many examples of species-specific ligand preference. For example, α-zearalenol ($IC_{50} = 1.5 \pm 0.7 \times 10^{-9}$ M) competed for binding with greater affinity than E2 for GST-rtERdef which was in contrast to all other GST-ERdef fusion proteins examined. However, the phytoestrogen coumestrol ($IC_{50} = 1.6 \pm 0.3 \times 10^{-6}$ M) bound with lowest affinity to GST-rtERdef, while genistein ($IC_{50} = 3.0 \pm 2.0 \times 10^{-8}$ M) bound with greatest affinity to GST-aERdef. These results demonstrate that ERs from different species exhibit differential ligand preferences and relative binding affinities for estrogenic substances and that these differences may be due to the variability in the amino acid sequence within the ER ligand binding among species.

1797 TRANSCRIPTIONAL PROFILING OF PHENOBARBITAL (PB) HEPATOTOXICITY IN THE MOUSE.

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The hepatic effects of 4 daily, 80 mg/kg (ip) doses of PB, an epigenetic rodent carcinogen, were examined by analyzing mRNA isolates at 6-hr post-dose (i.e., at 6-, 30-, 54-, and 78-hr) on Affymetrix Mu11k GeneChips®. This dosage regimen of PB produced the expected 20-40 fold increases in CYP2B (testosterone-16β-hydroxylase) and 5-fold increase CYP3A (testosterone-6β-hydroxylase) activities in the murine liver microsomes. In addition, the Mu11k GeneChip analysis demonstrated that a total of 341 unique gene transcripts were affected by PB in the mouse. Of the 12 genes previously shown to be induced in rodents, which include CYP450's, phase II drug-metabolizing enzymes, $\alpha 2\mu$-globulin, and δ-ALAS, 10 of these were also induced in the mouse. Only epoxide hydrolase and UDP-glucuronosyltransferase were not detected in the mouse experiment. These results compare favorably with similar studies of PB transcriptional effects in rat liver and in rat primary hepatocyte cultures in our laboratory, where a total of 323 unique transcripts were shown, by READS™ differential display analysis (Gene Logic, Inc.; Gaithersburg, MD), to be affected by 80 mg/kg/d ip doses of PB *in vivo*, and by exposure to 2 mM concentrations of PB *in vitro*. Many of these effects on hepatic mRNA levels were similar in the two species, typically slight (<5-fold) increases which were not maintained over the entire 78-hr time course of these studies, and involved previously unknown mechanisms of action of PB on the liver. PB affected rodent genes for apoptosis, DNA repair proteins, cell cycle/signal transduction pathways, fatty acid/lipid intermediary metabolic enzymes, cell surface receptors, cytokine effectors, and steroid hormone metabolic enzymes. Finally, a set of about 50 novel transcripts were identified by the "open" system in the rat (READS™ differential display), which did not match any known gene or EST sequences in the public databases. These studies are among the first to utilize such powerful new technologies to examine the mechanisms of drug and chemical hepatotoxicity on a genome-wide basis.

1798 EFFECTS OF A PEROXISOME PROLIFERATOR (WY-14,643) ON HEPATOCYTE TRANSCRIPTION USING MICROARRAY TECHNOLOGY.

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Peroxisome proliferators produce a number of hepatocellular changes that are mediated through transcriptional regulation. The cellular changes in rats and mice include increases in the number of peroxisomes, mitogenic effects, and induction of cytochrome P450, however humans lack these responses. Nonetheless, both species respond to the therapeutic effects of peroxisome proliferators on lipid metabolism. This study was designed to characterize the transcriptional response of isolated rat hepatocytes to the peroxisomal proliferator (WY-14,643). The goal of the study was to validate microarray data by evaluating expected biochemical and molecular changes, and identify novel genes that may be associated with the species-specific effects of peroxisome proliferators. F344 rat hepatocytes were isolated, incubated over night, and exposed to WY-14,643 (0.1-100 μM) for 4 or 48 hours. Peroxisomal beta-oxidation (Pbox), cytochrome P4504A (CYP4A) protein, and lactate dehydrogenase leakage were quantitated in the isolated hepatocytes. In addition, RNA was isolated from hepatocytes for evaluation using microarray technology (Affymetrix). WY-14,643 had no effect on Pbox after 4 hr of exposure but increased Pbox approximately 14 fold after 48 hours of exposure without affecting cell viability. Similarly, WY-14,643 did not affect CYP4A protein at 4 hr but produced a significant increase at 48 hours. These biochemical effects were a result of transcriptional regulation as determined by RT/PCR and microarray. Acyl coA oxidase and CYP4A transcription was increased at 4 hr (prior to biochemical effects) and continued to increase at 48 hr. In addition, a number of genes associated with oxidative damage (glutathione transferase, catalase, SOD dismutase) and apoptosis (BCL-2, topoisomerase) were induced in response to WY-14,643, suggesting a potential relationship between effects on redox potential and apoptosis in hepatocytes. Taken together, these data indicate that microarrays are capable of detecting and validating biochemical responses.

1799 TOXICOGENOMICS: ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

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In spite of the large amount of data collected in the past years, the mechanisms underlying the toxicity of acetaminophen (APAP) are not fully understood. So far, the involvement of metabolic activation and the conjugation of acetylbenzoquinoneimine (NAPQI) to GSH and selective protein arylation have been reported. The generation of oxidative stress and DNA fragmentation, the inhibition of apoptosis and the activation of Kupffer cells are other characteristics of APAP-induced hepatotoxicity. However, the molecular mechanisms involved in the initiation and progression of liver injury remain unclear. In this study, we sought to further investigate the mechanisms of APAP-induced hepatotoxicity on the gene expression level using DNA microarrays. Adult male mice (C57BL/6) were administered APAP at doses of 100 or 300 mg/kg. To differentiate toxicity-related gene expression from that unrelated to liver injury, a matched group treated with 300 mg/kg of the non-toxic positional isomer AMAP was also included. Eight hours after treatment, hepatic transcripts were analyzed and liver damage was assessed by increased liver enzyme release and histopathology. Gene expression analysis showed that a subset of genes involved in stress response, cell cycle regulation and apoptosis was regulated by APAP but not by AMAP. These findings were corroborated using RT-PCR. In the high-dose group, the regulation of the gene transcription correlated well with the extent of liver injury. Among the identified genes, the early and dramatic induction of the transcription factor LRG-21 in the animals exhibiting liver injury suggests the possible involvement of this gene in the initiation of APAP-induced hepatotoxicity. In conclusion, APAP-induced liver injury in mice positively correlated with the regulation of genes implicated in hepatotoxicity. Moreover, the gene expression profile of the animals in the low-dose group suggests that the modulation of some genes is detectable prior to the appearance of overt toxicity.

1800 GENOMIC AND PROTEOMIC INVESTIGATIONS OF ACETAMINOPHEN (APAP) TOXICITY IN MOUSE LIVER *IN VIVO*.

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APAP toxicity has been postulated to involve covalent modification of critical proteins and inhibition of their functions. In order to understand the molecular events associated with toxicity we have assessed mRNA and protein changes in mouse liver after APAP i.p. at 150 (subtoxic) or 500mg/kg (toxic). Mice were sacrificed at 15, 30, 60, 120 and 240 minutes. mRNA expression was conducted using Gene Discovery Array filters (Genome System Inc.) and in-house mouse and human micro-array filters. After 33P cDNA probe hybridization, the filters were scanned by phosphorimager and spot intensities quantified using ArrayVision (Imaging Research). Genes showing reproducible repression or induction of more than 2 fold were noted. Proteins in liver mitochondrial fractions were separated by fluorescent 2D-differential gel electrophoresis and differences identified by mass spectroscopy. Electron microscopy revealed swollen (at 30 min) and mega-mitochondria (at 120 min) after 500mg/kg. Mitochondrial specific or associated genes like protoporphyrinogen oxidase or sterol carrier protein 2 along with the proteins catalase, aldehyde dehydrogenase , HSP10 and HSP60 were up and down regulated in time and dose dependent fashion. In addition, mRNA expression of metallothioneins, beta-actin, cathepsin D, c-fos, glutathione peroxidase and MAP kinase phosphatase were induced with varying time course profiles. We have described the modulation of genes and gene products in APAP-induced mouse liver damage and these changes may be involved in the biochemical basis of this toxicity.

1801 CUSTOM MADE CDNA ARRAYS FOR TRANSCRIPT PROFILING: IMPLICATIONS FOR DRUG DISCOVERY AND EARLY SCREENING FOR THIAZOLIDINEDIONE TOXICITY.

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Thiazolidinediones (TZD's), recognised for improving insulin sensitivity and intended for the treatment of type II diabetes, have limited efficacy in humans due to the observed dose dependant toxicity. Animal exposure data suggests that one TZD-induced toxic effect is adipocyte accumulation within the bone marrow, perhaps as a consequence of stromal cell changes. TZD's are ligands for the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) which is involved in lipid and glucose homeostasis. The aim of our ongoing research programme is to identify novel compounds for treating type II diabetes that does not possess the undesirable toxicological profile seen with the TZD's. We have employed transcript profiling technology using a nylon-based cDNA array, with a view to assisting in the toxicological evaluation of insulin sensitizing compounds. Human genes were selected for an array that include adipocyte and osteoblast markers, and PPARγ activators. 170 such 'specific' clones were selected, together with 768 random clones for experimental normalisation. A human bone marrow stromal cell line was used in these experiments. Cells were left untreated or treated with vehicle, test compounds including the TZD's rosiglitazone and troglitazone, or with rabbit serum as a positive control. cDNA was prepared and radiolabelled, and hybridised to the arrays. Phosphorimager scans of array images were analysed using ArrayVision software. Using this system we are able to detect changes in gene expression patterns that correlate with differentiation of the stromal cells towards an adipocyte phenotype. Such changes include upregulation of G3PDH, adipsin and E2F4 transcript levels. Further work is in progress to extend and confirm these observations, and to determine whether specific gene 'fingerprints' are associated with different classes of insulin sensitizers.

1802 COMPARISON OF TOXICANT-INDUCED GENE EXPRESSION PATTERNS IN HEPG2 HUMAN HEPATOMA CELLS.

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Gene arrays may be used to distinguish different types of toxicities (eg., DNA damage, hepatic necrosis, peroxisomal proliferation). We show that gene arrays can identify toxic compounds that cause DNA damage, but only after isolating subsets of genes that respond consistently. HepG2 cells were treated with cisplatin, a DNA crosslinker, and gene array results were compared with those resulting from parallel treatments with equitoxic concentrations of transplatin (an inactive isomer) and two hepatotoxic NSAIDs, flufenamic acid and diflunisal. Cells in log phase growth were treated for 24 hours with compound concentrations eliciting an approximate ED_{30} cytotoxic response, based on inhibition of reductase activity or protein synthesis. Reproducibility was found to be critically important and initial experiments were performed multiple times to ensure statistically meaningful data. A dozen genes (from a total of about 400 on arrays enriched in DNA damage indicator genes) distinguished statistically between cisplatin and transplatin/flufenamic acid/diflunisal treatments. The analysis used a variety of computer algorithms designed to maximize differences between cisplatin-induced and transplatin/flufenamic acid/diflunisal-induced expression patterns. More importantly, the subsets of genes identified in these experiments correctly predicted the genotoxic compounds in Phase-1's proprietary database even though these were not part of the learning set. Moreover, clustering was observed for several other mechanistic classes of database compounds. In contrast, correlational analysis using all the genes on the arrays showed little if any difference between DNA damaging compounds and other drug classes. As we refine the approach, we expect to obtain subsets of genes showing representative responses for each major type of toxicity. Ultimately, by comparing the gene expression profiles to an established database, we should know what types of toxicity to expect with any compound.

1803 IDENTIFICATION OF ESTROGEN-INDUCED GENES DOWNREGULATED BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN BY COUPLING SUPPRESSION SUBTRACTIVE HYBRIDIZATION AND cDNA MICROARRAYS.

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The antiestrogenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is mediated through crosstalk between the aryl hydrocarbon (AhR) and estrogen receptor (ER) signaling pathways. Identification of 17β-estradiol (E2)-induced genes downregulated by TCDD utilized suppression subtractive hybridization (SSH) using poly(A)-RNA from MCF-7 cells treated with 1 nM E2 and 1 nM E2 + 1 nM TCDD as targets and references, respectively. Results of SSH confirmed that 43 clones isolated using this technique have sequence homology to previously described genes; in addition, three potential novel sequences were identified. The following genes were downregulated (>2-fold) after treatment with E2 + TCDD compared to treatment with E2 alone: nucleotide synthases, cell cycle regulators, X-chromosome related genes, protein synthesis regulators, homeotic FSH homology, calmodulin, and two unmatched clones. Similar expression profiles were observed after probing with cDNA from MCF-7 cells cotreated with E2 + diindolylmethane (DIM), although the magnitude of some of the decreased responses were less than that observed after cotreatment with E2 + TCDD (1 nM). Despite these quantitative differences between the inhibitory effects of TCDD and DIM, the overall pattern of antiestrogenic activity for 25 μM DIM was similar to that observed for TCDD. Some of the changes in levels of gene expression induced by TCDD may also be attributed to other activities (including toxicity) of this compound compared to DIM. In combination with high-throughput gene screening methods, SSH or SSH-array can be a powerful technique for studying crosstalk between two signaling pathways. (ES04176 and ES09106)

1804 THE EFFECT OF SHORT TERM TREATMENT OF VARIOUS HEPATOCARCINOGENS AS ASSESSED BY GENOMIC MICROARRAY TECHNOLOGY.

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Chronic administration of high doses of certain drugs/chemicals may induce hepatocellular tumors in rodents. Although the exact mechanism of tumorogenesis is unknown, technological advancements in examining genetic expression can be utilized to characterize chemical treatment and further the understanding of how hepatocarcinogens produce tumors. The objective of this study was to determine if chemically-induced changes in gene expression are altered during toxicity and/or are there expression patterns that may be predictive of carcinogenicity. Male Sprague Dawley rats were treated daily for 5 days with a variety of mutagenic or non-mutagenic carcinogenic or non-carcinogenic compounds which were bemitradine, clofibrate, phenobarbital, tamoxifen, 2-AAF, 4-AAF, doxylamine, methapyrilene, and isoniazid. In general, all doses were well tolerated. One 200 mg/kg phenobarbital animal died, but no other mortality was observed. Few clinical signs were noted during the

study except reduced activity in phenobarbital treated animals and altered gait in clofibrate treated animals. No gross abnormalities were noted at necropsy. However, the percentage body weight gain from Day 1 to Day 5 was decreased in a dose-responsive manner for bemitradine, clofibrate, phenobarbital, tamoxifen, 2-AAF, and methapyrilene. In addition, increased liver/body weight ratios were observed in a dose-responsive manner for bemitradine, clofibrate, phenobarbital, and 4-AAF treated rats. Expression analysis of nearly 8000 genes indicate a marked expression pattern change that is unique to specific carcinogen classes.

1805 GENE EXPRESSION IN LIVER TISSUES OF FEMALE RATS DURING THE EARLY STAGES OF DMBA-INDUCED MAMMARY TUMORIGENESIS.

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In addition to inducing tumorigenesis, DMBA (7,12-dimethylbenz[a] anthracene) has previously been reported to alter gene expression in both target and non-target tissues of exposed animals. However the alterations to liver gene expression throughout the early stages of female rat mammary tumorigenesis still remain largely unknown. Gene expression in liver tissue obtained from both control and DMBA-treated female rats at 6, 24, 72 hrs and 2 weeks post-exposure, was assessed using the branched DNA assay (bDNA). Among the 16 genes studied, WAF1/p21 appeared to be the only gene overexpressed in all the DMBA-treated rats at all tested time points (4 to 6 fold). Several genes exhibited peak expression at 72 hours, MDM-2, Cyclin G, Cyclin D1, and MDR-1 (up to 18 fold). The expression of Catechol-O-Methyltransferase and Calreticulin decreased significantly at 6 hours and recovered to control levels at later time points. Comparison of the bDNA assay results with a microarray study showed an 83% agreement between the two assays when comparing genes which showed significant changes in both methods (5/6 comparable genes). This study demonstrates comparable results between the bDNA and cDNA microarray assays in measuring differential gene expression in the livers of female rats in the early stages of DMBA-induced mammary tumorigenesis. Moreover, the identification of changes in gene expression in a non-target organ provides valuable information for evaluating tumor development and tissue-specific response to toxicological stress.

1806 EXAMINATION OF GENE EXPRESSION CHANGES OVER TIME IN MAMMARY AND LIVER TISSUES OF RATS EXPOSED TO A CARCINOGENIC DOSE OF DMBA.

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7,12-dimethylbenz[a]anthracene (DMBA) is a carcinogen widely used to induce mammary tumors in female Sprague-Dawley rats. In this study gene expression was examined in mammary tissues over time in order to identify the changes associated with DMBA induced mammary tumorigenesis. Gene expression was also examined in the liver tissues. RNA levels were measured with cDNA microarrays which contained DNA targets for 125 genes selected specifically for toxicological investigations. Fifty-one day old female rats were administered 10 mg/ rat of DMBA by gavage and RNA levels were measured at 6 hrs, 72 hrs, 6 weeks, and 12 weeks post-exposure. By 12 weeks all DMBA exposed animals tested had palpable mammary masses. Changes in liver gene expression were observed 6 hours post-exposure, with RNA levels of COMT, an enzyme involved in metabolism of catechol estrogens exhibiting decreased expression at 6 and 72 hours. In the mammary tissues of exposed animals numerous genes were differentially expressed in all animals 72 hours post-exposure including: C-reactive protein (+), capases 2, 6, and 7 (+), cyclin E (+), HGF receptor (+), hydroxysteroid sulfotransferase A (+), MDM-2 (+), and TGF β-3 (+). At the 6 week time point among the 7 genes differentially expressed were cyclin D3 (+) and EGF (+). Five genes exhibited altered expression at 12 weeks including, cyclin D1 (+), p53 (+), and TGF β-3 (+). In this study differential gene expression and patterns were observed over time for genes in both mammary and liver tissues that are associated with metabolism, cell cycle control, DNA damage, protein damage, and carcinogenesis. The gene expression results observed in mammary tissue appear consistent with tumorigenesis induced by a genotoxic carcinogen such as DMBA.

1807 EXPLORING GENE REGULATION BY IMMUNOSUPPRESSANTS USING AN IN HOUSE TOXICITY BASED CDNA MICROARRAY.

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We are interested in applying 'transcript profiling' technology to assist safety evaluation of development drugs. Using an in house filter array ('ToxBlot'), we have been able to monitor expression patterns of a large number of genes. This array has been custom designed to include major 'toxicologically relevant' genes including those associated with apoptosis, cancer, signal transduction and immunology. The 620 genes on the array are sequence-verified human cDNAs which have been spotted in duplicate. Initially we are performing transcript profiling experiments using reference immune suppressants including Cyclosporine A (CsA). CsA is widely used in the treatment of solid organ transplant grafting as well as T cell driven autoimmune diseases, but with nephrotoxic side effects including tubular injury. CsA principally inhibits renal phosphate calcineurin by binding to the intracellular immunophilin cyclophilin resulting in the inhibition of T-lymphocyte activation. It is intended to use transcript profiling to assist screening for novel immunosuppressants with reduced toxicity. To obtain transcript profiles, human HepG2 hepatoma cells were dosed at incremental concentrations from 0-125μM of CsA (n=3). In addition, profiling has been performed with populations of primary human peripheral blood mononuclear cells (PBMCs) to determine gene changes associated with proliferative arrest of activated T cells induced by immunosuppressants. Our data show that CsA causes reproducible transcript level changes, both in HepG2 cells and in PBMCs. Genes that are transcriptionally upregulated by CsA in HepG2 cells include transferrin and integrin b4, and downregulated genes include telomerase, ApoCIII and clathrin. Further work is in progress to extend and confirm these observations with CsA and other immune suppressants, and to determine whether specific gene 'fingerprints' can be ascribed to different classes of compounds.

1808 EXPRESSION ANALYSES OF THE EFFECTS OF RESERPINE ON HUMAN HEPATOMA (HEPG2) CELLS USING MICROARRAYS.

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Xenobiotic exposure often results in altered gene expression patterns, which may be predictive of subsequent adverse events. This study was conducted to evaluate reserpine-induced changes in gene expression patterns in human hepatoma (HepG2) cells. Reserpine (RES) is a rodent-carcinogen, but not a human carcinogen that is currently being evaluated in transgenic animal models. In order to understand how changes in gene expression correlate with cytotoxicity, initial studies determined the toxicity of RES in HepG2 cells by measuring changes in MTT activity and ATP levels following a 24-hr exposure. The TC50 for MTT and ATP was estimated at 50 μM respectively. These studies indicated alterations in mitochondrial functions and cell viability. To evaluate RES-induced changes in gene expression, HepG2 cells were seeded at a density of 300,000/ml in a final volume of 6 ml and were cultured for 96 hr prior to dosing. Following the 96-hr growth period, media was removed and the cells were dosed by adding media with 50 μM RES and 0.5% DMSO. After a 24-hr exposure the media was removed, cells were processed to recover mRNA and the target RNA was hybridized to human oligonucleotide microarrays. Preliminary results indicate alterations in expression of approximately 340 genes; based on changes of ±3 S.D as a level of significance for analysis. Upregulation of about 170 genes and down-regulation of approximately the same number of genes was observed. These and additional studies may lend themselves to a better understanding of the role of gene expression in mechanistic and predictive toxicity studies.

1809 DIFFERENTIAL GENE EXPRESSION IN BUTYRATE-TREATED HT 29 COLON CARCINOMA CELLS.

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Dietary compound sodium butyrate (NaB) is a potential chemopreventive agent. Treatment of HT 29 cells with NaB resulted in cell differentiation, apoptosis, together with increased activity of phase-II detoxification enzymes. To determine the mechansim of these effects, we performed differential gene expression array assays using Clontech human stress and toxicology arrays (234 genes). After 3 mM NaB treatment for 3 days, the most dramatically

induced mRNAs were members of heat shock protein (HSP) family, which included HSP 70, HSP 47, HSP 27, HSP 40 and HSP 110. The increased expression of hsps was confirmed by RT-PCR. HSF-1, a transcription factor that regulates hsp expression, was found to be activated. Similar effects could be achieved by tricostatin, an inhibitor of histone deacetylase. We conclude that the cellular response to butyrate includes upregulation of hsp expression, possibly by a mechanism that involves altered histone acetylation. This response may function in elimination of proliferative machinary as the cell becomes differentiated, or represent a response to the differentiation-associated cellular redox change. (Supported by NIH grants ES-09047 and GM-08248.)

1810 AN EVALUATION OF THE DNA ARRAY: PREDICTING TOXICOLOGICAL ENDPOINTS USING THE LOCAL LYMPH NODE ASSAY AS A MODEL.

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The DNA array technology is becoming a widely used method for associating gene expression changes with cellular phenotypic changes. The high-density DNA array is a powerful tool for evaluating changes in global gene expression. Arrays have the potential to address a number of key toxicological issues, including mode of action, dose-response relationships, hazard identification, and human exposure assessment. This study examines the reliability of the DNA array technology by performing intra- and inter-animal tissue reproducibility experiments. These experiments demonstrated that DNA arrays provide reproducible detection of expressed genes. Subsequently, the local lymph node assay (LLNA) was used as a model to evaluate the use of DNA arrays for predicting toxicological endpoints. We exploited the proliferative response and other salient conditions of this model to identify the underlying genetic mechanisms of dermal sensitization. Pooled draining auricular lymph nodes from 4:1 acetone:olive oil (AOO) vehicle-control and 1-chloro-2,4-dinitrobenzene-treated CBA mice were collected. Total RNA was purified and reverse transcribed using ^{32}P-dATP and gene-specific primers for probing a murine cDNA expression array from Clontech Laboratories, Inc. The LLNA proliferative response is known to correlate well with sensitization potential and we now report a concomitant gene expression pattern using this model. Treatment-induced changes in gene expression levels were detected in 4% of the arrayed genes. Of particular interest was an elevation in PD-1, an Ig gene superfamily member, integrin beta, and MHR23B, a Rad23 ultraviolet excision repair protein homolog. Also of interest was a down-regulation of CD2 antigen as well as GLYCAM1, an endothelial ligand for L-selectin. Thus, we demonstrate that the DNA array has the ability to detect genes that respond to treatment.

1811 ANALYSIS OF GENE EXPRESSION IN TRANSGENIC AND KNOCKOUT MICE USING GLASS MICRO-ARRAYS.

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Transgenic and knockout mice are used with increasing frequency to elucidate toxicological mechanisms but the influence of the specific gene involved in the transgene or knockout on the expression of other genes is largely unknown. A glass microarray containing over 4000 mouse cDNAs was used to analyze levels of gene expression in selected target tissues from Big Blue lacI transgenic and p53, Ahr and P450 knockout mice compared with normal controls of the same background strain. Among the groups of genes present on the array are representatives of those involved in cell cycle, oxidative stress, DNA replication and repair, xenobiotic-metabolizing enzymes, apoptosis and hormone responses. Cy3 and Cy5 labeled cDNA reverse transcribed from RNA isolated from the liver, kidney, g.i. tract, brain, muscle, lung and heart of young adult mice was hybridized to the microarray. For each cDNA element on the array the ratio of fluorescence intensity for each fluor was detected simultaneously with a twin laser scanner. A 16 bit gray scale image was generated for each fluor. The fluorescence intensity of each feature at each wavelength was normalized by reference to median fluorescence across the whole array. After normalization the array was reproduced using false color and the two images superimposed. Red represents increased and green decreased gene expression while yellow indicates no change. Finally the data was clustered within, and across, experiments to indicate genes with related expression patterns.

1812 MICROARRAY ANALYSIS OF 4500 HUMAN GENE EXPRESSIONS, AMPLIFICATIONS AND DELETIONS IN CELLS WITH LOW CYTOTOXIC DRUG SENSITIVITY.

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Differential sensitivity to xenobiotics is manifest in many forms including such responses as adverse drug reactions, and resistance to insecticides, antibiotics and cytotoxic drugs. In animals differential sensitivity is often species and strain specific, and in man variation is found between different population groups for some xenobiotics. Such variation can be due to the differential expression of one gene, but in most cases there is a contribution from many genes. It is our aim to elucidate the genes involved in xenobiotic sensitivity in a number of models, and the underlying genotype. To do this we have used glass based micro-array technology. These arrays are manufactured in our own facility using equipment we have built from published designs (DeRisi et al). The micro-arrayer has a 16 split pin print head capable of spotting up to 7000 spots/cm2 with an interspot distance of 125mm, and diameter of 90-100mm. We are using an Axon GenePix scanner with commercial and custom software for analysis. In this study we examined the differential gene expression of 4500 genes in human cell lines that had acquired a decreased sensitivity (i.e., resistance) to cytotoxic drugs. The patterns of differential gene expression have been clustered to reveal groups of genes that are co-regulated. In addition gene amplifications and deletions have been examined by micro-array technology and correlated to the differential gene expression.

1813 THE SELECTION OF AN ENDOGENOUS REFERENCE GENE IN QUANTITATIVE GENE EXPRESSION ANALYSIS CAN GREATLY AFFECT INTERPRETATION OF THE DATA.

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Real time PCR (TaqMan) is an accurate and relatively high-throughput method to analyze relative quantitative gene expression changes using a reference gene for sample normalization. Housekeeping genes, such as glyceraldehyde-3-phosphate dehydrogenase, beta-actin and 18S ribosomal RNA are commonly used reference genes. However, such genes may not be appropriate references because some embryo-toxic compounds cause general growth retardation and therefore may alter metabolic processes and/or cytostructural organization of various embryonic structures. We compared relative gene expression changes in cultured rat embryos treated with vehicle or tool compounds. The test compounds ranged from non-teratogenic, to mild, to severe teratogencity (growth retardation and disorganization of posterior axial structures at varying severity). The gene expression data was ormalized against several housekeeping reference genes as well as against krox20, a zinc finger gene which is expressed exclusively in rhombomeres 3 and 5, and S100-beta, which is expressed primarily in the notochord during early organogenesis. Normalization against the housekeeping and structure-specific genes produced dramatic differences in the data outcome. Normalization with krox-20 reflected graded changes of certain gene expressions, which would be expected in embryos which exhibited graded severities in axial developmental abnormalities, and these expression changes were also reflected by evaluation of the target gene expression by wholemount in situ hybridization. We have concluded that caution in reference gene selection is important in embryonic gene expression evaluations. The reference gene should not exhibit expression in a region or biological process that may be affected by the teratogen. Also, lower copy number reference genes may be advantageous since they are more suitable for multiplex PCR experiments and they may also reduce background expression variations in individual embryos.

1814 GENE EXPRESSION IN THE MOUSE TESTIS: DEVELOPMENT OF A MURINE TESTIS TRANSCRIPTOME BY MINING PUBLIC DATABASES.

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The murine testis transcriptome (MTT) represents a database of all genes currently known to be expressed in the murine testis, and will used to construct high density cDNA arrays for toxicogenomic analyses. The NCBI UniGene, Jackson Laboratories Mouse Gene Expression (GXD), and National Library of Medicine (MEDLINE) databases were searched to identify genes expressed in mouse testicular tissue. By combining these publicly available data, it was possible to create a more comprehensive MTT than by using any of these sources exclusively. 1767 known genes were identified. 1164 (66%)

were obtained from the UniGene database as clusters containing ESTs sequenced in libraries derived from murine testis. Analysis of the GXD and MEDLINE for genes expressed in the testis or component cell types identified an additional 335 (19%) and 268 (15%) genes, respectively. In addition to identifying genes experimentally determined to be expressed in the murine testis, the search of the literature also identified genes known to be involved in testis function in humans or rats, resulting in the selection of a significant number of homologous genes in the murine databases that would not have otherwise been included in the MTT. Of these 268 genes, 151 (56%) were derived from mouse, 98 (37%) were derived from rat, and the remaining nine (4%) were derived from human model systems. Gene expression analysis of 588 genes using a commercially available array identified 171 genes expressed at high levels in murine testis. Of these 171, 107 (63%) were already contained in the MTT. The remaining 64 (37%) were added to the MTT, bringing the final total of known genes in the MTT to 1831 (www.bch.msu.edu/~zacharet-/MTT.htm). This approach represents an inexpensive, efficient, and productive method for creating comprehensive cell type, tissue, or organism specific transcriptomes, and takes full advantage of the exhaustive cDNA sequencing efforts of other researchers.

1815 TIME RELATED TUMOR NECROSIS FACTOR-ALPHA (TNFα) EXPRESSION AND SIGNALING DUE TO FUMONISIN B_1 *IN VIVO*.

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Fumonisin B_1 (FB_1), a toxic metabolite of *Fusarium moniliforme*, is a carcinogen and causative agent of various animal diseases. Our previous studies indicated the involvement of TNFα in FB_1-induced toxic responses. To further investigate the time-course of TNFα production and signaling, mice (4/group) were treated subcutaneously (sc) or orally (po) with 25 mg/kg of FB_1 as a single dose and sacrificed at 0, 2, 4, 8, 12, 24 and 48 h after treatment. Both sc and po treatment increased alanine aminotransferase (ALT) and aspartate aminotransferase in plasma, with erythrocyte counts lowering at 4 h and 24 h after sc and po treatments, respectively. The leukocyte counts decreased at 2-8 h in sc, and at 8-12 h after po treatment. The TNFα mRNA expression in liver after sc treatment, measured by reverse-transcriptase polymerase chain reaction, showed a biphasic increase at 2 h and 12 h compared to control. This biphasic increase in liver was supported by a similar trend observed for inhibitor of apoptosis protein (c-IAP2) at 4 h and 24 h in liver, determined by western blot, and in ALT in plasma at 4 h and 24 h. The biphasic trend of c-IAP2 was related to the decrease in nuclear factor κB level (western blot) at 8 h. Kidneys showed an increase in TNFα expression at 2 h after po but no effect seen in spleen, suggesting that FB_1 acts locally on the tissue to induce TNFα expression without any contribution from peripheral macrophages. (Supported in part by NIEHS grant ES09403.)

1816 FUMONISIN TOXICITY IN TUMOR NECROSIS FACTOR RECEPTOR 2 (TNFR2)-KNOCKOUT MICE.

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Fumonisin B_1 (FB_1), a mycotoxin prevalent in corn, causes a variety of toxic effects in different mammalian species. The biochemical responses of FB_1 involve inhibition of ceramide synthase leading to accumulation of free sphingoid bases and expression of tumor necrosis factor α (TNFα). Cytotoxic effects of TNFα are mediated via two distinct membrane receptors. Toxic response to FB_1 was investigated in wild-type (WT) male C57BL/6J mice and a corresponding TNFR2-knockout strain (TRK). The hepatotoxic effects of 5 daily injections of 2.25 mg/kg/day of FB_1 were observed in WT but were considerably reduced in TRK, evidenced by circulating alanine aminotransferase and aspartate aminotransferase levels and histopathological evaluation of the tissue. FB_1 induced TNFα expression in the livers of both WT and TRK mice to a similar extent (3-4 fold over control); however, a corresponding increase of cellular NFκB, expected after the downstream cellular signaling of TNFα, was noted only in the WT. Accumulation of liver sphingosine after FB_1 treatment was similar in both WT and TRK, but the FB_1-induced increases in liver sphinganine and kidney sphingosine and sphinganine were lower in TRK than in WT. Results emphasized the role of TNFα in FB_1-induced hepatotoxicity in mice and the possible relationship of sphingoid base accumulation and TNFα induction. (Supported in part by NIEHS grant ES09403.)

1817 EXPRESSION ANALYSIS OF RAT MULTIPLE DRUG RESISTANCE (MDR1A, 1B AND 2) BY BRANCHED DNA SIGNAL AMPLIFICATION.

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Branched DNA (bDNA) signal amplification is a powerful tool for monitoring the expression of a selected gene in a high-throughput manner. We describe the tissue expression patterns of three rat mdr isoforms using bDNA technology. P-glycoprotein (encoded by mdr) is responsible for multidrug resistance to cancer chemotherapy and efflux of xenobiotics. The purpose of this study was to quantify mRNA in tissues that are known to express the mdrs and to assess the capability of the bDNA technique to detect mdr isoforms in low copy tissues. Oligonucleotide probe sets were designed to individual isoforms of the mdr family within this highly homologous family. Previously, mdr1a expression was detected to high levels in intestine and was reported in brain and kidney. The bDNA technique verifies that conclusion, but indicates that mdr1a is expressed 10-fold higher in intestine than brain, and detectable in essentially all tissues with concentrations in brain slightly higher than kidney, bladder, and lung. Most reports indicate that mdr1b is prevalent in lung, is expressed to a lesser extent in liver, and is possibly present in kidney and intestine. Using bDNA, mdr1b expression was 6-fold higher in lung than intestine, kidney, and other tissues but detected at low levels in liver. Expression of mdr2 has been reported to be most prevalent in the liver and spleen followed by heart. Using the bDNA technique, mdr2 expression was 2-fold higher in liver than in spleen, lymph node and heart, whereas mdr2 expression in other tissues was much lower. Therefore, bDNA is sensitive and specific for individual mdr isoforms and is capable of reliably quantifying expression in low copy tissues. (ES-07079; Lied Foundation 559086.)

1818 INDUCIBILITY OF THE RAT AND HUMAN MRP2 ('MULTIDRUG RESISTANCE PROTEIN 2') GENE AND IDENTIFICATION OF SEQUENCE ELEMENTS REGULATING THE EXPRESSION OF THE RAT GENE.

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The drug-resistance mediating transporter MRP1 and the biliary efflux transporter MRP2 mediate ATP-dependent efflux of e.g., drug conjugates through cellular membranes. So far four further MRP genes have been identified but little is known about their function. In this study we characterized the 5'-flanking region of the rat mrp2 gene with respect to basal expression and inducibility of the gene. Furthermore we demonstrated the inducibility of the human MRP2 gene. In rat hepatocytes and hepatoma cells mrp2 gene expression is inducible e.g., by the chemical carcinogen 2-acetylaminofluorene (2-AAF), the synthetic glucocorticoid dexamethasone, the barbiturate phenobarbital, and chemotherapeutic drugs like vinblastin and cisplatin. Sequencing of the 5'-flanking region of the rat gene and transfection assays with reporter gene constructs containing unidirectional deletions in the flanking region revealed sequences which might mediate these inducing effects. Site-directed mutagenesis experiments demonstrated that a Y-box and a GC-box play a decisive role for the basal expression of the gene. In conclusion the gene seems to be regulated in a similar way as related ABC transporter genes in which also a Y-Box (human MDR1) and a GC-Box (human MRP1) are involved in basal expression.Furthermore, the inducibility of the MRP2 gene might have clinical relevance i) with respect to pharmacokinetic properties of therapeutic drugs, and ii) with respect to the induction of multidrug resistance in malignant and non-malignant cells.

1819 INDUCTION OF ALANINE AMINOTRANSFERASE GENE EXPRESSION BY TACRINE IN HEPG2 CELLS.

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Tacrine (1,2,3,4-tetrahydro-9-acridinamine monohyrdochloride monohydrate; Cognex) is a drug for the treatment of Alzheimer's disease. The most common adverse effect of tacrine therapy has been increased serum levels of alanine aminotransferase (ALT) activity in approximately 50% of patients receiving the drug. The mechanism(s) by which tacrine elevates ALT is not known. In vitro isolated hepatocyte data showed mitochondrial damage as an early event, and ALT release near cytotoxic levels. In the present study, we explored whether tacrine induces ALT levels through activation of ALT gene expression. Human hepatoma cells, HepG2, were incubated with 0, 0.1, 0.6

and 1 mM tacrine for 4 and 24 hours. Cytotoxicity, assessed by determination of extracellular lactate dehydrogenase activity, was not observed during the 4hr treatment but at 24hr the LDH activity in the medium increased by 50% at 1 mM tacrine. ALT mRNA expression was quantitated using real-time quantitative reverse- transcriptase polymerase chain reaction (RT-PCR). Tacrine caused dose- and time-dependent increases in ALT gene expression with maximal elevations of 2 fold (4hr) and 10 fold (24hr) after 0.6 mM treatment. Co-treatment with actinomycin D had no effect on ALT mRNA induction at 4hr. Extracellular ALT levels were also increased in response to tacrine in these cells. These results indicate that tacrine induces ALT gene expression during non-toxic and toxic exposures which may play a role in the increased ALT activity seen clinically.

1820 TRANSCRIPTION OF MOUSE CYP1B1 DEPENDS ON AHR ACTIVITY AT A SINGLE RESPONSE ELEMENT THAT IS CRITICALLY MODULATED BY NOVEL PROXIMAL UPSTREAM COMPLEXES.

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Cyp1B1 is expressed constitutively under control of the Ah receptor (AhR) and is induced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). CYP1B1 enhancer region contains four XRE consensus sequences that typically form complexes with AhR and the dimerization partner Arnt. However a complex of similar size which contains neither AhR or Arnt and is unresponsive to TCDD forms at two elements (XRE1, XRE4). Only one element selectively binds AhR/Arnt (XRE5). Systematic mutation of XRE1 and XRE4 shows that this anomalous complex (anC) binds to a consensus sequence ACG-CACGCGGCG. Only the GG pair is not present in XRE5 which is therefore critical to selection between AhR or anC. This anC binding site overlaps with the XRE core sequence. Four XRE5 tandem repeats when linked to a 210 base pair Cyp1B1 proximal promoter and a luciferase reporter gene responded to TCDD to reach activity levels similar to those seen with the full enhancer. The equivalent XRE4 construct was ten fold less inducible. The full enhancer potentiates basal activity 3-4-fold whereas XRE5 and XRE4 tandem repeats were ineffective. Selective site mutations show that XRE1 and XRE4 function as potentiating elements particularly for basal activity whereas AhR/Arnt binding to XRE5 is essential for both basal and induced activity. The downstream half-region (with XRE4 and XRE5) was six-fold less effective in basal regulation but nevertheless exhibited a full induction by TCDD. An upstream half-region (with XRE1 and XRE3) was inactive when used separately.

1821 TCDD AND SUSPENSION ACTIVATE CYP1B1 EXPRESSION IN HUMAN KERATINOCYTES AND DERMAL FIBROBLASTS.

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Binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to AhR results in dimerization with ARNT and expression a number of AhR-responsive genes, including members of the cytochrome p-450 (CYP) family of monoxygenases. TCDD is a potent inducer of CYP1A1 expression in adherent human epidermal keratinocytes. Loss of adhesion and suspension of keratinocytes in the absence of known exogenous AhR ligands also induces CYP1A1 expression through the AhR pathway. However, neither TCDD treatment nor suspension induces the expression of CYP1A1 in normal dermal fibroblasts. To determine if the AhR/ARNT pathway is functional in this mesenchymally derived cell type; we performed northern and western analysis to show that AhR and ARNT are expressed in dermal fibroblasts. We found that another AhR-responsive member of the CYP superfamily, cytochrome P-450 1B1 (CYP1B1), was responsive in dermal fibroblasts. Northern analysis demonstrated that steady state mRNA levels for CYP1B1 were increased in fibroblasts and keratinocytes by either TCDD treatment or suspension. CYP1B1 mRNA levels were increased at 1.5 h after suspension or TCDD treatment, peaked at 12 h, and returned to basal levels by 24 h. We are using fibroblasts and keratinocytes from AhR null mice to investigate the requirement of the AhR signal transduction pathway in CYP1B1 expression in these two cell types. Our data suggest that AhR responsive gene expression may be cell type specific in the epidermis.

1822 IDENTIFICATION OF AN ENHANCER ELEMENT REQUIRED FOR EXPRESSION OF CLASS PI GLUTATHIONE S-TRANSFERASE GENE BY A CO-PLANAR POLYCHLORINATED BIPHENYL.

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Coplanar polychlorinated biphenyl (PCB) congeners and related compounds, e.g. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, are recognized as potent hepatocarcinogens. These compounds are teratogenic and have endocrine disrupting activities. 3,3',4,4',5-pentachlorobiphenyl (PenCB), one of the most toxic coplanar PCB congeners, specifically induces class Pi glutathione *S*-transferase (GSTP1), a marker enzyme for preneoplastic hepatic foci and hepatocarcinomas, as well as cytochrome P450 1A1 in primary cultured rat liver parenchymal cells. However, the 5'-flanking sequence of GSTP1 gene does not contain a xenobiotic responsive element, to which arylhydrocarbon receptor binds. To understand the mechanism for the induction of GSTP1, we have identified a 5'-upstream region required for GSTP1 induction by PenCB using chloramphenicol acetyltransferase (CAT) assay. Plasmids containing a 3.0 kb fragment of the 5'-flanking region of the GSTP1 gene and its deletion mutants fused with the CAT gene were transfected into primary cultured rat liver parenchymal cells, and CAT activity was determined after treatment of the cells with PenCB. We have shown that GSTP1 enhancer I (GPEI) located 2.5 kb upstream is required for PenCB stimulation of GSTP1 gene expression in primary cultured rat liver cells. Epidermal growth factor (EGF), an inducer of GSTP1, also stimulated GPEI-mediated gene expression. The sequence of GPEI is similar to that of antioxidant responsive element. It is suggested that a novel signal transduction pathway activated by PenCB contributes to the stimulation of GSTP1 expression.

1823 ESTROGEN AND ARYL HYDROCARBON RECEPTOR EXPRESSION AND CROSSTALK IN HUMAN ISHIKAWA ENDOMETRIAL CANCER CELLS.

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Ishikawa endometrial cancer cells express the estrogen and progesterone receptor, and 17β-estradiol (E2) induces proliferation of cells used in this study. Treatment of Ishikawa cells with [^{3}H] 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) gave a radiolabeled nuclear complex that sedimented at 6.0 S in sucrose density gradients, and Western blot analysis confirmed that Ishikawa cells expressed human aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (Arnt) proteins. Treatment of Ishikawa cells with 10 nM TCDD induced a 9.7-fold increase in CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity and a 10.5-fold increase in chloramphenicol acetyltransferase (CAT) activity in cells transfected with pRNH11c containing an Ah-responsive human CYP1A1 gene promoter insert (-1142 to +2434). TCDD and related AhR agonists exhibit antiestrogenic activity in human breast cancer cells, and inhibitory AhR-estrogen receptor (ER) crosstalk was investigated in Ishikawa cells using E2-induced cell proliferation and transcriptional activation assays. AhR agonists including TCDD, benzo[a]pyrene (BaP) and 6-methyl-1,3,8-trichlorodibenzofuran, inhibited 32-47% of the E2-induced responses. In contrast, neither estrogen nor progesterone inhibited EROD activity induced by TCDD in Ishikawa cells, whereas inhibitory ER-AhR crosstalk was observed in ECC-1 endometrial cells suggesting that the interactions were cell context dependent and probably governed by limiting concentrations of transcription factors. (CA64081 and ES09106.)

1824 THERAPEUTIC, DIETARY PHYTOCHEMICALS GENISTEIN, CATECHIN, RESVERATROL, QUERCETIN AND NARINGENIN ACT VIA THE ESTROGEN CELL-SIGNALING PATHWAY.

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Hepatic expression of apolipoprotein (apo) II is in part modulated by estrogen-mediated stabilization of its mRNA. This stabilization is due to the estrogen-regulated mRNA stabilizing factor (E-RmRNASF) expressed in the liver in response to estrogen (Ratnasab*apathy, 1995, Cell. Mol. Biol. Res, 41: 583-594*). E-RmRNASF protects the RNA from targeted endonucleolytic degradation. The hepatic expression of E-RmRNASF is modulated by certain estrogenic and antiestrogenic nonsteroidal environmental xenobiotics (*Ratnasabapathy et al. 1997, Biochem. Pharmacol., 53: 1425-1434*). To

determine whether dietary phytochemicals purported to prevent hormone-dependent breast and prostate cancers and atherosclerosis, acted via the estrogen cell-signaling pathway, roosters were administered increasing doses up to 1 mmol./kg of genistein, daidzein, resveratrol, quercetin, catechin or naringenin parenterally and tested for hepatic expression of E-RmRNASF. Besides estrogen, resveratrol, genistein and catechin stimulated the expression of E-RmRNASF in the liver, and hence were estrogenic. A lack of E-RmRNASF expression was seen with the roosters treated with the vehicle, naringenin, quercetin or daidzein. To determine whether the agents exerted partial agonistic or antagonistic effects, roosters were administered combinations of estrogen and increasing doses of the above phytochemicals. Resveratrol showed agonistic activity at all concentrations (10-1000 μmol./kg) tested. Genistein, and catechin showed partial agonistic activity, while quercetin and naringenin appeared to be antagonistic. At the concentrations used daidzein appeared not to affect estrogenic stimulation of E-RmRNASF expression.

1825 IDENTIFICATION AND CHARACTERIZATION OF POLYMORPHISMS IN THE 5'-REGULATORY REGIONS OF HUMAN CYTOCHROME P450 1A1 AND 1B1.

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The cytochrome P450 (CYP) superfamily plays an important role in phase I metabolism and detoxification of xenobiotics. However, these enzymes have also been implicated in the paradoxical activation of pro-carcinogens into carcinogenic species. Furthermore, many CYP substrates are also transcriptional inducers of CYPs. Our laboratory has used polymerase chain reaction single strand conformation polymorphism to scan the promoter regions of human cytochrome P450s 1A1 and 1B1 in order to identify single nucleotide polymorphisms that may affect constitutive expression and/or induction of these genes. Two novel polymorphisms have been identified in CYP1B1 ($T_{-350}C$ and $C_{-238}T$) and three polymorphisms have been identified in CYP1A1 ($A_{-469}G$, $A_{-562}G$, and $G_{-594}T$). Each polymorphism was confirmed by DNA sequencing and polymerase chain reaction restriction fragment length polymorphism. The promoter regions of these CYP polymorphisms have been cloned and the functional properties of allelic variants are being investigated by transient transfection and mobility shift assays. Allele-specific alterations in CYP induction and/or expression may be a contributing risk factor for the development of certain forms of cancer and other human diseases. (Supported in part by US EPA R825808 and Multidisciplinary Research Pilot Program, SUNY at Buffalo.)

1826 BRANCHED DNA (BDNA) SIGNAL AMPLIFICATION FOR ANALYSIS OF CYTOCHROME-P450 GENE EXPRESSION.

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The importance of the cytochrome-P450 enzyme (CYP) family in xenobiotic metabolism, as well as its differential expression and activity in response to a wide range of chemicals and drugs is well documented. In this report, we evaluate the design and specificity of a branched DNA (bDNA) assay for the detection of multiple isoforms of rat CYPs from hepatocellular RNA. Oligonucleotide probe sets were designed to various chemically inducible rat CYP mRNA transcripts, including CYP1A1, CYP1A2, CYP2B1/2, CYP3A1/23, and CYP4A2/3. The robustness of the bDNA assay was assessed with the CYP2B1/2-specific probe set and total hepatic RNA isolated from control and phenobarbital-treated rats. Analysis of these RNA samples by bDNA signal amplification resulted in a linear quantifiable signal from an RNA concentration range that spanned three orders of magnitude (0.1-100 μg). The fidelity of the bDNA assay was evaluated within a single assay and between assays where repeated measurements of a single sample were reproduced reliably. The specificity of each individual CYP probe set was evaluated by utilizing the well-known effect of specific chemicals on the expression of hepatic CYP isoforms. Male Sprague-Dawley rats were administered 3-methylcholanthrene (3MC), phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), or clofibric acid (CLO). bDNA analysis of chemical-induced differences in CYP gene expression indicated that 3MC induced CYP1A1 and CYP1A2 mRNA levels 670- and 11-fold, respectively; PB induced CYP2B1/2 expression 71-fold; PCN induced CYP3A1/23 expression 34-fold; and CLO induced CYP 4A2/3 expression 4.7-fold. Overall, these data support the use of bDNA signal amplification technology as a reliable, sensitive, and specific method to monitor the differential expression of multiple isoforms of the CYP gene family. (Support: NIH ES-03192, ES-07079; Kansas Health Foundation KHF-411606.)

1827 USE OF REAL TIME GENE-SPECIFIC POLYMERASE CHAIN REACTION (PCR) TO MEASURE RNA EXPRESSION OF THREE FAMILY MEMBERS OF CYTOCHROME P450 4A IN RATS.

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Exposure of rats to peroxisome proliferators induces members of the cytochrome P450 4A (CYP4A) family. In rats the CYP4A family consists of 3 related genes, CYP4A1, CYP4A2 and CYP4A3, each of which is expressed and induced in a tissue- and sex-dependent manner. While CYP4A1 is sufficiently different from the other two members to enable relatively easy gene-specific quantitation, the close similarity between CYP4A2 and 4A3 makes quantitative discrimination more difficult. We have combined a fluorescent real-time PCR assay (TaqMan®) with the gene-specific Mismatch Amplification Mutation Assay (MAMA) to allow us to carry out gene-specific quantitation of all three members of this family. We have designed the assay such that a single fluorescent TaqMan® probe binds to all three gene members while the specificity is conferred by gene-specific primers. The specific MAMA technique takes advantage of the ability of the Taq polymerase to discriminate between the two genes based on mismatches at the 3' end of a PCR primer. In the 84-base PCR product used for this assay, there is only a singe base difference between 4A2 and 4A3. Despite this similarity, there is at least a 1000-fold discrimination between the two sequences using authentic standards. Analysis of rat liver and kidney RNA from both sexes demonstrates that this discrimination is also achieved in complex RNA mixtures. The technique should be broadly applicable to other research areas such as allelic discrimination, detecting mutational hotspots in tumors, and discrimination among closely-related family members of other genes.

1828 BASAL AND XENOBIOTIC-INDUCIBLE EXPRESSION OF UGT1A6 IS MEDIATED BY THE XENOBIOTIC RESPONSIVE ELEMENT.

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UDP-Glucuronosyltransferases (UGT) isoform 1A6 facilitates the removal of simple phenolic compounds from the body, thereby preventing further metabolism and subsequent toxicity. In liver, UGT1A6 exhibits low basal levels of expression and high inducibility by various agents including dithiole thiones (oltipraz), phenolic antioxidants (tBHQ), and polycyclic aromatic hydrocarbons (PAH) (3MC). The inducibility of UGTs is thought to represent an adaptive response conferring increased liver detoxication capacity in response to chemical exposures and involves transcriptional activation. Transient transfection studies using a luciferase reporter plasmid under the control of the UGT1A6 gene promoter indicated the induction of UGT1A6 gene expression in response to 24 h treatment with 50μM oltipraz (3-fold), 50μM tBHQ (3-fold), and 2.5μM 3MC (20-fold) in primary rat hepatocytes or HepG2 cells. The mechanism of the 3MC effect on UGT1A6 has been proposed to involve the XRE (at position -134 to -129), however the mechanism of the oltipraz and tBHQ effect is unknown. To determine the mechanism(s), UGT1A6 promoter 5' deletion constructs and constructs with mutated XRE were prepared. Deletion to -161 maintained oltipraz, tBHQ, and 3MC inducibility. A single base pair mutation and deletion of the XRE reduced basal expression by 90% and resulted in loss of inducibility by all three compounds. Therefore, these data suggest that the XRE is involved in basal and inducible expression of UGT1A6 upon treatment by PAH and selective phase 2 enzyme inducers.

1829 CELL-TYPE SPECIFIC DIFFERENCES IN THE REGULATION OF HUMAN γ-GLUTAMYLCYSTEINE SYNTHETASE HEAVY (GCSh) AND LIGHT (GCSl) SUBUNIT GENES.

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γ-glutamylcysteine synthetase (GCS), the enzyme catalyzing the rate-limiting step of glutathione synthesis, is composed of a catalytic and a regulatory subunit, designated GCSh and GCSl respectively. Northern blot analysis using mRNA from human tissues has shown that the ratio of GCSl to GCSh mRNA is not uniform, suggesting tissue-dependent regulation. We used human cell lines derived from liver (HepG2), lung (A549), kidney (HEK 293) and skeletal muscle (RD) to examine cell-type specific regulation. We transfected luciferase reporter gene constructs containing the 5' promoter region of each gene into each cell line to study differences in transcriptional regulation. The ratio of GCSl to GCSh promoter-mediated transcription was approximately

4.0 in A549 cells, 1.6 in HepG2 and RD cells, but only 0.25 in HEK 293 cells. To examine cell-type dependent gene-induction, we treated each cell line with the GCS inducing agents pyrrolidine dithiocarbonate (PDTC), phenethyl isothiocyanate (PEITC), and b-naphthoflavone (b-NF) and measured changes in steady state RNA levels of GCSl and GCSh using an RNAse protection assay. Preliminary results suggest that although both GCSl and GCSh are induced in HepG2 cells by all three agents, in the other three cell lines there is little or no induction of either subunit by PEITC or b-NF while only GCSl is induced by PDTC. While some differences may reflect variations in metabolic capabilities (i.e., for b-NF), these studies indicate that GCSl and GCSh are not always coordinately regulated and that there are cell-type specific differences in basal and induced regulation of these genes. (This work was supported by ES09749, CA57549, T32-ES0715-21.)

1830 INDUCTION OF HEPATIC MICROSOMAL EPOXIDE HYDROLASE AND CYTOSOLIC RGSTA3/5 AND ACTIVATION OF ACTIVATOR PROTEIN-1 BY PROTEIN-CALORIE MALNUTRITION IN RATS: REVERSAL BY CYSTEINE SUPPLEMENTATION.

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Protein-calorie malnutrition (PCM), as one of global problems, arises during protein and/or energy deficit due to disease and nutritional inadequacy. Cellular adaptive responses and gene expression associated with PCM remain poorly understood. The present study was designed to assess the expression of hepatic phase II detoxifying enzymes in PCM rats and to investigate the molecular mechanistic basis. A 4 week-protein restriction of rats resulted in 4-fold induction of hepatic microsomal epoxide hydrolase (mEH) with a 21-fold increase in mRNA. Immunoblot and Northern blot analyses revealed that rGSTA3/5 was induced by protein deprivation. Supplementation of rats fed the low casein diet with cysteine prevented increases in hepatic mEH and rGSTA3/5 protein and mRNA levels. Gel shift analysis revealed that the nuclear hepatic activator protein-1 (AP-1) transcription complex was activated in PCM rats. AP-1 binding activity was reduced by anti-JunB, and anti-c-Jun antibodies, whereas antibodies directed against c-Fos, FosB, Fra-1 and JunD were not inhibitory. Cysteine supplementation prevented the activation of AP-1 and the decrease in hepatic GSH level by PCM. PCM caused marked decreases in cytoplasmic eosinophilic contents and nuclear shrinkage in hepatocytes, whereas PCM rats supplemented with cysteine showed only mild fat degeneration with well-preserved portal structures. The current study provided evidence that PCM induced mEH and rGSTA3/5 and that cysteine was active in preventing the enzyme induction and AP-1 activation as well as in preserving morphological structures of hepatocytes.

1831 SPECIES DIFFERENCES IN THE EXPRESSION OF OCULAR AND STOMACH ALDH3A1.

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The class 3 aldehyde dehydrogenase (ALDH3A1) is a cytosolic NAD(P)+-dependent enzyme that efficiently catalyzes the oxidation of medium-chain aldehydes, such as those produced during lipid peroxidation. The ALDH3A1 gene exhibits a tissue-specific expression pattern. It has been shown that it is constitutively expressed at high levels in corneal cells and to a lesser extent in stomach in various mammalian species. This study was undertaken to investigate the species distribution of ocular and stomach ALDH3A1 in pig, mouse, rat, rabbit, chicken, turkey, zebrafish, trout and frog, which represent different orders of mammals as well as other non-mammalian classes. High ALDH3A1 activity was detected in both ocular and stomach tissues in pig, mouse and rat. Immunohistochemistry on ocular and stomach sections of these species showed that ALDH3A1 is abundantly present in the corneal and stomach epithelial cells, detected as a 54 kDa protein in immunoblots. The ocular tissues contained higher ALDH3A1 levels as compared to the stomach tissues. Lack of ALDH3A1 protein and enzymatic activity was observed in rabbit, zebrafish, trout, frog, chicken, turkey, and frog ocular and stomach extracts. Interestingly, significant levels of the cytosolic ALDH1A1 and the mitochondrial ALDH2 were detected by immunoblot analysis in the ocular and stomach tissues of the above species. These data suggest that expression of ALDH3A1 occurs in a taxon specific manner. Furthermore, the presence of ALDH3A1 or ALDH1A1/ALDH2 in ocular and stomach tissue supports the proposed catalytic role of those ALDHs in the detoxification of lipid peroxidation aldehydes. (Supported by NEI 1 R29 EY11490.)

1832 DIFFERENTIAL GENE EXPRESSION IN RABBIT RENAL CORTICAL SLICES EXPOSED TO ARSENIC COMPOUNDS.

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Differentially expressed genes were determined in an *in vitro* system -precision-cut rabbit renal cortical slice- after 3 hr exposure to 0.1 μM inorganic arsenite (As III), arsenate (As V), or 0.1 and 100 μM dimethylarsenate (DMA). The high-density Gene Discovery Array System (Genome Systems, Inc.) representing 18,376 non-redundant human cDNA clones was used to determine gene expression pattern. mRNA from renal slices was reverse transcribed to [α-^{33}P]dCTP-labeled cDNA and hybridized to the membrane. The differentially expressed cDNA clones were identified using the Genome Systems website following phosphoimager analysis. Up-and down-regulated genes (>3-fold from control) were:

AsIII	(0.1	μM):	11	up	and	12	down
AsV	(0.1	μM):	13	up	and	19	down
DMA	(0.1	μM):	14	up	and	18	down
DMA	(100	μM):	22	up	and	18	down

There are 12 common genes, such as ubiquitin, metallothionein-II, thyroxine-binding globulin, and anion exchange protein. Nine genes were unique to AsIII, such as ATP synthase subunit 9, nuclear protein p68, and insulin-like growth factor binding protein 6. Five genes were unique to AsV, such as GDP-dissociation inhibitor, and HLA class II histocompatibility antigen DQ5. Only 2 genes were unique to DMA (0.1 μM): interlekin 1 receptor accessory protein and thy-1 glycoprotein, while the high concentration of DMA had numerous unique genes such as transcriptional activators hSNF2 and STAT5A, and tumor necrosis factor precursor 2 related protein. Although these genes need to be confirmed by Northern analysis, Gene Array technology has been proven to be a powerful tool to provide a global view of molecular alterations induced by specific arsenic species. (NIH ES04940.)

1833 GENE EXPRESSION AND CELL FUNCTION IN CISPLATIN TREATED RAT AND HUMAN KIDNEY AND LIVER SLICES.

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Cisplatin is a chemotherapeutic agent that induces cell death through an apoptotic mechanism. The role of apoptosis in cisplatin induced kidney and liver damage was investigated in precision-cut rat and human kidney and liver slices. Slices were incubated with cisplatin (10 - 100 μM) for up to 24 hours. Viability of the slices was monitored in conjunction with the expression of genes regulating cell cycle progression and apoptosis. In rat kidney slices, p53 gene and protein expression, early indicators of apoptosis, were induced by ≤20 μM cisplatin at 24 hours. Additionally, the expression of the early response gene c-fos was elevated at all concentrations. The expression of Gadd45, a gene involved in growth arrest and DNA damage, was induced at all concentrations of cisplatin, with the greatest expression change at 10μM. Genomic DNA laddering, one of the final events in the apoptotic cascade, was evident in slices treated with ≥60 μM cisplatin. This increase in DNA laddering coincided with a decrease in slice viability, as measured by ATP content and organic anion transport. In rat liver slices, at 24 hours, p53 gene and protein expression levels were below control at all concentrations. In work previously reported in this lab, p53 gene and protein expression in rat liver slices was shown to be transient and elevated prior to 24 hours of incubation. In this study, Gadd45 gene expression and DNA laddering was evident in rat liver slices incubated with all concentrations of cisplatin, while viability was decreased at concentrations ≥40 μM as assessed by ATP and GSH content. Human kidney slices were less sensitive to cisplatin (≥100 μM) than were human liver or rat kidney and liver slices out to 24 hours. In both species, cisplatin induced apoptotic events occur in liver and kidney slices, and these apoptotic events precede and may accompany the decrease in slice viability.

1834 QUINOL-THIOETHERS REGULATE A VARIETY OF GENES INCLUDING A NOVEL G PROTEIN BETA SUBUNIT IN HL-60 CELLS PRIOR TO APOPTOSIS.

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Benzene mediated hematotoxicity and bone marrow suppression may be due to the combined effects of hydroquinone and its glutathione conjugates. 2,3,5-tris-(glutathion–S-yl)hydroquinone (TGHQ) induces oxidative stress and apoptosis in human promyelocytic leukemia (HL-60) cells. Induction of apoptosis occurs 6-8 hrs after treatment of HL-60 cells with 200 μM of TGHQ. In order to understand the role of gene expression in the early phase of induction of apoptosis, a differential screening of cDNA libraries from TGHQ treated and untreated HL-60 cells was performed. Primary screening of 90,000 clones identified 385 differentially regulated cDNAs. Secondary screening by dot blot analyses revealed 111 cDNAs regulated by TGHQ. Sequencing of the cDNA clones identified 41 independent clones differentially regulated by TGHQ in HL-60 cells, and these were categorized into five groups: 1. Global modifiers of gene expression (transcriptional repressor GCF2, RNA polymerase II, elongation factor 1ß); 2. Tumor specific genes (TACC1, laminin binding protein); 3. Ribosomal proteins (RPLp1, L17); 4. Signalling and apoptosis related genes (clathrin associated protein AP-2, bax, thymosin ß-10, chaperonin containing TCP-1) and 5. Novel genes (novel G protein ß subunit, a protein kinase C receptor). The expression pattern and physiological significance of TGHQ regulated genes is being determined. Since many of these genes are not generally associated with apoptosis or oxidative stress, the results imply additional functions for these genes. (Supported by ES07784.)

1835 EFFECT OF THE EXPOSURE TO P-BENZOQUINONE AND HYDROQUINONE ON THE BINDING ACTIVITY OF THE TRANSCRIPTION FACTOR AP-1 IN PROMYELOCYTIC LEUKEMIA CELLS (HL-60).

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Benzene is one of the most heavily used chemicals in the world and has a wide environmental occurrence. The pathways of benzene metabolism are important in the bioactivation and therefore in the toxicity of this compound. Cytochrome P450-mediated oxidation of benzene to phenol and the polyhydroxylated metabolites: hydroquinone (HQ), catechol, and 1,2,4-trihydroxybenzene. The oxidation of these benzene metabolites generate reactive benzoquinone (BQ) and free radicals as well as other reactive oxygen species, which are involved in transcriptional regulation of some genes. Numerous studies have associated exposure to benzene with blood and bone marrow toxicity in occupational exposed workers, including lymphocytopenia, pancytopenia, aplastic anemia, acute myelogenous leukemia, and possibly lymphoma. This work evaluated alterations in the expression levels of c-Jun and c-Fos, and the binding activity of AP-1, using the mobility shift asay, in HL-60 cells exposed to HQ and BQ. Our results showed that BQ and HQ induced an increased gene expression of c-jun and c-fos, as well as an increased AP-1 binding activity when cells were treated with 1μM for 1 or 4h; however, we could not detect c-Jun in the AP-1 complex at 4 h. In contrast, when cells were treated for 1h with BQ or HQ, c-Jun was present in the AP-1 complex. Therefore our results suggest that another member of the Jun family is present in the AP-1 complex at 4 hours.

1836 EFFECT OF ACROLEIN ON AP-1 AND GENE EXPRESSION IN A549 CELLS.

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Acrolein, a reactive α,β–unsaturated aldehyde is a common environmental pollutant. Recently, it has been shown to be a byproduct of lipid peroxidation, and increases in acrolein production may be a marker for Alzheimer' disease, diabetic glomerular lesions and atherosclerosis. Although the overt toxicity of acrolein has been widely studied, the effects of low dosages are not known. We previously reported that DNA synthesis and NF-κB activation were inhibited by acrolein in the A549 lung adenocarcinoma cell line (*J. Biol. Chem.* **274**: 9200 [1999]). The current data demonstrate that AP-1 activation is decreased by 26 and 50% at 0.5 and 1 h, respectively, after exposure to 45 fmol/cell of acrolein. This was further confirmed by an AP-1 reporter assay. This effect may result from changes in the redox status of cells due to the depletion of glutathione as well as covalent modification of AP-1 protein. Differential gene expression was monitored using a cDNA microarray containing 4000 well characterized genes after treating A549 cells with 45 fmol/cell acrolein for 0.5 h followed by 0.5 h of recovery. Redox related genes including *quinone oxidoreductase, glutathione synthetase, NADH-quinone oxidoreductase* and *c-Jun* were elevated more than 9-fold. There was also an elevation in *cytochrome P450 IIE1* and some death-related genes. Based on the results of the microarray, several genes are being further studied to help understand the mechanism of toxicity of acrolein. (Supported by HL48035, ES09791 and Center Grant ES07784.)

1837 ETHIONINE INCREASES TUMOR NECROSIS FACTOR α EXPRESSION BY LIPOPOLYSACCHARIDE-STIMULATED MURINE MACROPHAGES.

W. H. Watson and R. K. Chawla. *University of Kentucky, Lexington, KY.* Sponsor: D. P. Jones.

Increased serum tumor necrosis factor-α (TNF) and hepatic deficiency of S-adenosylmethionine (AdoMet) are observed in patients with alcoholic liver disease as well as in experimental models of inflammatory liver injury induced by hepatotoxins. AdoMet is a major methylating agent and its deficiency is attributed to impaired hepatic synthesis. However, the biochemical mechanisms for elevated serum TNF are not fully understood. We previously reported that rats with hepatic AdoMet deficiency had ninefold more serum TNF upon lipopolysaccharide (LPS) treatment as compared to LPS- treated normal controls. The present study examined the effect of AdoMet deficiency on LPS-induced TNF production in the murine macrophage/monocyte cell line RAW 264.7. The cells were made AdoMet deficient by pre-incubating them with ethionine, a structural analogue of methionine. Ethionine, when converted to S-adenosylethionine (AdoEth), prevents utilization of AdoMet for methylation reactions and creates a functional deficiency of AdoMet. The effects of ethionine treatment on intracellular AdoMet and AdoEth concentrations, TNF protein and TNF mRNA levels, TNF mRNA stability, and transcription from the TNF promoter were determined. Ethionine treatment increased the intracellular concentration of AdoEth but did not significantly affect AdoMet concentrations. Ethionine treatment increased the amount of TNF protein released and the induction of TNF mRNA in response to LPS, but the stability of the TNF mRNA was unaffected by ethionine. A TNF promoter reporter construct was similarly affected by ethionine; LPS-induced CAT activity (reporter gene) and CAT mRNA levels were higher in ethionine-treated cells than in control cells. Therefore, ethionine acts at the transcriptional level to enhance LPS-induced TNF expression in RAW 264.7 cells.

1838 MODULATION OF THE EXPRESSION OF p53 DURING DIFFERENTIATION AND APOPTOSIS IN MURINE EMBRYONIC STEM CELLS WITH ALL-*TRANS*-RETINOIC ACID.

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The role of all-*trans*-retinoic acid (RA) in embryonic development has been established. RA is a morphogen but its excess can produce teratological effects. P53, a tumor suppressor gene that encodes for phosphoproteins, controls cellular proliferation, differentiation and apoptosis. The temporal modulation of p53 by RA was investigated in murine embryonic stem cells (ES) during normal differentiation. Undifferentiated ES cells express high levels of p53 mRNA and protein followed by a decrease in p53 as differentiation progressed. At 11-21 days of spontaneous differentiation the p53 level increased, presumably contributing to inhibition of proliferation in cells entering terminal differentiation. The addition of RA during 8-10 days increased the levels of p53 mRNA and protein, corroborated by increased apoptosis. RA-induced morphological differentiation provided specific, predominantly neural type of cells. The maximum increase in p53 mRNA in RA-treated ES cells was at day 17, whereas maximum protein expression occurred at 14-17 days. The level of p53 decreased in terminally differentiated ES cells. Marked apoptosis was observed at 10 and 20 hours after RA treatment. Results demonstrated that RA accelerated cellular differentiation and apoptosis in ES cells and increased the expression of p53. However, RA increased apoptosis earlier than it caused changes in p53 expression, suggesting that RA-induced apoptosis is not initiated by the induction of p53.

1839 IDENTIFICATION & CHARACTERIZATION OF A NOVEL TUMOR SUPPRESSOR GENE IN WILMS TUMOR.

K. P. Singh and D. Roy. *Department of Environmental Health Sciences, University of Alabama, Birmingham, AL.*

Wilms tumor or nephroblastoma is an embryonal malignancy of the kidney affecting about 1 in 10,000 children. It comprises about 6 % of all childhood cancers. Mutations mainly in two tumor suppressor genes, WT1 and WT2, do not account for all (hereditary as well as sporadic) cases of Wilms tumors. In an effort to screen mutations in the genome of wilms tumors, we previously reported a common mutations detected through AP-PCR analysis in 5 out of 15 (30 %) human Wilms tumor tissue samples. We also reported the chromosomal localization of this mutation at human chromosome $11q^{23.2}$. Here we report the further characterization of the amplified gene loci. We cloned and sequenced the variant AP-PCR amplified fragment and DNA sequence was compared with the known gene sequence in the Gene Bank database using the BLASTn program. The level of expression of this novel gene in tumor and control tissue from the same patient was done with the RT-PCR, and confirmed by Nothern as well as RNase protection assay. Sequence homology search did not reveal significant homology with any known gene. However it has 99% sequence homology with one of the human EST sequence in the gene bank. Both, RT-PCR and RNase protection assay indicated the down regulation of this gene in tumor as compared to their normal tissues. Findings of this study indicate the presence of the novel gene in Wilms tumors. Presence of normal copy of this gene and it normal expression in the control tissue samples and its downregulation of expression as a result of mutation in the tumor tissues indicates that it may be a novel tumor suppressor gene.

1840 GENOMIC IMPRINTING ANALYSIS OF THE MANNOSE-6-PHOSPHATE/INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR IN CANCER SUSCEPTIBLE MICE.

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The M6P/IGF2R functions in the activation of the potent growth inhibitor, transforming growth factor β, and the degradation of IGF2, a mitogen often overproduced in tumors. The M6P/IGF2R has also been shown to be inactivated in both human and rodent cancers, and is genomically imprinted in rodents (i.e., only the maternal allele is expressed). The purpose of this study was to examine the imprint status of the M6p/Igf2r in the mouse strains C3H and C57BL/6, which are relatively susceptible and resistant to liver cancer, respectively. To investigate, a sequence polymorphism must first be identified. RT-PCR followed by mismatch detection found no difference in the M6p/Igf2r sequence between C57BL/6 and C3H. Therefore, 12 additional strains of mice were analyzed for sequence polymorphisms. DNA sequencing revealed a C to A base change in the 3' UTR of M6p/Igf2r in the SKH2 strain with respect to C57BL/6 and C3H. Crosses of C57BL/6 x SKH2 and C3H x SKH2 were generated to introduce the polymorphism in the F1 hybrids. Liver, heart, and brain RNA samples from each F1 cross were then analyzed for monoallelic or biallelic expression at the M6p/Igf2r locus. Results indicated a maternally derived, monoallelic expression pattern of the M6p/Igf2r in the liver and heart, but not in the brain. Importantly, no difference was observed in the imprint status of M6p/Igf2r between the F1 crosses in each tissue analyzed.

1841 HUMAN KERATINOCYTE EARLY TRANSCRIPTIONAL GENE ACTIVITY FOLLOWING SULFUR MUSTARD.

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Sulfur mustard (SM) by intramolecular rearrangement forms a stabilized carbonium ion that alkylates DNA, RNA, protein, and membrane molecules through electrophilic reaction. SM exposure on human skin produces delayed skin vesication at the dermal-epidermal basal contact of the keratinocyte epidermal stem cell. The primary changes occurring within the basal keratinocyte layer producing the loss of its attachment are not known. We were interested in determining the predominant mRNA changes in cultured human epidermal keratinocytes (HEK) shortly following SM exposure. Our hypothesis is that gene transcription following exposure to SM should be indicative of the level and type of specific cellular damage. In this study, we produced subtraction libraries (PCR-Select, Clontech, Palo Alto, CA) containing the up-regulated gene transcripts at 30 min after exposure to SM. Cultured normal HEK (Clonetics, San Diego, CA) at 70% confluence (150cm² flask) were exposed to 25 μM SM, an estimated subvesicating skin exposure, or to 200 μM, a vesicating exposure of SM. The polyA mRNA was purified, and the untreated control HEK cDNA subtracted from the cDNA from SM-treated HEK. The up-regulated gene transcripts were PCR-amplified, and the library was cloned and transformed into bacteria for cDNA sequence determination from colony isolation. Considerable diversity was found at 25 μM SM with 60 different transcripts of the 83 sequenced. In contrast, 57 cDNA sequenced at 200 μM SM produced only 16 different transcripts containing 9 known genes. The predominant known transcripts identified at 200 μM SM included γ-filamin, poly(rC)-binding protein 1, prothymosin and RAB11A. The down-regulated gene transcripts at 30 min after exposure to these SM concentrations are presently under investigation.

1842 LEAD INDUCTION OF TRANSCRIPTION FACTORS AND VEGF EXPRESSION IN HUMAN FETAL ASTROCYTES.

M. A. Hossain and J. Laterra. *The Kennedy Krieger Institute and Johns Hopkins School of Medicine, Baltimore, MD.* Sponsor: J. Bressler.

Lead (Pb) is a widespread environmental neurotoxicant whose developmental neurotoxicity remains a major medical issue. High levels of lead exposure increases the permeability of the blood-brain barrier (BBB) the function of which is regulated by perivascular astrocytes. The exact mechanism(s) of action of lead have not been fully elucidated. We hypothesized that VEGF, an angiogenic growth factor and vascular permeability factor, plays a role in Pb effects on the BBB. Using immortalized human fetal astrocyte (HFA) cultures we examined the effects of lead on VEGF expression and on the activity of the transcription factors HIF-1 and AP-1 that are capable of regulating VEGF expression. Induction of VEGF and transcription factors were determined by Northern blot analysis and electrophoretic mobility shift assay, respectively, and quantified by phosphor imaging. Maximum induction of HIF-1, AP-1 and VEGF resulted from exposing cells to 10 M lead acetate for 4 h and 20 h, respectively, in medium containing 10% serum. Induction of HIF-1 was increased 86% by lead ($p<0.01$) and 96% ($p<0.05$) by the protein kinase C (PKC) agonist PMA (100 nM). Induction of AP-1 was increased ~2 fold ($p<0.01$). VEGF expression was increased ~4 fold by lead ($p<0.001$) and ~2.5 fold by PMA ($p<0.001$). However, overexpression of a dominant negative form of HIF-1 in HFA cells did not block the induction of VEGF by Pb. In contrast, pretreatment with GF109203 (2 M), a specific inhibitor of PKC, inhibited lead and PMA mediated activation of VEGF transcription. These findings suggest that lead induces VEGF via a PKC-dependent and HIF-1 independent pathway in human fetal astrocytes. (Supported by NIH grant PO1 ES08131.)

1843 DIFFERENTIAL EXPRESSION OF NEUROFILAMENT SUBUNITS IN DIISOPROPYL PHOSPHOROFLUORIDATE (DFP)-TREATED HEN SPINAL CORD AND THEIR PRESENCE IN AXONAL AGGREGATES.

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Diisopropyl phosphorofluoridate (DFP) is an organophosphorus ester,that produces organophosphorus ester-induced neuropathy (OPIDN) in hen and other sensitive species. A single dose of DFP (1.7mg/kg,sc.) produces mild ataxia in 7-14 days in hens, that develops into severe ataxia or paralysis with the progression of the disease. OPIDN is associated with axonal swellings and degeneration of axons. This study was carried out to investigate the expression of neurofilament (NF) subunits in spinal cord of DFP-treated hens. Hens were treated with a single dose of DFP and sacrificed 1,5,10,20 days post-treatment. Western blot analysis showed increased expression of NF-M and decreased expression of NF-H and NF-L in the 2 M urea extracts of spinal cord particulate fraction within 24 hr of DFP administration. Thus, there was increase in the stoichiometry of NF-M:NF-L in the spinal cord of DFP-treated hens. Immunoprecipitation, cross-linking, and two-dimensional polyacrylamide gel electrophoresis showed the presence of heterodimers in hen spinal cord extract. The formation of heterotetramers, however, was not detectable by disuccinimidyl suberate (DSS) mediated cross-linking. Immunohistochemical staining revealed the presence of all three NF subunits in the cytoskeletal aggregates in DFP-treated hen spinal cord cross-sections. The results suggest that each NF subunit probably gets accumulated by a different mechanism in the axonal aggregates of DFP-treated hen. (Supported in part by NIEHS Grant ESO 5154.)

1844 PRECLINICAL SAFETY EVALUATION OF THE LIPID-REGULATING AGENT CI-1027 IN CYNOMOLGUS MONKEYS.

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Subacute effects of CI-1027 (6,6'-oxybis[2,2-dimethyl-4-hexanoic acid]-Ca) were investigated in male and female monkeys (3/sex/dose) given 10, 30, 100, or 300 mg/kg daily by gavage for 4 weeks. No significant treatment-related clinical signs, and no changes in body weight and food consumption were observed at 10 to 100 mg/kg (Day 28 $AUC_{(0-24)}$: 954 to 8220 μg·hr/mL). At 300 mg/kg (Day 28 $AUC_{(0-24)}$: 10400 μg·hr/mL), 1 male and 2 females were symptomatic and were euthanatized for humane reasons during Week 3 or 4. Clinical laboratory changes consisted of decreased red blood cell counts, hematocrit, and hemoglobin concentration (10% to 30% from pretest) in females at 100 and 300 mg/kg, and increased activated partial thromboplastin time (41% to 72%) and prothrombin time (14%) in males at 100 and 300 mg/kg. Changes observed in the 300 mg/kg group reflected deteriorating clinical condition. Pathological changes included increased relative liver weight (58% to 93%) at 300 mg/kg, and hepatocellular hypertrophy and/or hepatocellular fatty change, and decreased fine vacuolation of the adrenocortical zona fasciculata at 100 and 300 mg/kg. There was no evidence of microsomal enzyme induction. Pharmacologic effects at 10 to 300 mg/kg consisted of a 44% to 88% decrease in lipoprotein(a) and up to 35% decrease in total cholesterol. No adverse effects were noted at 30 mg/kg.

1845 EVALUATION OF HEPATIC PEROXISOMAL PROLIFERATION AND MICROSOMAL ENZYME INDUCTION BY THE LIPID-REGULATING DICARBOXYLIC ACID CI-1027 IN RATS, DOGS, AND MONKEYS.

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CI-1027 (6,6'-oxybis[2,2-dimethyl-4-hexanoic acid]-Ca) is a lipid-regulating agent in experimental models of dyslipidemia. Hepatic peroxisome proliferation and microsomal enzyme induction were evaluated in beagle dogs receiving oral CI-1027 for 2 weeks, and Wistar rats and cynomolgus monkeys for 2 and 4 weeks. Peroxisomal enzyme activity markers were carnitine acetyltransferase, acyl CoA oxidase, β-oxidation, and catalase, and microsomal enzyme activity markers were ethoxy- and pentoxyresorufin O-dealkylase, aminopyrine (monkey only) and erythromycin N-demethylase, lauric acid hydroxylase, and UDP glucuronyl transferase (GT). No hepatic changes occurred in dogs given doses up to 1000 mg/kg. In monkeys, liver weight increased at 150 to 1000 mg/kg, but was unchanged at 10 to 100 mg/kg. While β-oxidation was higher than controls in some drug-treated monkeys, there was no pattern of enzyme changes indicative of peroxisomal proliferation or microsomal enzyme induction. In rats at all doses, 30 to 750 mg/kg, liver weight increased up to ~100% and peroxisomal enzyme activities up to 6-fold. Lauric acid hydroxylase, a marker for CYP4A1, increased up to 11-fold at all doses in rats and GT activity increased up to 1.5-fold at 100 to 750 mg/kg. Increased CYP4A concentrations were confirmed with an ELISA. Toxicokinetic profiles revealed that CI-1027 exposures were less than dose-proportional and higher in monkeys than in dogs at the same dose. In rats given 300 or 750 mg/kg, exposures were lower at study termination than on Day 1, which may reflect GT induction. It is concluded that CI-1027 is similar to related compounds that cause hepatic peroxisome proliferation and microsomal enzyme induction in rats, but not in nonrodent species.

1846 ACUTE TOXICIOLOGY AND PHARMACOKINETIC ASSESSMENT OF A RIBOZYME (ANGIOZYME™) TARGETING VEGF RECEPTOR mRNA IN THE CYNOMOLGUS MONKEY.

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The potential acute toxicity of a ribozyme (ANGIOZYME™) targeting the flt-1 VEGF receptor mRNA was evaluated in cynomolgus monkeys following iv infusion or sc injection. ANGIOZYME™ was administered as a 4 h iv infusion at doses of 10, 30 or 100 mg/kg or a sc bolus at 100 mg/kg. Endpoints included blood pressure, ECG, clinical chemistry, hematology, complement factors, coagulation parameters, and ANGIOZYME™ plasma concentrations. ANGIOZYME™ was well tolerated with no drug-associated morbidity or mortality. There was no clear evidence of ANGIOZYME™ related adverse effects in this study. Slight increases in spleen weight and lymphoid hyperplasia were observed in several animals; however, these changes were not dose-dependent. Average steady-state concentrations of ANGIOZYME™ were achieved during the 4 h infusion of 10, 30 or 100 mg/kg. Dose-dependent elimination of ANGIOZYME™ was observed in steady-state concentrations for the 9 h sampling period. Thus, monkeys in this toxicology study received significant plasma ANGIOZYME™ exposure by the sc and iv routes.

1847 SAFETY STUDIES IN DOGS AND CATS OF A FABRIC REFRESHER.

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The safety of Oust®, a commercially available fabric refresher containing water, isopropanol, propylene glycol, surfactants, fragrances, zinc chloride and preservatives, was studied in cats and dogs under exaggerated exposure. Eight dogs and 8 cats were orally gavaged with 2 g product or water/kg bw. Another 8 dogs and 8 cats were whole-body sprayed with the product or water every other day for 14 d; each had 5cm by 5cm of trunk clipped to assess dermal irritation. A sensitization assessment was performed on 6 cats with clipped 5cm by 5cm trunk areas occlusively patched with gauze saturated with 0.2ml product and another with saline (first 3 d) and 2ml product and saline (2 additional d) for 5 consecutive days. After 14 d 0.2ml product challenge patches were applied to virgin clipped skin for 24 h. All animals were observed for skin, respiratory and general behavior or physiological alterations for 14 d after each last exposure. Blood samples were evaluated for hematology, serum chemistry, and liver and kidney function throughout. None of the dogs or cats exposed by the various routes exhibited ill effects or dermal or respiratory tract irritation. No behavioral or physiological changes occurred in normal functions, and all blood parameters remained within normal species' ranges. Sensitization did not occur. After each phase of the study, each animal was humanely neutered, rabies vaccinated and adopted into an approved household. No ill effects resulted to dogs and cats from exaggerated oral exposure, repeated whole-body topical exposure, or repeated dermal patching with this fabric refresher.

1848 TWENTY-SIX-WEEK DAILY REPEATED DOSE INTRAMUSCULAR TOXICITY STUDY OF THE KAPPA OPIOID AGONIST CI-977 IN BEAGLE DOGS.

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The analgesic agent CI-977, a potent and selective kappa opioid receptor agonist, was characterized in beagle dogs as part of the preclinical safety assessment. CI-977 was administered daily by intramuscular injection to groups of 5 beagle dogs per sex per group at 1, 3, and 6 μg/kg. The high dose was lowered from 10 to 6 μg/kg during Week 5 or 6 because of progressive weight loss. Central nervous signs (ataxia, persistent circling to the right, hypoactivity, tremor) and gastrointestinal signs (salivation, emesis, and diarrhea) were observed at 3 to 10 μg/kg. The intensity and frequency of clinical signs increased with dose, but did not diminish or increase throughout the treatment period. One male at 10 μg/kg had a single convulsion during Week 2. Body weight loss in the high dose group was noted up to Week 5 in males (ranging from 11% to 29% of pretest) and up to Week 8 in females (ranging from 18% to 39% of pretest). CI-977 plasma concentrations increased with dose, and were similar in males and females and throughout the treatment period. Testicular changes (atrophy, depletion of spermatids, single cell necrosis of spermatocytes, and multinucleated giant cells in the lumen of seminiferous tubules) were observed at 3 and 6 μg/kg. In summary, intramuscular administration of CI-977 daily at 1 μg/kg did not induce toxicity. Clinical signs observed at 3 and 6 μg/kg have been reported after treatment with opioid analgesics. Contrary to most opioid analgesics, CI-977 showed no indication of tolerance with repeated dosing. CI-977 induced testicular toxicity at 3 and 6 μg/kg.

1849 ARTERIAL AND HEPATIC EFFECTS OF CONTINUOUS INFUSION OF SIX ENDOTHELIN ANTAGONISTS IN DOGS.

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Endothelin-1 (ET-1) is a potent vasoconstrictor interacting with at least two distinct receptors, endothelin A (ET_A) and B (ET_B), and its potential role in the circulatory system has elicited interest in endothelin antagonists as therapeutic agents. Butenolide analogs, PD 166673, 166004, 167088, 166309 and 170246 with selective affinity for the ET_A receptor and Bosentan, an ET_A and ET_B receptor antagonist were infused at 2 μmol/kg/hr for 96 hr to groups of 2 catheterized beagle dogs/sex. Controls received saline. Blood for determination of compound and ET-1 concentrations were collected predose and at 6, 24, 47 and 96 hr. All compounds were well tolerated and there were no effects on electrocardiograms or blood pressure. Hepatic microsomal erythromycin N-demethylase activity decreased in PD 166004 and 170246 treated dogs to ~ 20% of controls. ET-1 concentrations increased 6 and 40 times after PD 166309 and Bosentan, respectively. Bosentan steady state concentrations of 7.4 to 22 μM were achieved at 47 hr. Plasma concentrations of PD 166673, 167088, 166309 and 170246 approached steady state of 0.6 to 2.7 μM by 24 hr. PD 166004 concentration increased at each time point consistent with inhibition of hepatic CYP mediated-metabolism. Coronary arteriopathy was observed in all dogs given butenolide analogs and in 1 of 4 dogs given Bosentan. Arterial lesions was also present in other tissues in all drug-treated groups expect PD 166673 treated dogs. Arteriopathy may be a class effect of infusion of endothelin antagonists in dogs. Receptor selectivity, chemical structure and ET-1 concentrations were not predictive of the distribution and severity of arteriopathy.

1850 REPRODUCTIVE TOXICITY OF 1-BROMOPROPANE, A NEWLY INTRODUCED ALTERNATIVE TO OZONE-LAYER DEPLETING SOLVENTS, IN MALE RATS.

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1-Bromopropane has been newly introduced as a alternative to ozone-depleting solvents. We aimed to clarify its dose-dependent reproductive toxicity in male rats. Thirty-six Wistar male rats were randomly divided into four groups of nine. The groups were exposed to 200, 400, 800 ppm 1-bromopropane or only fresh air, eight hours per day for 12 weeks. Epididymal sperm indices were evaluated after 12 week's exposure. The testes, epididymides, seminal vesicle, prostate and other organs were weighed and examined histopathologically. Spermatogenic cells in the stage VII seminiferous tubules and degenerating spermatids in the stages IX-XI seminiferous tubules were counted. Plasma testosterone levels were measured by a radioimmunoassay. The testicular weight did not significantly change, but the weight of epididymides, seminal vesicle and prostate dose-dependently decreased. The weight of seminal vesicle decreased significantly at the lowest concentration of 200 ppm or over. 1-Bromopropane induced a dose-dependent decrease in epididymal sperm count and motility, and an increase in tailless sperm and sperm with an immature head shape. The spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and round spermatids at stage VII did not decrease significantly. Degenerating elongated spermatids at the postspermiation stages, stages IX-XI increased dose-dependently. Plasma testosterone level significantly decreased at 800 ppm. We revealed that 1-bromopropane impairs spermatogenesis by disturbing transformation process of spermatids in the testis. Its reproductive toxicity might be different from that of 2-bromopropane which specifically impairs spermatogonia. Thus this solvent may have serious reproductive toxic effects in men and should be used carefully in the workplace.

1851 1-BROMOPROPANE IS DOSE-DEPENDENTLY NEUROTOXIC TO RATS IN LONG-TERM INHALATION EXPOSURE.

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1-Bromopropane has been newly introduced as an alternative to ozone layer-depleting solvents. We aimed to clarify the dose-dependent effects of 1-bromopropane on the nervous system. Fourty-four Wistar male rats were randomly divided into four groups of eleven. The groups were exposed to 200 ppm, 400 ppm, 800 ppm 1-bromopropane or only fresh air eight hours per day for 12 weeks. Grip strength of forelimbs and hindlimbs, maximum motor nerve conduction velocity (MCV) and distal latency (DL) of the tail nerve were measured in nine rats of each group every four weeks. The other two rats of each group were perfused at the end of the experiment for morphological examinations. The rats of the 800-ppm group showed poor kicking and impossible standstill on the slope. Forelimb grip strength decreased significantly at 800 ppm and hindlimb grip strength decreased significantly at 400 ppm or over after 12 week's exposure. MCV and DL of the tail nerve deteriorated significantly at 800 ppm. Ovoid or bubble-like debris of myelin sheaths was prominent in the unraveled muscular branch of the posterior tibial nerve in the 800-ppm group. Swelling of preterminal axons in the gracile nucleus increased dose-dependently. Plasma CPK decreased dose-dependently with significant changes at 400 ppm and 800 ppm. 1-Bromopropane induced paralysis and deteriorated grip strength, MCV and DL dose-dependently with morphological changes in peripheral nerve and preterminal axon in the gracile nucleus. 1-Bromopropane may be so seriously neurotoxic to humans that it should be used carefully in the workplace.

1852 OVARIAN TOXICITY OF 1-BROMOPROPANE, AN ALTERNATIVE TO OZONE LAYER-DEPLETING SOLVENTS, IN RATS.

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This study was aimed at clarifying the effect of 1-bromopropane (1-BP), an alternative to ozone layer-depleting solvents, on female reproductive function in rats. Forty female Wistar rats were divided into four groups of ten. Each group was daily exposed to 0, 200, 400, or 800 ppm 1-BP for eight hours a day. After 7 weeks of exposure, all the rats in the 800 ppm group were debilitated, then they were decapitated during the 8th week. Other groups were exposed for 12 weeks. Vaginal smears were examined every day to monitor ovarian cyclicity during the experimental period. After 12 weeks of exposure, the rats were decapitated on the day of diestrus I during the 13th week. In the 800 ppm group, the body weight gain was significantly suppressed compared to the control, but body weight of the other two groups did not significantly differ from the control. Vaginal smear test showed that the number of irregular estrous cycles significantly increased dose-dependently. Both the weights of reproductive organs in the 200 ppm and 400 ppm groups showed no significant difference compared to the control. The present study indicates that relatively low concentration exposure to 1-BP affect reproductive organs in female rats. Ovarian cyclicity is probably a more sensitive index for monitoring the reproductive toxicity than the weight of reproductive organs in 1-BP intoxication.

1853 LACK OF PHOTOTOXICITY OF COSMETIC FORMULATIONS CONTAINING GLYCOLIC ACID IN AN *IN VITRO* HUMAN SKIN MODEL.

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Glycolic acid (GA) is a naturally occurring product found in sugar cane and is a member of a class of chemicals known as alpha-hydroxy acids. GA has been widely used in cosmetic and dermatological formulations for over a decade. These formulations are often used in dermal applications where they may be subject to ultraviolet radiation (UV) exposure from sunlight. We have investigated the potential for phototoxicity of typical cosmetic concentrations of GA (4% and 8%) in a generic cosmetic formulation with and without a

chemical sunscreen (octyl methoxycinnamate, SPF2). The assays were conducted following a cytotoxicity protocol using a human keratinocyte model, EpiDerm™ (Epi-200), utilizing a colorimetric thiazolyl blue (MTT) endpoint. Prior to formulation exposure, a maximum non-cytotoxic dose in EpiDerm was established for UVA/B radiation (290-400nm) utilizing a metal-halide lamp solar simulator. The maximal dose of UVA/B radiation that caused negligible cytotoxic effects was 1.35/ 0.135 J/cm² UVA/UVB, respectively. The maximal exposure for UVA treatment alone (320-400nm) was 6 J/cm². For subsequent assays, EpiDerm cultures were treated with the maximal non-cytotoxic dose of UVA/B or UVA. EpiDerm cultures were exposed to test materials or controls (negative control, vehicle; positive control, chlorpromazine) for two hours prior to UV exposure and returned to the incubator (0-45 hours) after exposure, followed by MTT cytotoxicity analysis. GA treated tissues exposed to UV showed relative survival percentages comparable to tissues that received no UV (89-109%); positive phototoxic control showed an expected cytotoxic response (7.5%). These studies indicate that glycolic acid contained in these cosmetic formulations is not phototoxic after UVA/B or UVA exposure and can be used in dermal cosmetic applications where UV radiation exposure may occur.

1854 MULTI-GENERATION REPRODUCTION STUDIES WITH GLYPHOSATE IN RATS.

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Glyphosate is a non-selective herbicide with activity on most annual and perennial plants. Glyphosate inhibits essential aromatic amino acid synthesis by blocking the activity of a plant specific enzyme. Glyphosate based-formulations are used in over 100 countries in virtually every phase of agricultural and residential weed control and are increasingly used in applications involving genetically modified plants tolerant to glyphosate. This expansive use necessitates a proactive product stewardship program ensuring a wide distribution of the health and safety information for glyphosate. To this end, the results from two multi-generation reproductive toxicity studies with glyphosate are presented. Glyphosate (98.7% pure) was fed to groups of CD rats (12 males and 24 females per dose group) for three successive generations at dose levels of 0, 3, 10, and 30 mg/kg/day. There were no treatment-related effects on mating, fertility or reproductive parameters. An equivocal increase in unilateral renal tubule dilation occurred at the top dose in the F3b pups, however, a more extensive evaluation in the subsequent multigeneration reproduction study conducted at much higher dose levels did not show the effect. This second dietary rat study (30 rats/sex/dose group) was conducted over two generations using 0, 2000, 10000 and 30000 ppm glyphosate (97.7% pure). Decreased body weight gains were seen at the highest dose in parental animals and in pups during the later part of lactation and there was an equivocal decrease in average litter size. No treatment related effects were observed at lower dose levels. The NOAELs for systemic and reproductive toxicity were 10000 ppm (740 mg/kg/day) and 30000 ppm (2268 mg/kg/day), respectively.

1855 A 2-MONTH TOXICITY STUDY OF PEN110-TREATED RED BLOOD CELLS FOLLOWING INTRAVENOUS ADMINISTRATION IN ALBINO RABBITS.

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PEN-110 (trade name INACTINE™) is a low MW electrophilic compound, which inactivates a wide range of viruses in whole blood and red blood cells (RBCs) through selective interaction and irreversible modification of nucleic acids. Following viral inactivation, PEN110 is removed to non-detectable levels (<0.03 μg/ml). The current study was conducted to evaluate the effects of infused PEN110-treated autologous RBCs in support of a Phase 1 clinical study. Blood was collected from New Zealand White Rabbits and 3 mL of a citrate phosphate dextrose solution was added to ~10 mL of isolated RBCs. After overnight-refrigerated storage, the resuspended RBCs were incubated for 6 h at room temperature in the presence of a sodium phosphate solution (pH 7.0) or a freshly prepared solution of PEN110 (12 mM). Subsequently, the RBCs were washed 4x with 0.2% dextrose/0.9% sodium chloride solution to partially remove PEN110. The supernatant was discarded and 3 mL of an RBC storage solution an Additive Solution Formula was added to the RBCs after which they were stored under refrigeration overnight. Treated RBCs containing residual PEN110 were administered via a lateral ear vein to achieve a dose of ~10 μg/kg PEN110 (i.e., >2,500 X the clinical Phase 1 dose). Animals were observed for 2 months. Clinical examinations were performed daily. Physical examinations were conducted weekly. Body weights were recorded weekly. Food consumption was determined daily. Hematology and serum chemistry parameters were analyzed prior to the initiation of dosing, 24 h following administration (day 1) and on days 3, 7, 29, and 59 (prior to necropsy). Ophthalmological examinations were performed prior to the initiation of dosing and during week 7. In addition, hematology parameters were evaluated on samples of treated RBCs collected prior to administration. A complete necropsy and a microscopic examination was conducted on all tissues from all animals. Treatment of isolated RBCs with ~12 mM PEN110 and subsequent administration of the treated RBCs in the presence of remaining PEN110 at a dose level of ~10 μg/kg to rabbits caused no test article-related effects over a 2-month monitoring period after two months.

1856 ASSESSMENT OF THE REPRODUCTIVE TOXICITY POTENTIAL OF GASOLINE VAPOR IN A TWO-GENERATION STUDY IN RATS.

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A 2-generation study was conducted on gasoline vapor to assess its reproductive toxicity potential. The test material was collected from vapor recovery units of a gasoline distribution terminal and was shown to be similar in composition to vapors to which humans are exposed through occupational or public contact. In the two-generation reprotoxicity study, groups of male and female Sprague-Dawley rats were exposed daily for 6 hours to gasoline vapor at concentrations up to 20000 mg/m³. This dose is approximately 50% of the lower explosive limit and the highest level considered safe to test. There were no treatment-related effects in parental animals, and no microscopic changes other than hyaline droplet nephropathy in the kidneys of male rats. There were no effects on fertility and reproductive parameters, and no fetal effects. Additionally, there was no evidence of endocrine modulating activity as indicated by a lack of effect on estrous cycling, sperm count and quality, quantification of primordial oocytes, and no changes in any specific landmarks related to sexual development in either generation. Thus, no toxicologically important findings were identified in this study, and the NOAEL for reproductive effects for gasoline vapor was 20000 mg/m³.

1857 TOXICITY DATA USE IN TEMPORARY EMERGENCY EXPOSURE LIMIT (TEEL) DERIVATION.

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Methodology for deriving Teels for chemicals without Emergency Response Planning Guidelines (ERPGs) uses published toxicity parameters (e.g., LC_{50}, LD_{LO}, where L=lethal, LO=low). Data precedence is human, rat, mouse, or other species, using Inhalation, oral (os), skin, intraperitoneal, intravenous, subcutaneous, or other routes. Current route adjustment factors (RAF) are independent of species and properties of chemicals. Non-inhalation data are transformed to concentration equivalent mass per unit volume units (mg/m³) using species-specific mean body weights (BW) and breathing rates (BR). The objective of this study was to test the adequacy of these adjustments. Toxicity data for 90 chemicals with ERPGs were extracted for six parameters for all animal species. Same species, different routes of intake (ssdr), and different species, same route of intake (dssr os) were analyzed for LD_{50} and LD_{LO} data. For LD_{50} and LD_{LO} ssdr data, ratios (R) were taken of the dose by route "i" to the oral dose for each chemical. The mean (MR_i), standard deviation (SD) and standard error (SE) were calculated for all R_is for each route of intake "i". There are some differences between revised RAFs ($RAF_{REV}=(1/MR_i)x0.25$, the oral RAF) from LD_{50} and LD_{LO} data, particularly for intravenous (0.41 and 1.96, RAF=1.00) and for subcutaneous (0.09 and 0.31, RAF=0.10) intake. No attempt was made to eliminate outliers from this data set. For LD_{50} and LD_{LO} dssr os data, ratios were taken of dose for species "j" to rat LD_{50}s or human LD_{LO}s. The mean (MR_j), SD and SE were calculated for all R_js. The BW/BR for rats (1.31) was divided by the LD_{50} MR_j. BW/BR for humans (3.50) was divided by LD_{LO} MR_j. These values are an indication of the adequacy of the species adjustments for this set of data. Although there are considerable scatter in the data, elimination of just 3 (of 37) mouse to rat LD_{50} ratios gives a BW/BR_{REV} of 0.76, close to the current value (0.714). The analyses need to be completed for other routes of intake and animal species before changes are made to the current TEEL methodology.

1858 SAFETY EVALUATION OF INTRAVENOUS GLYCINE.

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Solutions of the amino acid glycine are used for organ irrigation during abdominal and musculoskeletal surgeries, and significant systemic absorption of glycine during these procedures has been reported. In addition, glycine is used as an inactive ingredient in intravenous drug products and as a component of total parenteral nutrition amino acid injections. Post-surgical adverse events have included local and cerebral edema, visual effects (including blurring or loss of acuity) and immune-mediated symptoms. To more fully understand the potential risks, the safety of intravenous glycine has been evaluated. In clinical investigations conducted with healthy male volunteers, reversible visual effects (acuity and visual evoked potentials) were observed in one subject following a bolus intravenous dose of 4.4g glycine in 200ml over 5 minutes. Similar effects were also observed at doses of 15 to 22g, infused over a period of 20 minutes. The absence of a dose- or time-dependent relationship, and the mild, transient nature of the visual effects suggest that bolus intravenous doses of up to 22g of glycine are well-tolerated. Animal data for intravenous glycine administration support the reported effects of systemic glycine exposure in humans. Transient effects on visual parameters (diminished visual evoked potentials) were observed in dogs following single doses of 1g/kg, while single doses of glycine in sheep equivalent to 0.9g/kg produced reversible behavioural blindness and loss of pupillary response. Based on these data, it is concluded that serious adverse events are not likely to occur in response to a bolus intravenous dose of up to 22 g glycine (i.e., 0.44g/kg in a 50kg adult).

1859 NINETY-DAY ORAL GAVAGE TOXICITY STUDY OF C_9-C_{16} AROMATIC FRACTION OF JET-A IN FEMALE SPRAGUE-DAWLEY CD RATS AND MALE C57BL/6 MICE.

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This study was conducted to characterize the potential toxic effects of C_9-C_{16} aromatic fraction of Jet-A in female Sprague-Dawley CD rats and male C57BL/6 mice following daily oral administration for 90 days. Four groups of 15 female rats/group and 15 male mice/group were administered a daily oral gavage dose of the test substance at 0 (vehicle control), 20, 100, and 500 mg/kg in a corn oil vehicle. Mean hemoglobin, hematocrit, and red blood cell counts (HGB/HCT/RBC) were decreased in the mid- and high-dose female rats. Necropsy examinations revealed enlarged livers in the high-dose group rats. This correlated with the organ weight measurements indicating significant liver weight increases (absolute and relative to brain and body weight) in the high-dose rats compared with their controls. There were no pathological lesions observed or changes in serum chemistry parameters which were related to the test substance. Clinical observations included hunched posture in all groups of mice, with the frequency of the observation increasing with higher doses. Lethargy was recorded from all of the high-dose mice and half of the high-dose rats. Lethargy was also observed in 5/15 low-dose and 12/15 mid-dose mice. Salivation was observed in all of the high-dose rats and from 6/15 from the mid-dose rats. Rats in the mid- and high-dose groups were observed shoveling their contact bedding with their noses within minutes after being dosed. The shoveling and salivation observed in the rats is consistent with an irritation response in the mouth. There were no apparent effects of the test substance on the body weights during the study. Increased food consumption was observed in the rats that received test substance; with significant increases observed during most of the study in the high-dose group. Based on the results of this study a NOEL for the C_9-C_{16} aromatic fraction would be 20 mg/kg/day.

1860 SAFETY ASSESSMENT OF THE HUMAN FACTOR VIII RETROVIRAL VECTOR.

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A gene therapy product consisting of a retroviral vector expressing human Factor VIII (hFVIII(V)) has been developed for the treatment of Hemophilia A. To assess the acute toxicity of the retroviral vector particle, studies were conducted in rabbits and mice. Male rabbits (10/group) or mice (14/group) were injected intravenously with hFVIII(V) at doses of 0.67, 3.3 and 16.7 x 10^8 or 1.7, 6.7 and 26.7 x 10^8 TU/kg (transduction units), respectively, once daily on each of three consecutive days. Half of the animals in each group were sacrificed on day 4 and remaining animals on day 15. There was no mortality in mice treated with up to 26.7 x 10^8 TU/kg, or rabbits at doses of up to 3.3 x 10^8 TU/kg. At a dose of 16.7 x 10^8 TU/kg, 2/10 rabbits died of an anaphylactoid reaction following the second or third injection. Histopathologic evaluation indicated acute heart failure secondary to pulmonary artery constriction. In addition, there was a dose-dependent enhancement of pre-existing neutrophil infiltration to the walls of larger pulmonary vessels in rabbits treated with mid and high dose hFVIII(V). Resolution of these vascular changes was in progress by day 15. In contrast, no similar infiltration was reported in mice at doses as high as 26.7 x 10^8 TU/kg or in rabbits sacrificed 3 months to 2 years following treatment with hFVIII(V). The acute vascular effects observed in this study are similar to those reported in rabbits treated with adenoviral vectors. In mice there was a slight (~20%) increase in spleen weights in the high dose animals at both intervals, and a dose-dependent incidence of lymphocytic hyperplasia of generally minimal severity at day 15. There was no effect of hFVIII(V) in either species on body weight, food consumption, clinical pathology or macroscopic observations at necropsy. In summary, the acute reaction to hFVIII(V) gene therapy and the enhancement of pre-existing neutrophil infiltration in lung of rabbits may be a species-specific response to treatment with viral vectors. No similar findings were seen in mice.

1861 EVALUATION OF ANGIOZYME™, A RIBOZYME, IN 28 DAY TOXICITY STUDIES IN MONKEYS AND MICE.

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ANGIOZYME™ is an anti-angiogenic ribozyme (inhibiting the Flt-1 receptor mRNA for vascular endothelial growth factor (VEGF)), intended for treatment of cancer. Toxicity studies were conducted in CD-1 mice and cynomolgus monkeys. Monkeys were injected subcutaneously with doses of 2.5, 8.3, or 25 mg/kg/d and mice with 10, 33, or 100 mg/kg/d for 4 weeks followed by a 4-week recovery period. Doses in both species corresponded to 30, 100 or 300 mg/m^2/d, potential doses for clinical studies. Standard toxicity parameters were evaluated in both species and in addition ECG, BP and complement were examined in primates. In monkeys and mice sacrificed after 4 weeks, there was a dose-dependent accumulation of basophilic granules in renal tubular epithelial cells and local irritation/inflammation at the injection site accompanied by minimal to mild muscle fiber degeneration in monkeys only. In addition a minimal to mild hypertrophy of Kupffer cells was observed in monkeys treated with 8.3, or 25 mg/kg/d which correlated with a slight liver weight increase. No changes in serum chemistry parameters or urinalysis indicative of hepatic or renal dysfunction were seen. There were no other effects on any antemortem assessment including complement. In both species hepatic, renal and injection site effects recovered partially or completely. The hepatic and renal changes were consistent with changes reported with other oligonucleotides. With the exception of microscopic changes in kidney and liver and minimal local effects at the injection site, ANGIOZYME™ was well tolerated.

1862 CYTOTOXICITY TESTS FOR BIOCOMPATIBILITY ASSESSMENT OF MEDICAL DEVICES: A COMPARISON OF ISO 10993 AND MHW JAPAN METHODOLOGIES.

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Cytotoxicity tests are initial screening methods prior to more specific tests for assessing biocompatibility of medical devices. Cytotoxicity testing methodologies differ between the US Pharmacopoeia (USP), the International Standards Organization (ISO), and the Japanese Guidelines for Basic Biological Tests for Medical Devices and Materials-Notification 99 (MHW-Japan). We compared the sensitivities of the USP/ISO test (48 hour exposure) and MHW-Japan procedure (7 day exposure) using cell culture medium extracts (37°C/24 hours) of materials (Silicone, Polypropylene, Latex) used in medical devices and positive controls (Zinc-diethyldithiocarbamate/ZDEC, and Zinc-dibutyldithiocarbamate/ZDBC) utilizing standard evaluation criteria. We also evaluated protein content of the cell cultures as an additional index of cytotoxicity. Good correlation was observed in the toxicity assessments by visual quantitation and protein content in the V79 cell line (MHW-Japan) and in the L929 (USP/ISO) method. However the neat extract of sili-

cone was cytotoxic in the V79 (MHW-Japan) colony formation assay, while it was non-cytotoxic in the L929 (USP/ISO) assay, by both visual grading/colony counting and protein content. In addition, toxicity by Latex, ZDEC and ZDBC extracts was observed at a 2-fold lower concentration in the V79 assay as compared to the L929 test. These results suggest that the V79/MHW-Japan procedure may be more sensitive than the L929 USP/ISO assay, due to differences in test methodologies although the physiological relevance of these differences is uncertain. Current efforts at harmonization of international guidelines for pre-clinical evaluation of medical devices/materials should take into consideration such variations in test methods and their physiological relevance, since the choice of cytotoxicity methods used can influence the outcomes of submissions of test devices for regulatory approval.

1863 A DERMAL SAFETY EVALUATION OF EXTRACTS FROM TAGETES PLANTS USED IN FRAGRANCES.

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Extracts can be obtained from various species of Tagetes, particularly Tagetes minuta and Tagetes patula. Tagetes oil is obtained by steam distillation and Tagetes patula absolute and Tagetes minuta absolute are obtained by solvent extraction. Irritation, phototoxicity, sensitization and photosensitization were evaluated. None of the 3 materials produced irritation in hairless mice when applied undiluted. However, irritation was observed with undiluted Tagetes oil when applied via semi-occlusion on the clipped dorsal skin of 4 rabbits. Slight irritation was observed in guinea pigs when tested with a 3% sample of Tagetes patula absolute. Tagetes minuta absolute was slightly irritating in guinea pigs but not irritating in humans when applied under occluded or semi-occluded patches for 24 hours at dose levels ranging from 0.01% to 2%. All 3 materials produced phototoxicity in hairless mice at dose levels of 0.1 to 100%. A NOAEL for the oil was observed at 0.01%. Tagetes minuta absolute was also tested in guinea pigs and humans and produced phototoxic responses in both. In humans, a NOAEL was observed at 0.1% after 24 hour occluded applications followed by irradiation with UVA at doses of 16-20 J/cm. Tagetes minuta absolute produced no sensitization in 22 human volunteers (5 induction applications followed 2 weeks later by challenge) or in 25 human volunteers after six 24 hour occluded induction applications followed 2 weeks later by a 24 hour occluded challenge. No photosensitization was observed when these same 25 volunteers were evaluated for photosensitization using the same protocol as for sensitization but with UVB irradiation after each induction application and UVA irradiation after the challenge application.

1864 90-DAY ORAL TOXICITY STUDY ON *n*-NONANE IN FEMALE FISCHER 344 RATS AND MALE C57BL/6 MICE.

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Long chain petroleum hydrocarbons, ranging from C9 to C15 are pre-dominant constituents in weathered jet-fuel spills. To evaluate potential toxic effects of a C9 hydrocarbon following repeated oral gavage, *n*-nonane (neat) was administered to groups of 10 female Fischer 344 rats and 10 male C57BL/6 mice at daily doses of 5.0, 1.0, 0.1 and 0 (control) g/kg, 7 d/wk for 90 d. Clinical observations included urogenital wetness, perianal alopecia, diarrhea, and hunched posture at 5.0 g/kg in both species. Mean body weights were similar between control and treated groups. Significant differences in hematology and serum chemistry values were observed; however, values were within normal species limits. Rats had increased liver and adrenal weights and decreased spleen and ovary weights at 5.0 g/kg. Increased adrenal and decreased ovary weights were observed also at 1.0 g/kg. In mice, an increase in liver weight and a decrease in kidney weight were observed in the 1.0 and 5.0 g/kg groups. Individual rat blood levels of *n*-nonane, collected 2 hrs post dosing at 90 d, ranged from 0.44 (0.1 g/kg) to 9.53 μg/g (5.0 g/kg). Microscopic lesions consisted of varying degrees of hyperplasia and hyperkeratosis of squamous epithelium in the non-glandular stomach in all nonane-treated groups of both species. Mild inflammation of the proximal small intestinal mucosa was present in 20% of the high dose rats only. Mild perianal squamous hyperplasia was observed in high and mid dose rats and mice. The NOAEL for this study is 0.1g/kg in both species, for all biological endpoints except the lesions in the non-glandular forestomach. The lack of an analogous structure in the human stomach, and the absence of lesions in the glandular stomach of the study animals suggests that the proliferative forestomach lesions represent a species-specific response, of no clinical significance to humans.

1865 PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES IN CYNOMOLGUS MONKEYS USING A TELEMETRY SYSTEM (IV), AND THE VALUE OF USING CONSCIOUS MONKEYS IN SAFETY PHARMACOLOGY STUDIES.

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Safety pharmacology studies, as presently defined in ICHM3, require the evaluation of effects on vital functions such as the central nervous, cardiovascular and respiratory systems. We studied the effects of two well characterized drugs (Amiodarone and Acetazolamid) on the vital functions of conscious monkeys, in order to evaluate the potential of the telemetry system as a safety assessment bridging test between pre-clinical and clinical studies. Furthermore, circadian rhythms of normotensive and hypertensive monkeys were also investigated. Male cynomolgus monkeys (4-5 years old) were used for this study. The animals were anesthetized using ketamine anesthesia prior to the surgical implantation of a telemetry system transmitter. After surgery the animals were placed in a measuring cage mounted on a receiver board and allowed several weeks to recover prior to use in the study. All parameters such as blood pressure, heart rate, ECG, respiratory rate and body temperature were continually recorded utlizing the telemetry system. Amiodarone at 40 mg/kg/day for 4 days produced decreases in blood pressure by 20-25% and nocturnal heart rate by 10%, and also showed a tendency to prolong the QT interval. Amiodarone is known to induce QT interval prolongation, resulting in ventricular arrhythmia. Acetazolamid, administered once at 300 mg/kg, produced increases in the heart rate and respiratory rate with changes in blood gas parameters (HCO_3^-, BE). Acetazolamid is known to induce a metabolic acidosis. In order to examine the effects of hypertension on circadian rhythm in cynomolgus monkeys, renal hypertension was produced using the 2 kidneys 1 clip method. In the acute stage of renal hypertension, the circadian rhythms of blood pressure and heart rate disappeared. In the chronic stage, circadian rhythms were observed, however, hypertension was maintained. The above results clearly demonstrate the potential of telemetry system usage in safety pharmacology studies.

1866 EMBRYOTOXIC AND TERATOGENIC EVALUATION OF TNK-TPA IN RABBITS; A STUDY AROUND ANTIGENICITY ISSUES.

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The purpose of this study was to assess the effects of TNK-tPA in presumed pregnant rabbits and evaluate the development of the embryo and fetus following exposure of the doe from implantation to closure of the hard palate. To ensure exposure to TNK-tPA without interference of neutralizing anti- TNK-tPA antibodies, daily intravenous (IV) dosing for no more than 5 consecutive days during gestation days (GDs) 6–18 were administered. Cesarean-sectioning (C-sections) were performed on all surviving does on GD 29. The following parameters were evaluated in this study: antemortem observations and mortality, maternal body weights, maternal body weight change, food consumption, abortions, C-sections and litter observations, and fetal alterations (including fetal body weights, fetal anomalies and fetal ossification). Based on the results of this study, it was concluded that bolus IV administration of TNK-tPA at doses up to and including 5 mg/kg/day did not elicit maternal toxicity or developmental toxicity, including teratogenicity, when administered daily on gestation GDs 6 through 10. TNK-tPA Vehicle did not elicit maternal toxicity or developmental toxicity, including teratogenicity, when administered daily on GDs 6 through 10, 11 through 14, or 15 through 18. Daily bolus intravenous administration of TNK-tPA at doses greater than or equal to 0.5 mg/kg/day produced maternal and fetal toxicity when administered on GDs 11 though 14 or 15 through 18 in rabbits.

1867 AN ANIMAL MODEL FOR IDIOSYNCRATIC REACTIONS TO CHLORPROMAZINE.

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Neuroleptic malignant syndrome (NMS) is an idiosyncratic reaction to therapy with neuroleptic drugs such as chlorpromazine (CPZ). Rhabdomyolysis is associated with the development of NMS. Mortality from rhabdomyolysis is

approximately 50%, yet animal models for this drug-induced reaction are lacking. Risk factors for its appearance include conditions associated with inflammation, such as trauma and bacterial sepsis. Accordingly, we tested the hypothesis that an inflammatory stimulus, bacterial lipopolysaccharide (LPS), renders animals susceptible to rhabdomyolysis from CPZ. Male, Sprague-Dawley rats (150-200 grams) were fasted for 24 hours. A small dose of E. coli LPS (7.4 x106 EU/kg) or its vehicle (saline) was administered by tail vein two hours before an intraperitoneal injection of CPZ (70mg/kg) or its vehicle (saline). Serum enzymes indicative of striated muscle necrosis were measured. Neither creatine kinase (CK) nor aspartate aminotransferase (AST) were elevated in serum of rats treated with vehicle, LPS, or CPZ alone, but these were markedly increased in animals treated with LPS and CPZ. All animals receiving CPZ quickly became cataleptic and remained so for the duration of the experiment. The observation that serum enzymes were elevated only in the group given LPS plus CPZ suggests that LPS enhances striated muscle necrosis in the presence of the neuroleptic drug. These results may form the basis for an animal model with which to study drug-induced NMS and raise the possibility that concurrent, modest inflammation may underlie the susceptibility of individuals to this "idiosyncratic" reaction. (Supported by NIH grants ESO4139 and ESO8789.)

1868 TWO-YEAR TOXICITY/ONCOGENICITY STUDY OF α-DIFLUOROMETHYLORNITHINE IN $B_6C_3F_1$ MICE.

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α-Difluoromethylornithine (DFMO) is a suicide inhibitor of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis. DFMO demonstrates a broad range of chemopreventive activity in animals, and is being evaluated for chemopreventive efficacy in human trials. This study was designed to evaluate the chronic toxicity and potential oncogenicity of DFMO in mice. Beginning at 6 weeks of age, $B_6C_3F_1$ mice (50 Main Study mice + 35 Satellite mice/sex/group) were received DFMO at 0 (control), 100, 300, or 1000 mg/kg diet for two years. Plasma levels of DFMO were determined during months 3 and 18, and clinical pathology evaluations were performed during months 6, 12, and 24. Gross lesions from all Main Study mice, and all tissues from all Main Study mice in the high dose and control groups were evaluated histopathologically. DFMO had no effect on animal survival, and induced no gross toxicity identifiable by clinical observations. Small but statistically significant reductions in body weight gain were seen between weeks 25 and 85 in males at all DFMO doses; this effect was not seen in females. DFMO had no effect on clinical pathology, and induced no gross pathology or microscopic pathology. Within a sex, patterns of neoplastic lesions were similar in all groups. Aside from the transient reduction in body weight gain seen in male mice, chronic administration of DFMO at levels of up to 1000 mg/kg diet induced no evidence of toxicity. When administered at doses of up to 1000 mg/kg diet, DFMO is not oncogenic in $B_6C_3F_1$ mice. (NCI-N01-CN-25506-01.)

1869 DERMAL ONCOGENICITY STUDY OF BENZOYL PEROXIDE CARBOPOL GEL IN FISCHER 344 RATS.

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The oncogenic potential of benzoyl peroxide (BPO) was evaluated in male and female F344 rats by topical application in a carbopol gel vehicle. BPO gel was applied at doses of 5, 15 and 45 mg BPO/rat once daily for 104 weeks to a 3.5-x-5-cm treatment area on the dorsal skin. A discontinuous-treatment group received the high dose (45 mg of BPO/day) for 52 weeks and the vehicle for the remainder of the study. Vehicle-only and no-treatment groups served as controls. Rats were sacrificed at 52 (interim-sacrifice) or 104 weeks, and necropsies were performed. Treatment with BPO carbopol gel had no effect on survival, body weights, food consumption or gross pathology. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were mild-to-moderate degrees of hyperkeratosis, acanthosis, sebaceous gland hyperplasia and chronic subepidermal inflammation in all treatment groups. These effects were observed in the interim-sacrifice groups and in the rats sacrificed at 104 weeks. In the discontinuous-treatment group, hyperkeratosis was present approximately twice as frequently as in the controls, which suggests a partial but incomplete recovery. No findings indicative of oncogenicity resulted from daily topical application of BPO gel at doses up to 45 mg of BPO/day for 104 weeks. The absence of any skin tumors is particularly important considering the presence of chronic hyperplasia and other skin effects indicative of reaching the maximum tolerated dose in this study. These data together with chronic studies in mice support the conclusion that BPO lacks carcinogenic potential.

1870 DERMAL ONCOGENICITY STUDY OF BENZOYL PEROXIDE CARBOPOL GEL IN B6C3F1 MICE.

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The oncogenic potential of benzoyl peroxide (BPO) was evaluated in male and female B6C3F1 mice by topical application in a carbopol gel vehicle for 104 weeks. BPO gel was applied at doses of 1, 5 and 25 mg of BPO/mouse once daily to a 2-x-3-cm treatment area on the dorsal skin. A discontinuous-treatment group received 25 mg of BPO/day for 52 weeks and the carbopol gel vehicle for the remainder of the study. Vehicle-only and no-treatment groups served as controls. Mice were sacrificed at 52 (interim-sacrifice) or 104 weeks, and necropsies were performed. The high dose (25 mg of BPO/day) exceeded the maximum tolerated dose (MTD), in that it caused treatment-site ulceration, and was lowered to 15 mg of BPO/day at week 57. Because ulceration also occurred in mice receiving 15 mg/day, BPO treatment of the high-dose mice was suspended and vehicle was administered from week 93 until the end of the study. BPO had no effect on survival, body weights, food consumption or gross pathology, except for treatment-site ulceration in the high-dose group. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were dose-dependent induction of acanthosis, hyperkeratosis, sebaceous gland hyperplasia and subepidermal inflammation was evident at the interim sacrifice as well as at the terminal sacrifice in groups continuously treated with BPO. Ulceration, observed grossly, was confirmed microscopically. No findings indicative of oncogenicity resulted from topical application of BPO gel in any of the treatment groups. Importantly, 5 mg of BPO, administered in carbopol gel daily for 104 weeks, produced skin effects consistent with reaching but not exceeding the MTD. These data further support the conclusion that BPO lacks carcinogenic potential.

1871 TOXICOLOGICAL CHARACTERIZATION OF A NOVEL CIGARETTE PAPER.

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Through the use of novel papermaking technology a cigarette paper has been developed that possesses evenly spaced bands. These bands are comprised of paper with a higher basis weight than the paper in between the bands. Cigarettes made with this new paper are less likely to ignite certain test fabrics used in several laboratory testing methods. The toxicological evaluation of this change in cigarette paper design was determined under standard smoking conditions (35cc puffs of 2 seconds duration taken every minute). The effect of this paper on the delivery of up to ~50 analytes/parameters in cigarette smoke was investigated. The mutagenic activity of cigarette smoke condensate was evaluated in the Salmonella typhimurium reverse mutation assay using strains TA98, TA100, TA102, TA1535 and TA1537 with and without S9. The neutral red uptake assay with BALB/c 3T3 cells was used to assess the cytotoxicity of the particulate and gas/vapor phases of smoke. Changes in the respiratory tract of male Sprague-Dawley rats were assessed following 90 days exposure to smoke. The novel paper caused only minor changes in chemical smoke composition (the largest changes were a 20% decrease in phenol and a 27% increase in cadmium) and no changes in *in vitro* or *in vivo* biological activity.

1872 TOXICOLOGY OF BENZOFLEX®9-88 PLASTICIZER.

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Benzoflex®9-88 plasticizer (dipropyleneglycol dibenzoate) was evaluated in numerous safety studies. The acute toxicity ranged between 2 and <6.5 g/kg in rats. Other short term tests including skin sensitization were unremarkable. A battery of *in vitro* genetic toxicity tests including assays for gene mutations in bacteria (Ames *Salmonella* strains & *E. Coli*) and mammalian (mouse lymphoma) cells as well as for chromosomal aberrations in mammalian (Chinese hamster lung) cells showed no evidence of mutagenic or clastogenic activity. In a study to evaluate estrogenic activity, adult ovariectomized rats were dosed at 0, 500, 1000, 1500 and 2000 mg/kg/day for 7 days via oral gavage. No estrogenic activity was noted, using vaginal cornification and uterotrophic response as endpoints, at doses up to and including the maximally tolerated dose. A 13-week subchronic dietary study was conducted in male and female rats at doses of 0, 250, 1000, 1750 and 2500 mg/kg/day. Toxicity was observed only at the two highest doses. This included a decrease in bodyweight, increased plasma enzyme activity associated with slight liver cell hypertrophy, a moderate increase in the normal background hemosiderosis in the spleen, and minimal epithelial hyperplasia in the caecum only at 2500 mg/kg/day. Urinary pH was decreased in a dose-related manner consistent with a previous ADME study. All treatment related effects were reversible or showed a tendency to reverse in the four-week recovery period. The non-toxicologically significant NOEL was judged to be 1000 mg/kg/day. Finally, studies to date suggest a low order of mammalian toxicity for this plasticizer.

1873 TOXICOLOGY OF BENZOFLEX®2-45 PLASTICIZER.

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Benzoflex®2-45 plasticizer (diethyleneglycol dibenzoate) was evaluated in numerous safety studies. The acute toxicity ranged between 2 and 5 g/kg in rats. Other short-term tests including skin sensitization were unremarkable. A battery of *in vitro* genetic toxicity tests including assays for gene mutations in bacteria (Ames *Salmonella* strains & *E. Coli*) and mammalian (mouse lymphoma) cells as well as for chromosomal aberrations in mammalian (Chinese hamster lung) cells showed no evidence of mutagenic or clastogenic activity. In a study to evaluate estrogenic activity, adult ovariectomized rats were dosed at 0, 500, 1000, 1500 and 2000 mg/kg/day for 7 days via oral gavage. No estrogenic activity was noted, using vaginal cornification and uterotrophic response as endpoints, at doses up to and including the maximally tolerated dose. A 13-week subchronic dietary study was conducted in male and female rats at doses of 0, 250, 1000, 1750 and 2500 mg/kg/day. Toxicity was observed only at the two highest doses. This included decreases in body weight, slight liver cell hypertrophy and a moderate increase in the normal background hemosiderosis in the spleen. In addition, at 2500 mg/kg/day only, minimal epithelial hyperplasia was seen in the caecum and there were a few clinical signs and a single mortality resulting from the stress associated with treatment at this very high dosage. Urinary pH was decreased in a dose-related manner consistent with a subsequent ADME study. All treatment related effects were reversible or showed a tendency to reverse in the four-week recovery period. The non-toxicologically significant NOEL was judged to be 1000 mg/kg/day. Finally, studies to date suggest a low order of mammalian toxicity for this plasticizer.

1874 TOXICOLOGY OF BENZOFLEX®S-358 PLASTICIZER.

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Benzoflex®S-358 plasticizer (triethyleneglycol dibenzoate) was evaluated in numerous safety studies. The acute toxicity ranged between 2 and 6.5 g/kg in rats. Other short term acute toxicity tests including skin sensitization were unremarkable. A battery of *in vitro* genetic toxicity tests including assays for gene mutations in bacteria (Ames *Salmonella* strains & *E. coli*) and mammalian (mouse lymphoma) cells as well as for chromosomal aberrations in mammalian (Chinese hamster lung) cells showed no evidence of mutagenic or clastogenic activity. In a study to evaluate estrogenic activity, adult ovariectomized rats were dosed at 0, 250, 700, 1400, 2100 and 2800 mg/kg/day for 7 days via oral gavage. No estrogenic activity was noted, using vaginal cornification and uterotrophic response as endpoints, at doses up to and including the maximally tolerated dose. A 13-week subchronic dietary study was conducted in male and female rats at doses of 0, 400, 1000, 1600 and 2200 mg/kg/day. Toxicity was observed only at the two highest doses. This included a decrease in body weight, slight liver cell hypertrophy, a moderate increase in the normal background hemosiderosis in the spleen. Urinary pH was decreased as with other analogous chemicals. All treatment related effects were dose-related, reversible or showed a tendency to reverse in the four-week recovery period. The NOEL was judged to be 1000 mg/kg/day. Finally, studies to date suggest a low order of mammalian toxicity for this plasticizer.

1875 LY3353381, A SELECTIVE ESTROGEN RECEPTOR MODULATOR (SERM): ^{32}P-POSTLABELING ANALYSIS OF IN VIVO DNA ADDUCTS IN F-344 RAT LIVERS.

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LY353381 is a SERM, acts as a potent estrogen antagonist in uterine and mammary tissue while acting as an estrogen agonist to maintain bone density and lower serum cholesterol. Groups of female and male Fischer 344 rats were administered 20, 60, and 140 mg/kg LY353381 hydrochloride by oral gavage once a day for 3 days. The positive control group of rats received 45 mg/kg tamoxifen. At 2-3 hours after the last dose, the rats were euthanized and livers collected. Nuclear DNA isolated from livers of female and male rats of vehicle control, high dose, and positive control groups were analyzed by the ^{32}P-postlabeling assay using both butanol extraction and nuclease P1 digestion for enrichment of adducts. The low- and mid-dose groups were analyzed by the nuclease P1-enhanced postlabeling assay alone. Autoradiograms or scanned images of thin layer chromatograms obtained for LY353381-treated groups at the three dose levels were similar to those obtained for the corresponding vehicle control group. There were no extra spots indicative of adducts on the chromatograms of DNA from LY353381-treated animals. Determination of radioactivity associated with the background spots showed no statistically significant increase in background adduct levels in DNA samples of LY353381-treated animals compared with the vehicle control DNA samples. The positive control, tamoxifen, produced detectable levels of DNA adducts in the livers of both male and female rats. The results indicate that LY353381 does not produce detectable levels of DNA adducts at dose levels of 20, 60 and 140 mg/kg after oral administration for 3 days. The high dose is approximately 70% of a maximum tolerated dose (MTD).

1876 SAFETY AND PHARMACOLOGICAL EFFECTS OF LOCALLY ADMINISTERED CCI-1004 AFTER BALLOON ANGIOPLASTY IN RABBITS.

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CCI-1004, an antithrombotic that forms covalent bonds with vascular proteins, can potentially be applied to various situations where thrombotic complications arise, including the prevention of post-surgical thrombosis following angioplastic surgery. The safety profile of CCI-1004 was therefore investigated in a model of local delivery in rabbits to simulate the therapeutic setting for this agent. CCI-1004 (12.5, 50.0, or 312.5 nmole/rabbit) or vehicle was administered once by direct vascular injection to an isolated segment of the carotid artery, which was injured by balloon angioplasty prior to dosing or left intact. After a 3-min incubation, the blood flow through the carotid artery segment was re-established and the test article systemically released. Group mean values of APTT showed no statistically significant differences between control or treated animals, although elevated values (26.3-32.8 sec) were observed in 3 high dose rabbits. This observation was consistent with the mechanism of action of CCI-1004, and measurements in these animals decreased within 24 hours to levels comparable to those in controls. No gross or microscopic findings were attributed to CCI-1004 administration. It was concluded that CCI-1004 is well tolerated in rabbits following single intra-arterial local incubation with systemic release at doses of 12.5, 50.0, and 312.5 nmoles.

1877 CHARACTERIZATION OF ATYPICAL HISTOPATHOLOGY NOTED IN A 4-WEEK INTRAVENOUS INFUSION STUDY IN RATS.

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Identical validation studies were conducted at our Vienna, VA and Madison, WI laboratories, to determine the feasibility of using vendor-supplied precannulated rats, to compare the use and manageability of Fisher-344 and CD® rats, to evaluate the jugular and femoral routes of intravenous infusion, and to assess survival when a known vascular irritant is infused. The rats were precannulated by the supplier (Charles River Laboratories) using their custom-made polyurethane (Microrenathane®) catheter, which included silicone retention beads adhered with a cyanoacrylate adhesive. Eighty animals (10/sex/strain/route, for a total of 4 groups) were assigned to each study. A standard battery of in-life evaluations were performed, to include mortality, clinical signs, body weights, food consumption, and clinical pathology. After completion of the treatment period, all animals were necropsied, select organ weights were recorded, and the heart, kidneys, liver, lungs,lymph nodes, spleen, thymus, catheterization sites, and infusion sites were preserved. Tissues were embedded in paraffin, sectioned at approximate 5μ, stained with hematoxylin and eosin, and examined microscopically. Neutrophil counts were considerably higher in the jugular-Solutol males at Day 30, consistent with the higher incidence of infusion/catheter site abscesses these rats. Mean total cholesterol values were significantly lower in both strains of rats exposed to Solutol, regardless of route of administration. Microscopically, reactions to the presence of the catheter were variable and were within a fairly defined spectrum of changes,typified by the developed of a fibrous capsule and an accompanying chronic inflammatory response. Foreign body reactions to suture material were typically granulomatous, often with foreign body giant cells and mineralization. A clear,spicule-shaped material was seen frequently within or adjacent to the lumen of small arterioles in the lungs of many rats, characteristically eliciting a substantial inflammatory reaction and development of multiple microgranulomas. Thematerial appeared to originate from the silicone retention bead at the catheterization site, where a mass of similar appearance was found. Lesions of this kind may pose serious problems in safety assessment studies, particularly where the lung is a target organ. Based on these findings, it is suggested that one use caution when usingcustom-made implanatable materials for chronic infusion studies in rats.

1878 ASCORBIC ACID-STIMULATED DEPOSITION OF COLLAGEN IV IS ASSOCIATED WITH REPAIR OF RENAL CELL FUNCTIONS FOLLOWING SUBLETHAL INJURY.

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Pharmacological concentrations of L-ascorbic acid phosphate (AscP), a stimulator of collagen synthesis, have been shown to promote proliferation and repair of physiological functions in renal proximal tubular cells (RPTC) sublethally injured by S-1,2-dichlorovinyl-L-cysteine (DCVC). We hypothesized that AscP promotes this proliferation and repair by stimulating collagen synthesis and deposition. Primary cultures of rabbit RPTC were grown to confluence in the presence of physiological (50 μM) or pharmacological (500 μM) concentrations of AscP and exposed to 200 μM DCVC to produce ~50% cell death and loss 24 hrs after exposure. Proliferation, mitochondrial function (oxygen consumption, QO_2), and collagen IV synthesis and deposition were determined over the following six days. On day 1 after DCVC exposure, total monolayer protein content and QO_2 were ~50% of controls in cells grown in both 50 and 500 μM AscP. On day 6 after injury, protein content in cells grown in 500 μM AscP was significantly higher (89±9% of control) than the 50 μM AscP group (69±9% of control). On day 6, QO_2 in the 50 μM AscP group was still inhibited (64±7% of control) whereas QO_2 in the 500 μM AscP group was equal to control. Collagen IV synthesis and deposition was determined by immunoprecipitation and autoradiography of ^{35}S-methionine-labeled collagen IV. Autoradiography showed that DCVC exposure inhibited deposition, but not synthesis, of collagen IV in cells grown in 50 μM AscP at days 1, 4, and 6 post-injury. In contrast, synthesis and deposition of collagen IV by DCVC-injured cells grown in 500 μM AscP was equal to that of control on days 1, 4, and 6 post-injury. These results demonstrate the novel findings that 1) DCVC inhibits deposition, but not synthesis, of collagen IV and 2) pharmacological concentrations of AscP protect against the inhibition of collagen IV deposition in DCVC-injured RPTC. This study shows that repair of physiological functions in RPTC injured by DCVC is associated with AscP-stimulated deposition of collagen IV and suggests that collagen IV plays a role in RPTC regeneration and repair following toxicant injury.

1879 LINOLEIC ACID METABOLITES INDUCE RENAL CELL DEATH AT PATHOLOGICALLY RELEVANT CONCENTRATIONS.

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Linoleic acid is metabolized *in vivo* to a variety of important products. The epoxides of linoleic acid can be formed by specific cytochrome P450 isoforms and have been associated with increased mortality of severely burned patients. Recent *in vitro* studies using methyl ester derivatives suggested a soluble epoxide hydrolase (sEH) dependent pathway for linoleic acid epoxide mediated toxicity and have shown the diols of linoleic acid to be the toxic intermediate. In the present study, we tested the hypothesis that the free acid metabolites of linoleic acid would have a similar mechanism of toxicity in rabbit renal proximal tubular cells (RPT). Linoleic acid, cis-9,10-epoxyoctadecenoic acid (9,10-EOA), cis-12,13-epoxyoctadecenoic acid (12,13-EOA), 12,13-dihydroxyoctadecenoic acid, and 9,10-dihydroxyoctadecenoic acid produced concentration- and time-dependent cell death (LDH release) at pathologically relevant concentrations (100-500 μM). 12,13-EOA (500 μM) was the most toxic metabolite of linoleic acid and induced approximately 35% LDH release after a 30 min exposure. All other metabolites showed no difference from diluent controls at 30 min (10% LDH release). However, the diols and epoxides were equally toxic at 3 hr (50% LDH release). Glycine (2 mM), deferoxamine (500 μM), N,N'-diphenyl-1,4-phenylene-diamine (2 μM), and nifedipine (100 μM) did not block linoleic acid, 9,10-EOA and 12,13-EOA mediated LDH release. Metabolism studies demonstrated that less than 1% of ^{14}C-linoleic acid was metabolized to the epoxide, diol, and HODE metabolites after 1 hr. Minimal metabolism of linoleic acid prior to cell death suggests that toxicity is due to the parent compound. These results show that linoleic acid and the free acid epoxides and diols of linoleic acid are equally toxic in RPT. Also, the mechanism of toxicity is different than the methyl ester derivatives and is not mediated through a sEH dependent pathway or oxidative stress.

1880 THE ROLE OF HEME OXYGENASE IN OCHRATOXIN A MEDIATED NEPHROTOXICITY.

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Ochratoxin A (OTA) is a mycotoxin which has been shown to have nephrotoxic and nephrocarcinogenic potential. A proposed mechanism for OTA-toxicity is induction of oxidative stress. Heme oxygenase (HO) is a stress protein and its induction has been proposed to protect against oxidative stress caused by heme and hemoprotein. Increased HO expression has been shown in renal cell carcinoma. In the current study, renal cells in culture (primary rat proximal tubular cells and LLC-PK1 cell line) were used to investigate the role of HO in OTA-mediated toxicity. A specific inhibitor (zinc-protoporphyrin: ZnPP) and an inducer (hemin) of HO activity were used to manipulate HO activity in cell cultures exposed to different concentrations of OTA. In the presence of hemin, no difference in the production of reactive oxygen species (ROS) by OTA was observed, while ZnPP appeared to decrease both basal as well as OTA-induced ROS production. The presence of hemin did not alter the cytotoxic effect of OTA in these cells, while ZnPP appeared to exacerbate OTA-induced cytotoxicity. Western blot analysis showed a clear increase in HO expression in primary PTC after incubation with hemin (almost 2-fold) and a considerable decrease after incubation with ZnPP (about 25 % of control HO expression). Incubation for 2 h with OTA (50 μM) slightly decreased HO expression (79 % of control). These preliminary data suggest that induction of HO expression by hemin did not protect these cells against OTA-induced oxidative stress and cytotoxicity. Furthermore, it may be proposed that OTA decreases HO expression and thereby facilitates development of oxidative damage.

1881 FUMONISIN B_1 IS HEPATOTOXIC AND NEPHROTOXIC IN MILK FED CALVES.

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Fumonisins are a group of mycotoxins that alter sphingolipid biosynthesis and

induce leukoencephalomalacia in horses and pulmonary edema in pigs. Experimental administration of fumonisin induces acute hepatotoxicity in all species, including cattle, and nephrotoxicity in rats, rabbits, sheep, and goats. We further investigated the toxicity of fumonisin to calves. Ten milk-fed male Holstein calves aged 7 to 14 days were instrumented to obtain blood and urine. Treated calves (n = 5) were given fumonisin B_1 at 1 mg/kg, IV, daily and controls (n = 5) 10 ml 0.9% NaCl, IV, daily. All calves were euthanized on day 7. Fumonisin treated calves were lethargic and had a decreased appetite from day 4 to 7, and had serum biochemical evidence of severe liver and bile duct injury (increased serum sorbitol dehydrogenase, aspartate aminotransferase, gamma-glutamyl transferase [GGT], and alkaline phosphatase activities), and impaired hepatic function (increased serum bile acid and total bilirubin concentrations). Fumonisin treated calves also had biochemical evidence of renal injury (increased serum creatinine concentration) that functionally involved the proximal convoluted tubules (increased urine protein and GGT concentration, increased fractional clearance of potassium and phosphorus, increased urine GGT to creatinine ratio). Sphinganine and sphingosine concentrations in the liver, kidney, lung, heart, and skeletal muscle were increased in fumonisin treated calves. Sphinganine, but not sphingosine, concentration was increased in the brain of treated calves. In fumonisin treated calves, hepatic lesions were characterized by disorganized hepatic cords, variable severity of hepatocyte apoptosis, increased hepatocyte proliferation, and proliferation of bile ductular cells. Renal lesions in fumonisin treated calves consisted of apoptosis, karyomegaly, and increased cell proliferation of proximal renal tubular cells, as well as dilation of proximal renal tubules which contained cellular debris and protein. This is the first report of fumonisin induced renal injury and organ sphingolipid alterations in cattle.

1882 URINARY ANTIGENS AS MARKERS OF PAPILLARY TOXICITY.

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Renal papillary toxicity of xenobiotics is generally determined by inspection of necropsy specimens obtained from treated laboratory animals. This procedure is invasive and provides information on the state of the tissue at the day the organ is taken for inspection. It does not allow a day-by-day follow-up during the treatment period. To overcome this obstacle we have prepared monoclonal antibodies specific for antigens localized in functional (collecting duct, loop of Henle) and structural (extracellular matrix) components of the rat renal papilla. Applying these monoclonal antibodies in ELISA-type tests antigens with known localization in the tissue were determined in urine samples collected during day-by-day follow-ups. The results obtained with ELISA tests for 3 different antigens have shown that extent and profile of antigen release depend on the kind of drugs applied and on the application route. Whereas a single intraperitoneal application of 2-bromoethanamine or propylenimine resulted in a strongly increased release of one of the antigens (Papillary Antigen 1), single or repeated oral applications of bromoethanamine had minor effects. In contrast, both single intraperitoneal or repeated oral applications of indomethacin resulted in an increased release of all the three antigens. In most cases, histopathology findings correlated with the ELISA results. The observation of papillary alterations that led to enhanced antigen release but not to permanent damage indicate the potential of the new method. After daily application of ipsapirone in the diet or in drinking water release of Papillary Antigen 1 increased incrementally during the application period, but returned to normal pre-treatment levels after discontinuation of the treatment. Release of the other antigens was not affected. These results show that with the tests developed changes in the rat renal papilla caused by xenobiotics can be detected early by urinary analysis and monitored during follow-up studies. Moreover, the different antigen release patterns obtained after application of the different compounds indicate that they differ in their mode of action.

1883 A 28-DAY METHYL *tert*-BUTYL ETHER (MTBE) PALATABILITY STUDY AND THE EVALUATION OF PROTEIN DROPLET ACCUMULATION IN THE KIDNEYS OF RATS.

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MTBE is added to fuel to increase its oxygen content thereby reducing certain toxic emissions. MTBE has been found to contaminate groundwater in certain urban areas. Limited information exists to characterize the possible health risks from exposure to MTBE via drinking water (DW). The objective of this study was to assess the palatability of MTBE in DW as a basis for longer-term studies, and its ability to cause protein droplet nephropathy in male rats. Male and female F-344 rats were given DW containing 0, 1.5, 3, 7, or 15 mg MTBE /ml for 28 days, euthanized with CO2 and kidneys collected. Fixed sections of kidney were stained with Mallory's Heidenhain, a nonspecific protein stain, or for α2u-globulin (α2u) immunohistochemically. The concentrations of MTBE and its metabolite tert-butyl alcohol (TBA) were quantitated in the kidney by GC/MS. Body weight gain, and food and water consumption (FC and WC) was evaluated in each rat on a daily basis. Female rats drank ~3-5 ml less water/day throughout the study at all levels of MTBE. Initally WC was less (~3 ml) in male rats given 7 or 15 mg MTBE/ml compared to the control group. After the second week of the study no differences in WC were observed between the control and exposure groups. Throughout this study rats received ~110 to 1234 mg MTBE/kg/day. Body weight gain and FC was not affected in rats given MTBE DW. MTBE (15 mg/kg) caused a mild accumulation of protein droplets that stained positive for α2u in male rat kidneys. MTBE was detectable in male, but not female rat kidneys with male rats having a 7 to 35-fold higher kidney level of TBA compared to the female rat. MTBE was found to be palatable in DW. MTBE given in DW induced a male rat kidney response similar to what has been previously observed following inhalation exposure to MTBE. (Sponsored in part by the American Petroleum Institute.)

1884 CHARACTERIZATION OF *TERT*-BUTYL ALCOHOL BINDING TO α2U-GLOBULIN.

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tert-Butyl alcohol (TBA) is used as a chemical intermediate with a wide range of uses, including solvents for pharmaceutical products, paint removers, and personal care products. In both rodents and humans, TBA is a major metabolite of methyl *tert*-butyl ether, an oxygenated fuel additive. Chronic TBA exposure causes protein droplet nephropathy, α2u-globulin (α2u) accumulation, renal cell proliferation, and tumor formation in male, but not female, rats, suggesting an α2u-mediated mechanism for renal tumors. The objective of the present study was to determine whether TBA or a metabolite of TBA binds to α2u. Male and female F344 rats were administered a single gavage dose of 500 mg/kg TBA, 500 mg/kg [^{14}C]TBA, or corn oil. [^{14}C]TBA-derived radioactivity was detected at a higher percentage, relative to dose administered, in the male kidney, liver, and blood compared with the female tissue 12 h following [^{14}C]TBA administration. Gel filtration and anion-exchange chromatography demonstrated that [^{14}C]TBA-derived radioactivity coeluted with α2u from male, but not female, kidney cytosol. Protein dialysis studies demonstrated that the interaction between the [^{14}C]TBA-derived radioactivity and α2u was reversible. The low-molecular-weight protein fraction (LMWPF) from the kidney of [^{14}C]TBA-treated animals was isolated and incubated with *d*-limonene oxide, a chemical with a high affinity to α2u. *d*-Limonene oxide displaced the [^{14}C]TBA-derived radioactivity from the LMWPF. GC/MS analysis of the LMWPF confirmed that TBA was present in this fraction. These results demonstrate that TBA interacts with α2u, which explains the accumulation of α2u in the male rat kidney following exposure.

1885 BISMUTH BIOKINETICS AND NEPHROTOXICITY AFTER ACUTE COLLOIDAL BISMUTH SUBCITRATE OVERDOSE IN RATS.

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Bismuth(Bi)-induced nephrotoxicity has been reported to occur after acute overdoses of Bi-containing therapeutic drugs, used to treat peptic ulcers and *Helicobacter pylori* infections. We studied bismuth biokinetics and development of Bi-induced nephropathy in rats. Bi nephropathy was induced at t=0 hr by feeding a single overdose of colloidal bismuth subcitrate containing 3.0 mmol Bi/kg to 33 ten- to twelve-weeks-old female Wistar rats. The control group consisted of seven rats fed the vehicle. Kidney function parameters and Bi concentration in blood and urine were followed until the animal was sacrificed between t=1 and 48 hr to determine kidney histology and Bi content. Plasma creatinine increased during the whole experimental period. Proximal tubular cells of the S3 segment had vacuolated at t=1 hr and had become necrotic at t=3 hr. Cells of the S1/S2 segment started to vacuolate at t=3 hr to

become necrotic at t=12 hr. Biokinetics of bismuth in blood could best be described with a one compartment model characterized by an absorption half-life of 0.32 hour and an elimination half-live of 16 hours. The peak concentration of about 7.0 mg Bi/l was reached at t=2 hr. Conclusion: Of all proximal tubular segments, S3 necrotized first after an oral CBS overdose, followed by the S1/S2 segment. The Bi in blood curve for the first 48 hours was best described by a one compartment model.

1886 EFFECT OF ORAL ADMINISTRATION OF MANGANESE ON URINARY SYSTEM IN RATS.

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Oral toxicity studies were conducted to identify the toxicologic and reproductive performance in Sprague-Dawley rats during manganese administration. Both sexes were dosed in two-phase trials at 0, 360, 612, 1225, and 1838 mgMn/kg for 63 days. At the end of the dosing, 50% of animals were sacrificed, and the remaining cross-mated to evaluate reproductive efficiency with no dosing. After two weeks, the remaining animals were sacrificed. Male animals were noted to have viscous, gritty urine in the urinary bladder, and in the high dose groups had urinary bladder stones (uroliths). Proliferative masses associated with genitourinary structures were found in 66% of male rats from the 1838 mg/kg group. Phase I female liver weights were increased in all treatment groups, while male liver weights decreased in all groups. Phase I female kidney weights were not affected with treatment, while males in all treatment groups had decreased kidney weights. The most striking lesions were observed in the kidneys and prostate gland of male animals. Mild to moderate tubulointerstitial nephritis with tubular proteineous and glomerulosclerosis was observed in animals of all treatment groups. Moderate to severe changes were seen in combination in animals receiving 1225 and 1838 mgMn/kg. Urolithiasis in the urinary bladder was confirmed in 33-66% of treated animals. Prostatic changes included focal to locally extensive suppurative inflammation within tubules and interstitum, primarily in the two high-dose groups without correlation to testicular lesions. Female animals did not show a significant difference above controls in renal tissues. (Supported by ATSDR #U50/ATU398948-08.)

1887 ASSYMETRIC TRANSEPITHELIAL TRANSPORT AND ACCUMULATION OF CISPLATIN-N-ACETYLCYSTEINE IN S_1, S_2, AND S_3 SEGMENTS OF THE RABBIT RENAL PROXIMAL TUBULE.

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We measured the lumen-to-bath and bath-to-lumen transport, cellular accumulation and assessed the toxicity of cisplatin-N-acetylcysteine (cDDP-NAC) in isolated perfused segments (S_1, S_2, and S_3) of the rabbit renal proximal tubule. We observed the tubular epithelial cells under the light microscope for signs of cellular injury. Cellular swelling, blebbing, and the uptake of the vital dye FD & C green were indicative of cellular necrosis. Visible cellular damage occurs within 20 minutes after exposure of renal proximal tubular cells to 2mM cDDP and 4 mM N-acetylcysteine (presumably forming a 2 mM cDDP-NAC complex) at the basolateral and luminal membranes. This is in contrast to previous experiments that demonstrated that tubules bathed and perfused in the same concentration of cDDP only, displayed minimal toxicity. Lumen-to-bath transepithelial transport of cDDP-NAC was uniform for all segments at approximately 20 pMol min^{-1} (mm tubule length)$^{-1}$ while the bath-to-lumen transepithelial transport was much less at approximately 1 pMol min^{-1} (mm tubule length)$^{-1}$. Cellular accumulation of cDDP-NAC was greatest for the lumen-bath transepithelial transport as compared to bath-lumen transepithelial transport, 40 and 4 pMol (mm tubule length)$^{-1}$, respectively. When 2 mM p-aminohippurate was added to the bathing solution in the bath-to-lumen experiments, cellular accumulation decreased to non-detectable levels and elevated the apparent visual toxicity. We conclude that a potential "metabolite" of cisplatin, cDDP-NAC, is primarily sequestered by the proximal tubule at the luminal membrane. However, there is substantial uptake at the basolateral membrane which appears to be mediated by the PAH sensitive organic anion transporter.

1888 ENDOTOXIN AUGMENTED MERCURY-INDUCED NEPHROTOXICITY.

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Both endotoxin (LPS) and mercury are nephrotoxic compounds. The effects of either toxicant alone on the kidney have been well investigated but their toxic interaction has not. Endotoxin is a product of gram-negative bacteria cell walls. Humans are exposed to LPS through sepsis, by increased translocation across the gastrointestinal wall, or in the event of compromised liver function. Mercury is a food contaminant and the highest risk of human exposure is by consumption of contaminated fish. We have recently demonstrated that endotoxin potentiates mercury-induced nephrotoxicity in endotoxin-sensitive mice. In this study we tested the hypothesis that endotoxin augments mercury-induced nephrotoxicity in rats. Thirty two 41-43 day old, male Sprague-Dawley rats were randomly allocated to 4 groups of 8 rats each as follows: Group I (control) received 0.9% sodium chloride once IV, Group II received 2.0 mg of *E.coli* 0128:B12/kg once IV, Group III received 0.5 mg of mercury/kg (as mercuric chloride) once IV, Group IV received 2.0 mg endotoxin/kg (as in group II) once IV 4 hours before receiving 0.5 mg mercury/kg (as in group III) once IV. Rats were monitored for 48 hours. Serum and urine were assayed for markers of renal failure. Immunohistochemistry was done on kidneys for neutrophils. Rats given LPS plus mercury had a significant increase in serum creatinine and urea nitrogen concentrations than the rest of the groups. Kidneys from rats given endotoxin alone were normal but the most severe lesions were found in rats given endotoxin plus mercury. These kidneys also had a significant increase in neutrophils. Endotoxin significantly increased renal mercury concentration ($p \leq 0.05$). In conclusion, LPS augmented mercury-induced nephrotoxicity. This was associated with increased neutrophil infiltration and mercury content.

1889 COMPARATIVE EVALUATION OF NEPHROTOXIC RESPONSE OF YOUNG AND OLD RATS TO MERCURIC CHLORIDE.

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Degenerative changes in kidney function over the life span have been well documented. Furthermore, the changes in renal structure and function in aged rats are qualitatively similar to those in humans (Levi, Rowe, 1992; Mayersohn, 1994). However, how the degenerative processes of aging affect toxic vulnerability to metals has not received much experimental attention. This study was therefore carried out to evaluate age-dependent susceptibility of male Fisher-344 rats to nephrotoxicity due to subchronic treatment with mercuric chloride (HgCl2). Groups (5-6 rats/group) of both very young (7 weeks) and old (25-30 months) rats were treated by gavage with 0, 2 and 4 mg HgCl2 per kg b.w. once daily for 4 consecutive weeks. At the end of such treatment, the urines were collected at 0°C for 24 hours after each of 2 and 4 weeks of treatment and the animals were then immediately sacrificed for blood withdrawal and kidney removal. Both dose- and age- dependent increase in nephrotoxicity and accumulation of renal mercury were observed. A significant 5-fold increase in the urinary excretion of proteins, a 3-fold increase in the urinary excretion of N-acetyl-ß-D-glucosaminidase and gamma-glutamyltransferase and a 2-fold increase in BUN (measured by the ratio of treated/control) were observed in old rats compared to young rats when treated with 4 mg HgCl2 per kg for 4 weeks. These data demonstrate that very old rats are more susceptible than very young rats to the nephrotoxicity due HgCl2 caused by, in part, age-dependent difference in mercury accumulation in the rat kidney.

1890 EXPRESSION OF HEAT SHOCK PROTEIN 60 IN HUMAN PROXIMAL TUBULE CELLS EXPOSED TO ACUTE AND CHRONIC DOSES OF CADMIUM CHLORIDE.

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The heat shock proteins (hsps) are members of a large superfamily of stress response proteins that provide protection from physical and chemical insults including heavy metals. The goal of this study was to determine if hsp 60 has a protective role against toxicity from the heavy metal pollutant cadmium (Cd^{+2}) in human proximal tubule (HPT) cells. The expression of hsp 60 mRNA and protein were determined in HPT cells exposed to lethal and sub-lethal concentrations of Cd^{+2} under both acute and chronic conditions. Acute exposure to Cd^{+2} involved a 4 hr exposure followed by a recovery period in

Cd^{+2}-free growth medium. Chronic exposure to Cd^{+2} was modeled by continuous treatment with Cd^{+2} at both lethal and sub-lethal levels over a 16 day time course. Expression of hsp 60 gene and protein was determined using RT-PCR and western analysis. Acute exposure of HPT cells to 53.4 μM $CdCl_2$ resulted in an increase in hsp 60 mRNA and protein. Chronic exposure to Cd^{+2} failed to increase either hsp 60 mRNA or protein expression in HPT cells even at concentrations that were lethal to cells during the time course. Lethal exposure to Cd^{+2} resulted in a decrease in expression of hsp 60 protein compared to control levels. These findings suggest that hsp 60 expression may have a protective role when HPT cells are acutely exposed to Cd^{+2} and a deleterious role when hsp 60 protein is down regulated during extended exposure to Cd^{+2}.

1891 ACETAMINOPHEN-CYSTEINE (APAP-CYS) INDUCED RENAL GLUTATHIONE (GSH) DEPLETION.

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Acetaminophen (APAP) nephrotoxicity has been observed both in man and research animals. Inhibitors of either gamma-glutamyl transpeptidase (GGT) or the probenecid sensitive organic anion transporter protected against APAP-induced nephrotoxicity but not hepatotoxicity in mice (Hart et al. *Toxicol. Appl. Pharmacol.* 136:161-169, 1996) and suggested that transport of an APAP - cysteine conjugate (APAP-CYS) may contribute to the toxicity. APAP-CYS, is an ideal candidate for involvement in APAP nephrotoxicity since it is both a product of the GGT pathway and a likely substrate for the organic anion transporter. Administration of APAP-CYS (50 mg/kg, ip) to 18-hr-fasted, 3-month-old male CD-1 mice resulted in rapid depletion of renal GSH to <60% of control at 30 min with recovery to control levels by 2 hr. Hepatic GSH levels were unaffected, and no changes in blood urea nitrogen or plasma sorbitol dehydrogenase activity were observed. Renal-specific GSH depletion has been demonstrated in mice and rats following administration of amino acid glutamyl acceptor substrates for GGT. To determine if APAP-CYS might be acting similarly as a glutamyl acceptor, 10 units of bovine GGT/ml were incubated with 1mM APAP-CYS and 2mM GSH. HPLC analysis documented a time dependent loss of APAP-CYS with a corresponding, stoichiometric increase in glutamyl-cysteinyl APAP. These findings are consistent with the hypothesis that APAP-CYS contributes to APAP nephrotoxicity by depletion of renal GSH stores through interaction with the gamma-glutamyl cycle. (Supported by NIEHS ES07163.)

1892 THE EFFECT OF LOW CONCENTRATIONS OF ACETAMINOPHEN AND P-AMINOPHENOL ON PGE2 PRODUCTION IN THE HUMAN AND RAT KIDNEY.

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Co-oxidation of acetaminophen (APAP) and p-aminophenol (p-AP) by prostaglandin H synthetase (PHS) was investigated in human and rat kidneys. APAP and p-AP serve as reducing co-substrates by supplying electrons to the peroxidase of PHS, and thereby undergoe an oxidation to reactive intermediates. In this study, microsomes were prepared from the cortex, outer medulla and inner medulla of human kidneys, and from cortex and inner medulla of rat kidneys. Microsomes were incubated with arachidonic acid (AA) and different concentrations of APAP and p-AP (0, 0.1, 0.3, 0.5, 1.0, 3.0 and 5 mM) to assess the formation of prostaglandin E2 (PGE2). The formation of PGE2 during APAP incubations was significantly higher in inner medulla microsomes than in cortex microsomes for both human and rat kidney. An increase in PGE2 formation in the rat inner medulla was observed at low APAP concentrations (0.1, 0.3, 0.5, 1 and 3 mM) compared to 5 mM, where an inhibition of PGE2 was observed. Such inhibition was not observed in human inner medulla microsomes incubated with 5 mM APAP. Microsomes incubated with different concentrations of p-AP, showed a significantly higher level of PGE2 production in inner medulla versus cortex microsomes. An concentration dependent inhibition of PGE2 production in inner medulla microsomes was observed with 5 mM p-AP in both human and rat samples. Levels of total CYP450 were significantly higher in rat renal cortex (0.29 ± 0.01 nmol/mg) compared to inner medulla (0.21 ± 0.01 nmol/mg). No significant difference of total CYP450 between cortex and inner medulla was observed in the human kidney. CYP2E1 polypeptide levels in human inner medulla microsomes were 52% lower than the levels in cortex microsomes. The same trend was observed in rat kidney, where CYP2E1 polypeptide levels were 57% lower versus cortex. No significant difference was observed in the catalytic activity of CYP2E1 between cortex and inner medulla in both human and rat kidney. Furthermore there was no CYP1A2 and CYP3A catalytic activity in human and rat kidneys. These results correlate with the absence of CYP1A2 and CYP3A polypeptide levels in these tissues.

1893 EFFECTS OF ENZYME INHIBITION ON THE TOXICITY OF 4-AMINOPHENOL IN LLC-PK_1 CELLS.

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4-aminophenol (PAP) is selectively toxic *in vivo* to the S3 segment of the proximal tubule. However, the mechanisms responsible for PAP cytotoxicity are not entirely clear. PAP may undergo enzymatic as well as nonenzymatic oxidation to form reactive intermediates. To investigate the role of specific enzymes in the mechanism of PAP cytotoxicity, we coincubated LLC-PK_1 cells for four hours with 0.1 mM PAP and various concentrations (0.10-1.0 mM) of inhibitors of cytochrome P450 (CYP450), flavin monooxygenase (FMO), or prostaglandin sythase (PHS). Twenty hours later, viability was measured using Alamar Blue. In previous studies, 0.1 mM PAP reduced cell viability to 50-60% of control using this protocol. Cells coincubated with methimazole, an inhibitor of FMO, and 1-aminobenzotriazole, an inhibitor of CYP450, showed no change in viability as compared to cells incubated with PAP alone. PHS inhibitors included nonselective (phenylbutazone, indomethacin, piroxicam), and COX2-selective (nabumetone) agents. Only 0.10 mM phenylbutazone protected cells from PAP toxicity. Coincubation of cells with other PHS inhibitors had no effect on PAP-induced cytotoxicity. These data suggest that (1) neither CYP450 nor FMO play a role in the bioactivation or detoxification of PAP (2) metabolism of PAP or its metabolites by PHS may be involved the detoxification of PAP. (Supported by PHS GM51361.)

1894 COMPARISON OF CYTOTOXICITY INDUCED BY *PARA*-AMINOPHENOL IN ISOLATED RAT RENAL EPITHELIAL CELLS AND HEPATOCYTES.

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para-Aminophenol (PAP) produces proximal tubular necrosis in the absence of hepatic damage within 24 h of administration (300 mg/kg) to rats. Studies in our laboratory using LLC-PK_1 cells suggest that PAP-induced cytotoxicity depends on oxidation. We reasoned that since PAP is directly cytotoxic, both renal epithelial cells and hepatocytes should be injured when incubated with PAP. Therefore, we compared oxygen consumption and ATP content in rat renal epithelial cells and hepatocytes induced with various concentrations of PAP. Renal epithelial cells and hepatocytes were isolated from female SD rats by collagenase digestion. Cell suspensions were incubated with PAP (0-2.5 mM) in a shaking water bath at 37°C in Erlenmeyer flasks gassed with 95% O_2/5% CO_2 for up to 4 h. Aliquots of cells were collected hourly for determination of oxygen consumption ATP content. Cells incubated in the absence of PAP showed no time-dependent differences in oxygen consumption or ATP content. Renal epithelial cells or hepatocytes incubated with 0.5 mM PAP showed no differences in oxygen consumption or ATP content over the 4 h incubation period. Renal epithelial cells but not hepatocytes incubated with 1.0 mM PAP had a 50% decrease in oxygen consumption and a 37% decrease in ATP concentration after 4 h. At 2.5 mM PAP, hepatocytes showed a 55% decline in oxygen consumption and 29% decrease in ATP content after 4 h while renal epithelial cells had a 28% decrease in oxygen consumption and 33% decline in ATP content in the same time interval. These data suggest that renal epithelial cells are more susceptible to PAP cytotoxicity than are hepatocytes. However, at high concentrations of PAP, hepatocytes are injured and the biochemical responses are similar to those observed in renal cells. (Supported by PHS GM51361.)

1895 EVIDENCE FOR NEPHROTOXIC SULFATE CONJUGATES OF N-(3,5-DICHLOROPHENYL)SUCCINIMIDE METABOLITES.

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The agricultural fungicide, N-(3,5-dichlorophenyl)succinimide (NDPS), induces nephrotoxicity *in vivo* characterized by proximal tubular necrosis and

acute polyuric renal failure. However, previous *in vitro* studies have failed to reproduce the *in vivo* nephrotoxicity seen with NDPS or its nephrotoxic metabolites N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA). The purpose of this study was to examine the nephrotoxic potential of NDPS, its known non-conjugated metabolites, the O-sulfate conjugate of NDHS (NSC), and the putative metabolites N-(3,5-dichlorophenyl)maleimide (NDPM) and its hydrolysis product N-(3,5-dichlorophenyl)maleamic acid (NDPMA) using isolated renal cortical cells (IRCC) obtained from untreated male Fischer 344 rats. IRCC cells were obtained following collagenase perfusion of the kidneys. Cells (2-3 x 10^6 per flask) (N = 4) were incubated with up to 1.0 mM NDPS or an NDPS metabolite or vehicle for up to 120 min. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) release into the media. Only NSC (> 0.5 mM) and NDPM (≥ 0.5 mM) exposure increased LDH release from IRCC. NSC 1.0 mM increased LDH release within 15 min of exposure. NDPS and the remaining NDPS metabolites did not increase LDH release at bath concentrations of 1.0 mM for exposures of 120 min. These results demonstrate that sulfate conjugates of NDPS are fast-acting nephrotoxicants and could contribute to NDPS nephrotoxicity. These results also suggest that the kidney accumulates toxic sulfate conjugates on NDPS metabolites rather than forming the conjugates. (Supported by NIH grant DK31210.)

1896 ATTENUATION OF 4-AMINO-2,6-DICHLOROPHENOL (ADCP) NEPHROTOXICITY IN RENAL CORTICAL SLICES BY ASCORBATE, AMINOOXYACETIC ACID (AOAA), INDOMETHACIN AND GLUTATHIONE.

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ADCP is a potent acute nephrotoxicant *in vivo* inducing prominent renal corticomedullary necrosis. *In vitro*, ADCP exposure increases lactate dehydrogenase (LDH) release from rat renal cortical slices at 0.05 mM or greater. The purpose of this study was to examine the ability of antioxidants, CYP and FMO inhibitors, indomethacin, glutathione, and inhibitors of glutathione conjugate metabolism to attenuate ADCP nephrotoxicity *in vitro*. Renal cortical slices prepared from untreated male Fischer 344 rats (N=4/group) were preincubated at 37°C under a 100% oxygen atmosphere with an inhibitor or vehicle for 5-30 min. ADCP (0.1 or 0.5 mM) or vehicle was added and incubations continued for 120 min. At the end of the incubation period, LDH release was measured as an index of nephrotoxicity. ADCP nephrotoxicity was partially attenuated by ascorbate (1.0 or 2.0 mM), but not by DPPD, α-tocopherol or deferoxamine. Inhibitors of CYP450 (metyrapone, piperonyl butoxide, isoniazid) and FMO inhibitors (methimazole, N-octylamine) had no effect on ADCP nephrotoxicity. Indomethacin or glutathione 1.0 mM completely and partially blocked ADCP 0.1 and 0.5 mM nephrotoxicity, respectively. AOAA 1.0 mM, but not AT-125 1.0 mM, partially attenuated ADCP 0.1 mM nephrotoxicity. These results suggest that reactive metabolites may be produced from ADCP primarily via a cyclooxygenase-mediated mechanism. The difference in the ability of ascorbate and glutathione to attenuate ADCP nephrotoxicity could indicate that the reactive benzoquinoneimine metabolite might be the ultimate toxicant *in vitro* rather than a free radical-mediated mechanism.

1897 GENERATION OF PUTATIVE CYTOTOXIC METABOLITES OF N-(3,5-DICHLOROPHENYL)SUCCINIMIDE (NDPS) IN A HEPATIC/RENAL COINCUBATION SYSTEM.

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Previous studies implicated a role for glucuronide and sulfate conjugates in NDPS nephrotoxicity. This study was designed to investigate the possibility of generating cytotoxic phase II metabolites from the NDPS metabolite, *N*-(3,5-dichlorophenyl)hydroxysuccinimide (NDHS), using a hepatic/renal coincubation system. LLC-PK_1 cells (5x10^4/well) were plated in 24-well plates on the day prior to the experiment. NDHS (200-800 μM) and cells were then coincubated with rat liver microsomes (0.5-3 mg protein/ml) or cytosol (0.25-2 mg protein/ml). Incubations also contained the appropriate cofactors, either UDPGA (2 mM) or PAPS (1-4 mM). After 4-8 hr, the coincubation medium was removed and analyzed for the presence of glucuronide and sulfate conjugates by HPLC-tandem mass spectrometry. Cell viability was measured cytofluorometrically with alamarBlue. The cells were then reincubated in fresh media and viability was reassessed at 24 and 48 hours. A known cytoxicant, *N*-(3,5-dichlorophenyl)maleimide (NDPM, 2-50 μM), was incubated with LLC-PK_1 cells as a positive control. LC-MS/MS confirmed that glucuronide and sulfate conjugates were generated in the coincubation system. NDPM dramatically decreased cell viability at concentrations above 25 μM. Cell viability declined in coincubations with hepatic microsomes, even in the absence of substrate, suggesting that some component of the microsomes was damaging the cells. In contrast, cell viability was not affected in cytosolic coincubations, even in the presence of PAPS and substrate. These results suggest that glucuronide and sulfate conjugates of NDHS may not be toxic to the LLC-PK_1 cell line. It is also possible that the conjugates were not produced in sufficient quantities to cause cytotoxicity or are not transported into the cells. However, these experiments have shown that it is possible to generate Phase II metabolites in the coincubation system, which may be applicable to other compounds. (Supported by USPHS grant ES05189.)

1898 2-AMINO-4,5-DICHLOROPHENOL (2A45CP) TOXICITY IN RENAL CORTICAL SLICES FROM FISCHER 344 RATS.

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2A45CP is an aromatic ring hydroxylated metabolite of 3,4-dichloroaniline. 3,4-Dichloroaniline is nephrotoxic with primary damage located in the proximal tubules. The purpose of this study was to first assess the *in vitro* toxicity of 2A45CP in renal cortical slices and second, to determine the effect of antioxidants and sulfhydryl containing agents on the severity of 2A45CP toxicity. Renal cortical slices were prepared from male Fischer 344 rats (200-245 g). Renal slices were rinsed 3 times for 3 min each in 5-ml renal Krebs buffer. Tissues were then incubated for 90-120 min with 0-1 mM 2A45CP. In a separate series of experiments, the slices (50-100 mg tissue) were preincubated for 30 min with 0 or 1 mM dithiothreitol (DTT) or glutathione (1mM) prior to exposure to 0, 0.25, 0.5 or 1 mM 2A45CP. Loss of membrane integrity was monitored by measurement of lactate dehydrogenase (LDH) leakage. 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. Pretreatment with DTT did not alter 2A45CP toxicity as indicated by comparable changes in pyruvate directed gluconeogenesis and LDH leakage between vehicle and DTT pretreated slices. Preincubation with 1 mM GSH reduced the extent of LDH leakage to levels that were similar to control values. Pretreatment with 1 mM ascorbic acid also reduced 2A45CP toxicity. These findings indicate 2A45CP is directly toxic to renal cortical slices inducing a rise in LDH leakage, diminished pyruvate directed gluconeogenesis and diminished glutathione. Pretreatment with ascorbic acid or glutathione prevented 2A45CP toxicity in renal slices. (Supported by NIH grant ES08834.)

1899 INHIBITION OF A MICROSOMAL CA^{2+}-INDEPENDENT PHOSPHOLIPASE A_2 INCREASES OXIDANT-INDUCED APOPTOSIS IN RENAL PROXIMAL TUBULAR CELLS.

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Phospholipase A_2(s) are esterases that hydrolyze the *sn-2* ester bond in phospholipids releasing a fatty acid and a lysophospholipid. While reports have examined the role of PLA_2s in the genesis of apoptosis in a number of cell types, the exact role of PLA_2 and the isoforms involved are not clear. We have shown previously that approximately 90% of PLA_2 activity in rabbit renal proximal tubular cells (RPTC) was Ca^{2+}-independent and located in the microsomes. We hypothesized that this microsomal Ca^{+2}-independent PLA_2 (m-$iPLA_2$) plays a role in oxidant-induced RPTC apoptosis. Using an antibody against Ca^{2+}-independent PLA_2 ($iPLA_2$) and immunoblot analysis an 85 kDa protein band was identified in rabbit RPTC microsomes but not the cytosol. Immunocytochemistry using the same antibody revealed punctate staining in RPTC cytosol but not in the nucleus, supporting the microsomal localization of m-$iPLA_2$. Oxidant (tert-butylhydroperoxide) exposure caused both time- (0.5 to 24 hr) and concentration- (50 to 500 μM) dependent increases in annexin V binding (an early marker for apoptosis) prior to increases in propidium iodide (PI) DNA staining (a late marker for apoptosis and oncosis). Treatment of RPTC with bromoenol lactone (BEL) (1 to 10 μM), a specific inhibitor of $iPLA_2$, decreased m-$iPLA_2$ activity in RPTC in a concentration-dependent manner. Pretreatment of RPTC with increasing concentrations of BEL prior to TBHP exposure increased both annexin V and PI staining compared to TBHP alone. These results show that oxidants can cause RPTC apoptosis and that inhibition of m-$iPLA_2$ potentiates oxidant-induced apoptosis. Thus, m-$iPLA_2$ may act in a protective manner in RPTC by inhibiting apoptosis.

1900 TRANSLOCATION OF μ-CALPAIN MEDIATES MITOCHONDRIAL INHIBITOR-INDUCED CELL INJURY IN RENAL PROXIMAL TUBULES (RPT).

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Calpains, Ca^{2+}-activated cysteine proteases, play a role in physiological and pathological processes. Previous data from our laboratory demonstrated the translocation of calpain activity from the cytosol to the membrane fraction during the late phase of RPT cell injury. The goals of this study were to determine 1) the expression of calpain isoforms in rabbit RPT; 2) the alterations of each calpain isoform during mitochondrial inhibitor-induced RPT cell injury; and 3) the effects of various cytoprotectants on cytosolic calpain levels during RPT cell injury. RNA was isolated from RPT S_2 segments and subjected to RT-PCR with either rabbit μ- or m-calpain large subunit specific primers. Sequencing of the 700 bp PCR product of the m-calpain large subunit and the 500 bp PCR product of the μ-calpain large subunit confirmed their presence. Freshly isolated RPT were exposed to the mitochondrial inhibitor antimycin A (AA 10 μM) for up to 60 min in the absence or presence of glycine (2 mM), strychnine (1 mM), muscimol (100 μM), or pregnenolone sulfate (PS 100 μM). AA induced significant cell death (40±2% LDH release vs 6% control at 60 min). Immunoblot analysis showed that AA treatment resulted in a time-dependent reduction in cytosolic 80 kD μ-calpain (39±7% of control at 60 min). In contrast, immunoblot analysis did not demonstrate a significant decrease in cytosolic 80 kD m-calpain (90±3% of control). Glycine, strychnine, muscimol, and PS improved cytosolic μ-calpain levels to 66±%, 101±8%, 89±9% and 89±% of control, and reduced LDH release to 7±%, 10±%, 9±% and 12±%, respectively. While rabbit RPT express μ- and m-calpains, only the reduction of cytosolic μ-calpain correlates to the previously observed translocation of calpain activity to the membrane during mitochondrial inhibitor-induced RPT cell injury. The cytoprotective mechanism of action of glycine, strychnine, muscimol, and PS involves the blockade of the translocation of μ-calpain.

1901 CALRETICULIN REGULATES BRANCHING MORPHOGENESIS AND CELL PROLIFERATION OF RENAL PROXIMAL TUBULAR EPITHELIAL CELLS.

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Renal regenerative repair is a common event that follows nephrotoxic damage to proximal tubular epithelial cells. The exact molecular mechanisms that regulate the regenerative process are unclear. Calreticulin is a 46-kDa protein that binds to a specific consensus sequence (KXGFFKR) present on α-integrin subunits (1,2). Previous studies have shown that calreticulin appears to regulate the activation state of α integrins resulting in modulation of cell attachment and spreading. We wanted to determine if calreticulin regulates the ability of LLC-PK1 cells to undergo growth factor-induce branching morphogenesis and cellular proliferation. Thus, LLC-PK1 cells were transfected with an expression vector, pRC/CMV, containing a full-length (1.9 kb) human calreticulin cDNA. After transfection, G-418 resistant clones for calreticulin (pkCRT) or pkNEO were selected and processed for Western blot analysis. LLC-PK1 cells transfected with calreticulin exhibited increased calreticulin expression relative to pkNEO cells. When grown on monolayer, calreticulin overexpressing cells exhibited no differences in cellular proliferation rates, relative to pkNEO clones. In addition, migration rates of pkCRT were similar to those of the pkNEO clones. Attachment rates between pkCRT clones and pkNEO clones were also comparable. Cells were then seeded into collagen type I matrix and treated with growth factors to stimulate branching morphogenesis. Treatment of the various clones with 10% serum alone did not induce a proliferative response in any of the clones tested. pkNEO clones treated with growth factors exhibited a dramatic increase in cellular proliferation, a response blocked by co-treatment with TGF-β1. In contrast, overexpression of calreticulin resulted in a complete inhibition of growth factor-induced cellular proliferative rates. Furthermore, unlike the pkNEO clones, the pkCRT clones failed to undergo growth factor-induced branching morphogenesis. These findings demonstrate that calreticulin plays a critical role in the ability of renal proximal tubule cells to undergo growth factor-induced branching morphogenesis.

1902 CYTOPROTECTION AGAINST QUINONE-THIOETHER-MEDIATED CYTOTOXICITY BY AN "ENDOTHELIAL-LIKE" THROMBOXANE A2 RECEPTOR (TP) ALTERNATIVE SPLICE VARIANT COUPLED TO NUCLEAR FACTOR KAPPA B (NF-κB).

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11-deoxy-16,16-dimethyl prostaglandin E2 (DDM-PGE2) induces cytoprotection against 2,3,5-(trisglutathion-S-yl)hydroquinone [TGHQ]-mediated cytotoxicity via a receptor that is pharmacologically distinct from the known PGE2 receptor subtypes (EP) in renal epithelial cells (LLC-PK1). The present studies were conducted to determine whether the cytoprotective response to DDM-PGE2 is mediated by a TP receptor. DDM-PGE2-mediated cytoprotection against TGHQ-mediated cytotoxicity can be reproduced by TP agonists (U46619; IBOP), and is inhibited by TP antagonists (SQ 29,549; ISAP). DDM-PGE2-mediated 12-O-tetradecanoyl phorbol-13-acetate (TPA) responsive element (TRE)-binding activity is inhibited by a TP antagonist (SQ 29,548), but not by cyclooxygenase (aspirin, indomethacin) or thromboxane synthase (sulfasalazine) inhibitors, indicating that the cytoprotective response is not dependent on de novo TXA2 biosynthesis. DDM-PGE2- and U46619-mediated NF-κB-binding activity is inhibited by SQ 29,548, suggesting that the renal epithelial TP receptor couples to multiple transcription factors. Sulfasalazine inhibits DDM-PGE2-mediated cytoprotection and NF-κB-, but not TRE-binding activity. RT-PCR coupled with Southern blot indicates that porcine (LLC-PK1) and human (HK-2) renal epithelial cells express an "endothelial-type" TP receptor transcript, but not a placenta-type TP receptor. Collectively, these observations suggest that a cytoprotective TP receptor is expressed in renal epithelial cells, and that the cytoprotective response is mediated by NF-κB. (This work was supported in part by an award from the National Institute of Environmental Health Sciences [T32 ES 07247] and [GM56321 to SSL].)

1903 COMPARISON OF THE STRESS RESPONSE BETWEEN CULTURED HUMAN PROXIMAL TUBULE CELLS AND THE DERIVED CELL LINE HK-2.

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Heat shock protein (hsp) 27 is a member of a large superfamily of proteins collectively referred to as the heat shock or stress response proteins. These proteins are believed to provide protection against environmental insult, both physical and chemical. The goal of this study was to define expression of hsp 27 in an immortalized human proximal tubule cell line, HK-2, and compare it to cultured human proximal tubule cells (HPT). Cells were exposed to elevated temperature, sodium arsenite, and cadmium chloride under both acute and chronic conditions. HK-2 cells were more sensitive than HPT cells to cadmium and lower doses were used to achieve lethal and sub-lethal effects. Expression of hsp 27 gene and protein was determined using RT-PCR and western analysis. Upon exposure to elevated temperature of 42.5°C for 1 hour, the expression of hsp 27 mRNA and protein increased in HK-2 and HPT cells, however the level of induction in HK-2 cells was lower than the HPT cells. Acute exposure to sodium arsenite and cadmium chloride resulted in an increase in hsp 27 mRNA in HK-2 and HPT cells without a significant increase in protein levels in HK-2 cells. Chronic exposure to cadmium failed to increase either hsp 27 mRNA or protein expression in HK-2 and HPT cells. These results demonstrate that Hsp 27 expression is reduced in the immortalized cell line as compared to normal proximal tubule cells.

1904 PENTAMIDINE-INDUCED ALTERATION IN MITOCHONDRIAL MEMBRANE POTENTIAL IN LLC-PK1 CELLS.

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Nephrotoxicity is the therapy-limiting toxicity associated with pentamidine, a second line agent used in the treatment of AIDS-associated Pneumocystis Carinii Pneumonia. Inhibition of oxidative phosphorylation has been proposed as a possible pharmacologic mechanism of action in some protozoa. This effect could be related to the mechanism of nephrotoxicity. Previously, we have demonstrated that pentamidine inhibits basal and uncoupled respiration in LLC-PK1 cells (a proximal tubule epithelial cell line) after a 1 hour incubation. The purpose of this study was to examine the effect of pentami-

dine on mitochondrial function. LLC-PK1 cells grown in 35cm2 dishes, were treated with 0.005, 0.05, 0.125 and 0.5mM pentamidine for 1 hour and 20 hours. Then the cells were exposed to Rhodamine 123 (mitochondrial fluorescent dye) for 15 minutes at 37°C. The cells were harvested and washed once with phosphate-buffered saline(PBS) and resuspended in 1ml of PBS. The distribution of rhodamine 123 was determined using a Coulter Flow Cytometer . Pentamidine decreases mitochondrial membrane potential in a concentration and time dependent fashion. After treating the cells with pentamidine for 1 hour there was a significant decrease in membrane potential at 0.5mM. After 20 hours of incubation with pentamidine, there was a significant decrease in potential at 0.05 and 0.125mM and the shift at the 0.5mM concentration increased more than the 1 hour results. Preliminary data suggests that the decrease in membrane potential is not reversed when media containing pentamidine is replaced with fresh media without the agent for 24 hours. These experiments in conjunction with the data showing the inhibitory effect on cellular respiration demonstrate that pentamidine does alter mitochondrial function in LLC-PK1 cells. Further research is required to establish a link between altered mitochondrial function and nephrotoxicity. (Supported by NIEHS Training Grant T32-ES-07290 [RLB].)

1905 MODULATION OF THE BENZODIAZEPINE (BZD) RECEPTOR/GAB COMPLEX AFFECTS CELL DEATH INDUCED BY OXIDANT STRESS IN MADIN DARBY CANINE KIDNEY (MDCK) CELLS.

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The role of the central benzodiazepine receptor has been widely studied in the central nervous system (CNS) and has been linked to the GABA receptor, potentiating Cl-movement into the cell. The peripheral benzodiazepine receptor (PBR) has been studied as well, and it has been found mainly, but not exclusively, on the mitochondria in myriad cell types. BZD receptors found in the kidney are mainly localized in the distal tubule of the nephron. Renal BZD receptors have yet to be fully characterized with respect to the exact nature of their type (CBR vs. PBR) and function. The purpose of this study was to determine if modulation of BZD receptors by agonists or antagonists would alter MDCK cell responses to oxidant stress. Cells were pretreated for one hour with a nonspecific GABA antagonist and possible PBR antagonist (Lindane 1X10-6 M), a PBR specific antagonist (PK11195 1X10-6 M), and a CBR specific agonist (Flurazepam 1X10-4 -1X10-6 M). Following treatment with Lindane and PK11195, the cells were exposed to Menadione (0.125-1.0 mM). Cells pretreated with Flurazepam were conincubated with Menadione and Flurazepam. Necrotic cell injury was determined by lactate dehydrogenase (LDH) leakage and apoptotic cell injury was determined by propidium iodide (PI) exclusion. Pretreatment with Lindane caused a 37% reduction in Menadione (1.0 mM) induced necrotic injury. PK11195 had no effect on Menadione (0.25 mM) induced necrotic injury. Flurazepam (1X10-4 M) caused a 44% increase in Menadione (0.25 mM) induced necrotic injury and a 528% reduction in apoptosis. These results suggest that modulation of the CBR can alter the cell death response to an oxidative injury, possibly through altering Cl-movement into the cell.

1906 INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITION DOES NOT PREVENT PROTEINURIA IN PUROMYCIN AMINONUCLEOSIDE INDUCED NEPHROTIC INJURY.

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Puromycin aminonucleoside (PAN) administration in rats causes glomerular epithelial cell injury and proteinuria. The purpose of this study was to examine the role nitric oxide (NO) in this model of minimal change glomerular disease. Aminoguanidine (AG) was used to inhibit inducible nitric oxide synthase (iNOS). Sprague-Dawley rats were divided into Control (N=9), PAN (N=14), and PAN+AG (N=12) treatment groups. Control animals received saline (i.v.), PAN animals received PAN (75 mg/kg, i.v.), and PAN+AG animals received a PAN plus AG (50 mg/kg, i.p., administered twice daily). All animals were kept in metabolic cages, and urinary protein excretion and NO_2- excretion were measured daily. At day 7, plasma was collected and kidneys were harvested. PAN administration increased urinary NO_2- excretion by day 2, and levels remained elevated through day 7. AG prevented this PAN induced increase in urinary NO_2- excretion. Plasma nitrate (NO_3-) and NO_2- (NOx) concentrations were also increased in PAN and PAN+AG. iNOS protein expression was not detected in either the glomeruli or cortex at day 7. Proteinuria developed in PAN animals on day 4 and increased steadily through day 7. PAN+AG animals had a pattern of urinary protein excretion similar to the PAN group. These results indicate that in contrast to models of proliferative glomerulonephritis, NO formation during PAN-induced nephrotic syndrome is increased but does not participate in the development of glomerular injury as measured by proteinuria.

1907 ANTAGONIST NEPHROTOXIC INTERACTIONS IN BINARY AND TERNARY MITURES OF POLYCYCLIC AROMATIC HYDROCARBONS.

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Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants present in multiple sources of environmental pollution. Although the hepatic and pulmonary toxicities of PAHs have been extensively characterized, limited data concerning their nephrotoxic potential are presently available. In previous studies we have shown that rat glomerular mesangial cells (rGMCs) are preferentially injured by benzo(a)pyrene (BaP) and anthracene, while porcine cortico-tubular epithelial cells (LLCPK-1) are affected by chrysene. Recognizing that exposure to environmental chemicals, even at a single contaminated site, is rarely limited to a single compound, the present studies were conducted to evaluate the nephrotoxic potential of binary and ternary mixtures of these chemicals. Mixtures of BaP, chrysene and anthracene yielded unexpected antagonistic nephrotoxic interactions in both rGMCs and LLCPK-1 cells. Northern and reverse transcription polymerase chain reaction analyses revealed that *CYP1A1* inducibility decreased in LLCPK-1 cells, but not rGMCs, treated with binary mixtures of BaP and chrysene (0.3-30μM). Although binary treatment of rGMCs did not alter *CYP 1A1* expression, reduced *CYP 1B1* inducibility was observed. Therefore, the profile of *CYP* inducibility in both cell types was consistent with antagonistic nephrotoxic interactions between these chemicals. However, a direct correlation between nephrotoxicity and *CYP* induction cannot be inferred because anthracene effectively inhibited BaP and chrysene toxicity in rGMCs, but did not inhibit *CYP1A1* inducibility. We conclude that significant cell-specific nephrotoxic interactions occur upon exposure to PAH mixtures, with some of these interactions involving *CYP* expression. (Supported in part by ATSDR Grant TU68450502 and NIEHS Center Grant 09106.)

1908 MODULATION OF GLOMERULAR CELL FUNCTIONS bBENZO(a)PYRENE: IMPLICATIONS FOR MESENCHYMAL/ EPITHELIAL INTERACTIONS *IN VITRO*.

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Epidemiological and experimental studies have implicated aromatic hydrocarbons in the onset and/or progression of kidney diseases. Recent studies in this laboratory have shown that single or repeated *in vitro* exposure of glomerular cells to 3.0μM benzo(a)pyrene (BaP) inhibits mesangial cell proliferation and affords a proliferative advantage to visceral epithelial (i.e. podocyte) cells. On the basis of these findings we hypothesized that BaP differentially modulates glomerular cell function leading to disruption of critical mesenchymal/epithelial interactions *in vitro*. To study these relationships, single cell clones of mesangial cells and podocytes were established in serial culture. RT-PCR analysis revealed that CYP1A1 is expressed constitutively in mesangial cells, but not podocytes. Using TGFβ1 as a mesangial cell-derived candidate inhibitory factor of podocyte proliferation affected by BaP, single cell clones were challenged with TGFβ1 (0.01-0.5nM). TGFβ1 markedly reduced DNA synthesis in mesangial cells at all concentrations examined, but was without effect in podocytes under the same experimental conditions. Therefore, differences in the rate of oxidative metabolism between mesangial cells and podocytes may contribute to differential toxicant susceptibility, but this response is not likely mediated by interference with mesangial cell-derived TGFβ1. The paracrine signals affected by BaP and responsible for altered mesenchymal/epithelial cell interactions in our *in vitro* model system remain to be identified. (Supported in part by NIEHS Grant ES 09106.)

1909 MITOCHONDRIAL FUNCTION IN RAT KIDNEY AFTER COMPENSATORY HYPERTROPHY.

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Suspensions of mitochondria were isolated from renal cortical homogenates from control rats and rats that had undergone uninephrectomy and compensatory renal growth (NPX) to test the hypothesis that renal cellular hypertrophy is accompanied by increases in mitochondrial metabolism. This increase in metabolism involves increases in rates of electron transport, which may then lead to release of reactive oxygen species. Consequently, mitochondria from the remnant kidney may exhibit oxidative stress or be more susceptible to injury from oxidants and agents or conditions that deplete ATP and inhibit mitochondrial function. Specific activities of malic and succinic dehydrogenase were significantly increased in mitochondria from NPX rats. While rates of state 3 respiration, normalized to protein, were elevated, rates of state 4 respiration, normalized to protein, and respiratory control values were unaffected by compensatory hypertrophy. Although specific activities of glutathione (GSH) redox cycle enzymes were unchanged relative to protein in mitochondria from NPX rats, rates of uptake of GSH into these mitochondria, which occur by the dicarboxylate and 2-oxoglutarate carriers, were elevated approximately 2.5-fold as compared to those in control mitochondria. Susceptibility to toxicants was assessed by measurements of effects on respiration and lipid peroxidation. *tert*-Butyl hydroperoxide, methyl vinyl ketone, and *S*-(1,2-dichlorovinyl)-L-cysteine (0.1-2 mM) all produced more inhibition of state 3 respiration and larger decreases in malondialdehyde formation in mitochondria from NPX rats than in control mitochondria, indicating that mitochondria from hypertrophied cells are indeed more sensitive to oxidants or mitochondrial toxicants. Baseline levels of malondialdehyde were also significantly elevated in NPX mitochondria, suggesting that a basal oxidant stress is present in mitochondria from hypertrophied cells.

1910 COMPARATIVE ANALYSIS OF SIGNAL TRANSDUCTION PROTEINS IN TUMOR *VERSUS* NON-TUMORIGENIC RENAL EPITHELIAL CELLS.

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Several new cancer chemotherapeutics are designed to induce cell death by targeting signal transduction pathways that mediate the death response. Genetic alterations that lead to tumor cell phenotypes may cause functional alterations in cell signaling pathways that can influence efficacy of therapies. For this reason, we have examined the expression and activities of several key signal transduction kinases in tumor and non-tumorigenic renal epithelial cells. Tumorigenic renal epithelial cell lines that have a homozygous mutation in the tumor suppressor gene TSC-2 (ERC-18) or are expressing oncogenic H-ras (NRK-H/6.1) were compared to non-tumorigenic renal epithelial cells (TRKE, NRK 52E). We examined protein expression and activities of ERK-1, ERK-2, AKT, PKCa, Raf-1, and p38 in ERC-18, NRK H/6.1, TRKE, and NRK 52E renal epithelial cells. We also examined overall serine/threonine kinase activities in cell lysates. We found that the renal epithelial tumor cells appear to have higher basal levels of serine/threonine kinase activities and ERK-1 and ERK-2 protein than non-tumorigenic cells. In addition, ERC-18 tumor cells appear to have higher basal levels of PKCa protein than all cell types examined. Remarkably, ERC-18 and NRK H/6.1 tumor cells are more susceptible to apoptotic cell death induced by detachment (anoikis). These results indicate that kinase profiles of tumor versus non-tumorigenic epithelial cells may be a useful screen in the discovery phase of development to test the efficacy of potential death-inducing chemotherapeutics.

1911 ROLE OF MITOCHONDRIA IN S-(1,2-DICHLOROVINYL)-L-CYSTEINE (DCVC) INDUCED APOPTOSIS.

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DCVC, the cysteine conjugate of trichloroethylene, is a potent nephrotoxicant. In this study, we investigated the mechanism of DCVC-induced apoptosis in the cultured porcine proximal tubular cell line, LLC-PK1. DCVC exposure induced caspase activation after 10 hr followed by DNA fragmentation at 12 hr. Apoptosis was completely inhibited by aminooxyacetic acid, suggesting the toxicity of DCVC was caused by β-lyase-dependent bioactivation. When labeled with JC-1 and analyzed by flow cytometry, a decrease of mitochondrial membrane potential ($mt\Delta\psi$) was detected at 4 hr. The loss of $mt\Delta\psi$ was accompanied by release of mitochondrial cytochrome *c*, as measured by Western analysis. Immunostaining of mitochondrial HSP 60 showed its localization was not changed after DCVC treatment, indicating the general structure of mitochondria was preserved. Both DCVC-induced cytochrome c release and caspase activation were observed in cells lacking mitochondria DNA (ρ^0 cells). Because mitochondria in ρ^0 cells do not produce ATP, these experiments indicate that DCVC-induced toxicity is not due to disrupted mitochondrial ATP production. We conclude that DCVC induces cell death in proximal tubular cells by selectively disrupting the mitochondrial outer membrane, releasing cytochrome c, and activating caspase-mediated apoptosis. (Supported by NIH grant ES 0904.7.)

1912 ACETYL-L-CARNITINE REDUCES TAXOL EFFECTS ON MYELOID CELL LINEAGE IN THE DOG.

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One of the most significant side-effects of Taxol, a major anticancer drug, is severe neutropenia. Acetyl-L-Carnitine (ALCAR) is an agent investigated to counteract the cytostatic effect of Taxol on myeloid proliferative cells. This hypothesis was studied in 16 female Beagle dogs treated with a single dose of Taxol (100 mg/m^2) administered via slow intravenous infusion. Dogs were divided into two groups of 8 animals each. One group was treated intramuscularly twice a day with 10 mg/kg of ALCAR for at least 7 consecutive days before Taxol treatment and for 15 days after. The second group received physiological saline in place of the ALCAR solution. Standard haematological parameters were monitored for 15 days after Taxol administration. Bone marrow samples were obtained by aspiration from the iliac bone before and 24 hours after Taxol treatment. Complete myelogram counts and cell ratios, with a detailed morphological examination, were carried out on bone marrow smears. This study showed that: (i) all animals treated with Taxol were affected by severe neutropenia; (ii) the nadir for circulating neutrophils was about 5 days after Taxol administration; (iii) a high intra-group variability was found in the haematological analysis compared to a lower standard deviation observed in the bone marrow counts; (iv) in myelogram counts, proliferative cell pool values of the animals treated with ALCAR were higher than the control group. This study suggests a positive action of ALCAR on the myeloid side-effects of Taxol.

1913 BIOLOGICAL ACTIVITY, PHARMACOKINETICS, AND SAFETY ASSESSMENT OF HUMAN GROWTH HORMONE (HGH) DELIVERED *VIA* A SUBCUTANEOUS DEPOT.

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The objectives of these studies were 1) to determine the biological activity of hGH delivered in an injectable depot formulation in hypophysectomized (hypox) male rats; 2) to determine the pharmacokinetic profile for hGH release; and 3) to assess the potential toxicity of the depot formulation in rats after 28 days. The ALZA depot is a non-aqueous, biodegradable poly(lactide-glycolide) copolymer (PLGA)/benzyl benzoate solution. The biological activity of hGH delivered from the subcutaneous depot was confirmed in hypox rats injected on Day 0 with 400 mg depot containing hGH (20 mg), as determined by body weight gain, and elevated IGF-1 levels for 28 days. The pharmacokinetics of hGH over 28 days from a subcutaneous depot was assessed in immunosuppressed, non-hypox rats (cyclosporin, 10 mg/kg, IP). Analysis of serum hGH profiles over 28 days revealed that hGH was released with a low acute drug burst and with sustained delivery (with serum hGH levels above 10 ng/mL) through 28 days. To assess potential toxicity, rats were injected SC with 100, 350 or 700 mg depots (containing 4.9% hGH). No significant adverse effects were noted in rats after injection of up to 700 mg depot as assessed by body weight gain, clinical pathology, tissue weights, and histopathological evaluation of injection sites and representative tissues. In conclusion, a biodegradable depot platform has been developed which releases biologically active hGH, with a low acute drug burst and sustained drug delivery through 28 days.

1914 A SAFETY ASSESSMENT OF ORAL GLYCOLIC ACID IN RODENTS.

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Glycolic acid (GA; CAS 79-14-1) is a potential counter ion for pharmaceutical agents, but is also a toxic intermediate in the biotransformation of ethylene glycol to oxalic acid. The safety of orally administered GA at doses relevant for pharmaceutical use was assessed in a 2-week rat study and in acute safety pharmacology studies in rats or mice. In the 2-week Fischer 344 rat study (n=10/sex), daily oral doses of 240 mg GA/kg had no important effects on clinical signs, body weight, food consumption, ophthalmic effects, liver cytochrome P450, hematology, clinical chemistry, urinalysis, organ weights, gross pathology or histopathology. In safety pharmacology studies in rats or mice, single oral doses of 48 mg GA/kg had no effect on renal function, central nervous system (CNS) function, and gastrointestinal motility. Renal function was assessed in male Fischer 344 rats (n=8) based on urine volume, pH, sodium, potassium, chloride, creatinine, and osmolality, creatinine clearance and fractional excretion of sodium. CNS and behavioral effects assessed in male CD-1 mice (n=10) were spontaneous activity, clinical signs, temperature, grip strength, hexobarbital-induced sleep times, acetic acid-induced writhing, auditory startle responding, and convulsive thresholds. Gastrointestinal motility was evaluated in fasted male CD-1 mice (n=10) by assessing the transit rate of charcoal meal. Although no effects were observed, this result should be interpreted with caution because rodents may be less sensitive than humans to GA toxicity as is well established for ethylene glycol toxicity.

1915 SAFETY ASSESSMENT OF RECOMBINANT BASIC FIBROBLAST GROWTH FACTOR (RFGF-2) FOR THE TREATMENT OF CORONARY ARTERY DISEASE.

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The safety assessment program for rFGF-2 included efficacy, safety pharmacology, pharmacokinetics (PK), pharmacodynamics (PD), and toxicological evaluations. Single intravenous (IV) and intracoronary (IC) doses were studied in healthy pigs and IV, IC, and intrapericardial routes were used in pigs with experimental coronary disease (ameroid constriction model). Multiple doses (IV once per week for 3 or 6 weeks) were studied in healthy rats and pigs. The effect of heparin on toxicity, PK and PD was also examined, since heparin is used during cardiac catheterization procedures and it is known that heparin and heparin-like molecules (heparan sulfates) can play a significant role in the biological activity of basic FGF in some systems. Doses of 30-300 and 0.65-20 μg/kg of rFGF-2 were studied in rats and pigs, respectively. Heparin doses of 40-70 U/kg were used in interaction studies. The only toxicity identified for rFGF-2 was a slight, reversible tachycardia in conscious telemetered rats (1 dose/week for 3 weeks), dose-related hypotension in unconscious pigs (single dose), and slight increases in selected organ weights in rats (1 dose/week for 6 weeks). Heparin given ~10 minutes prior to rFGF-2 did not have a significant effect on PD parameters (blood pressure, heart rate, ECG) in pigs. However, heparin decreased plasma clearance and steady-state volume of distribution in both species. The effect of heparin on rFGF-2 PK was more pronounced in pigs. No predictable effect on terminal elimination half-life was seen. **Conclusion:** With the exception of transient hypotension, which is a known pharmacological effect of the molecule, single and multiple doses of rFGF-2 were well tolerated in rats and pigs.

1916 DETERMINATION OF TISSUE SPECIFIC DRUG TOXICITY USING EPITHELIAL CELLS FROM EIGHT DIFFERENT NORMAL HUMAN TISSUES.

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An important concern in drug development is unexpected toxicity in one or more tissues during clinical trials. This concern is amplified for cancer prevention agents where repeated dosing will occur over the course of the patient's lifetime. To address this issue, we have developed the Human Epithelial Cell Cytotoxicity Assay that uses epithelial cells from normal human skin, kidney, breast, prostate, oral mucosa, cervix, bronchus, and liver to determine changes in growth, mitochondrial function, and PCNA expression or albumin synthesis (hepatocytes). We will present data on twenty potential chemopreventive agents for each of the endpoints for all cell types. Our data indicate that epithelial cells from each tissue source are unique in their response to agent exposure. The data can be used to compare tissue specific differences in agent toxicity and as well as differences between structurally related agents.

1917 HYDROXYAPATITE-CEMENT AS A CARRIER FOR ANTIBIOTICS IN A 6-WEEK TOXICITY STUDY IN THE RABBIT.

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A study was performed to investigate the effectiveness of a new carrier - active substance system [Hydroxyapatite-Cement (HAC) as carrier for antibiotics] in treatment of chronic, posttraumatic osteomyelitis research. In the *in vivo* study, bone infection was induced in the tibia of 29 *New Zealand White Rabbits* by injection of 1mL Na-morrhuat and $3x10^6$ CFU *Staphylococcus aureus*. After 3 weeks, when chronic stage of infection was obtained, 17 animals were treated by debridement and filling the marrow either with HAC alone or HAC mixed with gentamicine (32 mg/g). Animals of the control groups were left untreated. After 6 weeks, all animals were sacrificed. 28 of 29 rabbits developed a chronic osteomyelitis, which could be confirmed by radiological and histopathological investigations. Best evidence of the efficiency of treatment was observed in histopathological and microbiological findings. In all swabs of the control groups taken 6 weeks following infection *S. aureus* were detected which was clonal to the strain used for induction of osteomyelitis. In HAC/gentamicine treated animals no colonies were detectable. In the HAC/gentamicine treated group, there was no histopathological evidence of infection. In all other groups different stages of chronic osteomyelitis were found. No side effect was observed, neither locally nor systemically by HAC or gentamicine. Therefore HAC is considered to be a very effective carrier for antibiotics in treatment of chronic, posttraumatic osteomyelitis.

1918 THE TWENTY-EIGHT DAY ORAL TOXICITY OF NITAZOXANIDE IN THE CANINE.

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Nitazoxanide (N) is a broad spectrum antimicrobial. It also possesses a degree of antiinflammatory activity associated with its potential to inhibit PGE2 and preferential inhibition of COX-2 over COX-1. The purpose of this study was to evaluate the toxicity of N administered orally by gavage for 28 consecutive days to Beagle Dogs and focus on the potential for adverse gastrointestinal effects associated with its NSAID activity. The study was comprised of five total groups. Three groups were composed of three dogs/sex/group and received N at dosages of either 7.5, 15 or 30 mg/kg/day. Two groups were composed of five dogs/sex/group and received either a dosage of N at 60 mg/kg/day or the vehicle, 0.25% carboxymethylcellulose at 5ml/kg/day. Two dogs each from the vehicle control and high dose groups were retained for an additional 14 days following the last dose to be observed as recovery animals. In all cases, the total daily dose of N was divided into two equal portions (3.75, 7.5, 15 and 30 mg/kg) 12 hours apart. No mortality or overt clinical signs of toxicity were observed in any animals during the course of the study. There were no test article associated effects noted on body weights, body weight changes, food consumption values, hematological parameters, clinical chemistry values or coagulation times. Similarly, both quantitative and qualitative urinalyses data were not remarkable in their comparison to controls. Necropsy revealed no treatment-related effects on absolute organ weights, relative organ-to-body weight ratios or relative organ-to-brain weight ratios. Histomorphological evaluation of tissues demonstrated occasional focal congestion of the gastrointestinal tract. This lesion was interpreted as not being associated with the administration of N, since no dose-response relationship was established. Several other incidental lesions were also infrequently noted and are typical of those found in dogs. The findings from recovery animals did not differ from those generated from the main study. Based upon the results of this study, N administered via oral gavage, daily at dose levels of 7.5, 15, 30 and 60 mg/kg/day, divided into two equal portions given at 12 hr intervals for 28 consecutive days produced no abnormal findings at any dose level tested.

1919 SPECIES DIFFERENCES IN TARGET ORGAN EFFECTS OF BENZOYLPHENYLUREA (BPU) IN RATS AND DOGS.

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Toxicity studies were conducted on BPU, a promising antineoplastic therapeutic, in Fischer-344 rats and Beagle dogs. BPU was administered orally to rats at 0, 3, 6, or 12 mg/kg/dose for 5 consecutive days. At the highest dose level, there were significant decreases in WBC (↓79%), RBC (27%)↓, and neutrophils (↓96%). These parameters remained depressed 14 days after the final dose. Myeloid, erythroid, and megakaryocyte depletion of bone marrow and lymphoid depletion of lymph nodes, spleen and thymus were observed at the 12 mg/kg/dose level, but these effects generally resolved after two weeks. In addition, significant testicular toxicity was observed which was not resolved 14 days after treatment ended. A regimen of 0, 5, 10 and 20 mg/kg every 4 days x 3 (q4dx3) did not alter toxicity significantly and testicular lesions were present 4 weeks after the last dose; therefore, effects are dependent on total cumulative dose, irrespective of dosing schedule. Oral administration of BPU to dogs at 0, 0.4, or 4.0 mg/kg/dose given q4dx3 resulted in deaths in 2 of 4 dogs within 7 days of the last 4 mg/kg/dose. Substantial depressions in RBC, WBC, neutrophils and lymphocytes, accompanied by bone marrow depletion of erythroid, myeloid, and megakaryocyte elements; thymic lymphoid depletion; and lymphoid depletion of the tonsils, but no testicular lesions were observed in dogs. No effects were observed at the 0.4 mg/kg/dose. In summary, the total maximum tolerated dose of BPU is ~60 mg/kg (360 mg/m^2) in rats and <12 mg/kg (<240 mg/m^2) in dogs. Myelotoxicity was dose-limiting in both rats and dogs and irreversible testicular lesions were observed in rats only. (Supported by NCI Contract N01-CM-87101.)

1920 DOSE-DEPENDENT HYPERLIPIDEMIA IN RABBITS FOLLOWING ADMINISTRATION OF POLOXAMER 407 GEL.

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Poloxamer 407 (P-407) is a tri-block polymer that exhibits concentration-dependent reverse thermal gelation, a characteristic potentially useful for developing sustained release injectable drugs. While some reports suggest that P-407 is 'non-toxic', rodent studies demonstrate that P-407 induces hyperlipidemia, an action that makes this polymer a questionable drug delivery vehicle. Unfortunately, the majority of earlier studies employed supraphysiologic doses of P-407. The present study examined if lower, clinically useful, doses of gel-forming concentrations of P-407 induced hyperlipidemia in rabbits. Male and female rabbits were injected with 5.5 mg/kg (0.025 mL/kg), 27.5 mg/kg (0.125 mL/kg), or 137.5 mg/kg (0.625 mL/kg) of 22% P-407 and the actions of this polymer on blood chemistry were assessed at 6h, 1d, 2d, 7d, and 14d following injection. Control rabbits received no injection. The highest dose of P-407 (137.5 mg/kg) significantly increased serum triglycerides and cholesterol in both male and female rabbits with the maximum increase observed at 2 d after injection. Male rabbits were more sensitive to P-407 than females following injection of 137.5 mg/kg P-407. The lower doses of P-407 did not alter serum triglycerides or cholesterol. In all groups, serum triglycerides and cholesterol were at baseline levels by 14 d. P-407 did not affect other blood chemistry parameters. Although P-407 induces a dose-dependent hyperlipidemia in rabbits, low doses of this polymer may be used in controlled release drug delivery applications without the untoward hyperlipidemic effect.

1921 CLINICAL STUDY OF HEPATIC EFFECTS OF PHENOBARBITAL IN EPILEPTIC DOGS.

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Hepatotoxicity is a potentially fatal complication of phenobarbital (PB) therapy in dogs. PB may also induce liver enzyme activities. Our study goal was to determine if healthy PB-treated dogs with liver-related serum biochemical (LRSB) abnormalities >2X upper limit of normal (>2XULN) had histopathological evidence of liver injury. Serum PB concentrations (sPB) and LRSB profiles were obtained for 100 epileptic dogs receiving PB. Elevations >2XULN of alkaline phosphatase (AP) and alanine aminotransferase (ALT) occurred in 26% and 11% of PB-treated dogs, respectively. Liver biopsies were obtained from 15 dogs (cases) with at least one LRSB >2XULN (AP and ALT ranged from normal to 40X and 3.3X ULN, respectively); from 8 healthy dogs (controls) not receiving PB; and from 2 PB-treated epileptic dogs (PB-controls) with no LRSB >2XULN. All cases demonstrated induction-associated changes (hepatocyte swelling, ground-glass cytoplasmic appearance), and 14/15 had evidence of hepatic injury (inflammation, necrosis, fibrosis, and/or vascular changes). There was no correlation between severity of histopathological injury and magnitude of AP, ALT, or sPB (range: 37-192 μmol/L), but a significant correlation with duration of therapy (range: 2 months to 6.5 years). Histopathological evidence of liver injury was present in 7/8 control dogs; however, median scores for fibrosis and inflammation were significantly higher ($P<0.05$) for cases. The 2 PB-control dogs also had histopathological evidence of liver injury. No diagnostic or histopathological markers specific for PB-induced liver injury were identified.

1922 TWENTY-ONE-WEEK INTRAVENOUS TOXICITY STUDY OF MGI 114 IN DOGS.

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MGI 114 (6-hydroxymethylacyfulvene, irofulven), a semi-synthetic derivative of the mushroom toxin illudin S, has potent preclinical antitumor activity and is currently in Phase II clinical trials for several solid tumors. The toxicity and reversibility of toxicity in dogs resulting from intravenous administration of MGI 114 for four treatment cycles (daily X 5 days followed by 23 non-treatment days) was assessed. Groups of dogs (4M/4F) were given doses of 0, 0.06, 0.15 and 0.3 mg/kg (1.2, 3, and 6 mg/m^2). Death of 2 high-dose males during the first treatment cycle due to hematologic toxicity resulted in a 20% dose reduction to 0.24 mg/kg (4.8 mg/m^2) for the remaining 3 courses of the study and 2 replacement males were added. Food consumption and body weights were recorded throughout the study. Blood samples were collected for hematology and clinical chemistry on Days 4, 8, 15, 22, and 28 of each course and approximately day 14 and 30 of the reversal period. Electrocardiograms, blood pressure measurements, and samples for urinalysis were collected prior to the initial dosing and the scheduled sacrifices (2/sex on Day 8 and Day 65 of the fourth dosing cycle). Selected organ weights were determined and tissues examined microscopically. Dose-related, but reversible toxicity, observed at doses ≥ 0.15 mg/kg consisted of emesis, decreased body weight and food consumption and hematopoietic toxicity (primarily thrombocytopenia and neutropenia). These effects did not increase in severity from one cycle to the next. No clinically significant hematopoietic toxicity was observed at 0.06 mg/kg. Testicular atrophy and degeneration was the only toxic effect that was not reversible within 60 days of the last dose. Bone marrow toxicity is the only dose-limiting effect of MGI 114 when administered over multiple cycles of a clinically relevant dose schedule to dogs.

1923 DOSE RESPONSE OF A NASAL DOSING SYSTEM FOR MICE.

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A nasal dosing technique was developed to facilitate the local and systemic delivery of conventional and biotech pharmaceuticals in rats, using the large surface area and the rich vascular system within the nasal cavity for drug delivery. This procedure now has been studied for its applicability in mice by conducting a dose response study. Different volumes of radiolabelled 14C-glucose were administered to mice. The volumes were 1, 3, or 5 microL, and the animals were sacrificed at 2.5 or 5 minutes after dosing. These animals were then processed for whole-body autoradiographic imaging in order to assess the distribution of the radiolabelled 14C-glucose within a variety of tissues. The brain, liver, blood, myocardium and lungs were selected and evaluated for their radioactivity concentration. After 2.5 minutes, the radioactivity levels between 1, 3, and 5 microL groups were increased for the blood (38 and 217% respectively) and myocardium (158 and 161%, respectively). A dose response increase was not observed after 2.5 minutes for the remaining tissues. However, 5 minutes after dosing the radioactive concentrations for all tissues had a dose response increase, except for the lung. The mean percentage increase of the tissues between 1 and 3 microL-treated animals ranged from 26 to 114% and between 3 and 5 microL-treated animals the range was 95 to 186%. Thus, a relationship exists between the volume of a test substance and the tissue distributions using mice and small dosing volumes.

1924 MASS BALANCE AND TISSUE DISTRIBUTION OF LEFLUNOMIDE (SU101) IN THE RAT.

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SU101 (Leflunomide), N-(4-[Trifluoromethyl]phenyl)-5-methylisoxazole-4-carboxamide, is an inhibitor of PDGF receptor signaling and is being developed as an anti-cancer drug for the treatment of glioblastoma. The absorption, distribution, metabolism, and excretion (ADME) of [14C]SU101, labeled with 14C in either phenyl or isoxazole ring, was investigated in male and female rats. Recovery of total radioactivity from urine, cage rinse, feces, expired air and tissues up to 168 hours post-dose were determined after bolus intravenous injection at 25 mg/kg. The parent drug, SU101, was readily converted *in vivo* to SU0020 primarily, followed by further conversion to other metabolites including trifluoromethylanaline (TFMA) and TFMA-related derivatives. Total recovery ranged from 92-98% in males and 93-100% in females, with ~7% as 14CO2 (derived from isoxazole ring labeled compound). Tissue distribution was studied at 0, 6, 12, 24, and 48 hours post-dose following a thirty minute intravenous infusion (via jugular vein cannula) at a nominal dose of 25 mg/kg body weight. At 48 hours after dosing, very low concentrations of radioactivity remained in all tissues, representing about 3% of the total dose. Radiochromatographic data indicated that SU101 was rapidly transported into the brain and existed as the parent molecule (SU101), even though some amounts of the drug had been converted to SU0020 in plasma. These data support the use of SU101 in the treatment of glioblastoma.

1925 A CHRONIC REPEAT DOSE TOXICITY STUDY OF A NOVEL ANTHRACYCLINE IN DOGS.

J. Brightwell. *Research Toxicology Centre, Rome, Italy.* Sponsor: A. I. Nikiforov.

MEN 10755 is a new disaccharide anthracycline in early clinical development. It possesses a notable antitumor activity in a wide panel of human tumors xenografted in nude mice, including tumors naturally resistant to other anthracyclines. As part of the non-clinical development program, a repeat dose intravenous (iv) study has been conducted in dogs. Groups of 4 male and 4 female beagle dogs were given iv injections of MEN 10755 at dosages of 0, 1.0, 2.5 and 3.0 mg/kg. Injections were given once every 2 weeks for a total of 8 injections. Treatment was followed by a 13 week treatment-free period. The 2 week interval between treatments was determined on the basis of complete recovery from drug-induced leukocytopenia. The high dose level was selected to be close to the single dose MTD (maximum tolerated dose), which was determined to be in the region of 3.75 mg/kg. Principal *in vivo* signs were diarrhea and/or emesis in the first days after each treatment cycle; severe hair-loss, from which there was complete recovery during the 13 week treatment-free period; reduced body weight gain in the mid and high dose groups; leukocytopenia after each treatment cycle. Major clinical chemistry changes were only seen towards the end of the study and were probably related to late occurring cardiotoxicity. Testes and spleen weights were reduced. The study demonstrates that the pattern of toxicity of MEN 10755 in dogs is similar to that seen with existing drugs of the same class. However, these toxic effects occurred at markedly higher dosages of MEN 10755 compared with other anthracyclines.

1926 CLINICAL MANIFESTATIONS IN DIAZEPAM AND ETHANOL INTOXICATED WHITE RATS: TOLERANCE TO ANAESTHETICS.

M. R. Basha and G. R. Reddy. *Sri Venkateswara University, Department of Zoology, Tirupati, India.* Sponsor: P. S. Kodavanti.

It is well known that in the medical practices the chronic alcoholic patients are resistant to usual doses of anaesthetics. However, age-related changes in the development of tolerance are still unclear. The present study was designed to elucidate the age-related changes in tolerance development to various doses of anaesthetics such as Diazepam and Thiopental in rats following chronic intake of Diazepam or Ethanol or both. Anaesthetic responses were measured using righting reflex test (RRT) for induction of anaesthesia, hot plate test (HPT) for analgesia, and sleep monitoring test (SMT) for duration of anaesthesia. The experiments were performed following the administration of Diazepam and Thiopental. The data suggests that the both young and old control rats were sensitive to both Diazepam and Thiopental as compared to Ethanol and Diazepam treated rats. The combined intake of both Ethanol and Diazepam enhanced the tolerance level as compared to the rats treated with Ethanol or Diazepam alone. In general, the old rats were more affected by the anaesthetic drugs which could be attributed to reduction in the drug metabolizing mechanism(s) during senescence.

1927 EFFECT OF CRF RECEPTOR-1 INHIBITION ON IMMUNE SYSTEM FUNCTION.

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A role for the Corticotrophin Releasing Factor receptor-1 (CRF R1) in host defense, based on leukocyte expression and a central role in the HPA axis, has been postulated. The effects of CRF-R1 antagonism on host defense were examined in a series of rat studies with the potent CRF R1 antagonist, DMP 904 (Ki=1 nM human, 0.6 nM rat). DMP 904 was dosed (80 mg/kg/day) to provide continual high level receptor inhibition. The effect of CRF-R1 inhibition on lipolysaccharide (LPS)-induced toxemia was examined in rats sensitized with D-galactosamine. LPS-induced toxemia, as assessed by serum transaminase activities, was not altered by DMP 904. The sheep red blood cell antibody-forming assay was used to assess potential effects on T- and B-lymphocyte function. Rats dosed with DMP 904 or vehicle demonstrated comparable responses. Finally, we investigated the effect of CRF-R1 inhibition on the clearance of a facultative intracellular bacterium, Listeria monocytogenes. Bacterial clearance was not impaired in the presence of DMP 904. Collectively, results of these experiments have not demonstrated a clear role for CRF-R1 in host defense. Pharmacologic inhibition of this receptor is, therefore, unlikely to compromise immune system function.

1928 SHORT PHOSPHOROTHIOATE OLIGOMERS FOR CHELATION AND ENHANCED IRON EXCRETION.

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Iron overload is a significant human health problem that will benefit from effective, nontoxic chelation therapy. We previously observed an increase in urinary iron excretion following systemic therapy with a phosphorothioate oligodeoxynucleotide (PS-ODN) in myelodysplastic patients. In vitro binding studies with a 27 base PS-ODN confirm metal binding properties with a higher affinity for mercury, iron, tin, lead and cadmium. These observations lead to the hypothesis that a short PS-ODN may be designed to evade toxicities associated with PS-ODNs but would also possess properties which would enhance the excretion of iron. A 10 base poly-cytidine PS-ODN with a 3'-ribose modification was synthesized and purified by HPLC. Four groups of eight rats each were injected with 10 mg of iron dextran bi-weekly for 4 weeks to produce iron overloaded animals. Animals were then treated with saline, desferoxamine, one injection of 100 µg of poly-C PS-ODN, or three consecutive days of 30 µg of poly-C PS-ODN. Urinary excretion, histology and modulation of metal responsive genes in the liver were evaluated. Analysis of liver tissues reveals diminished iron per milligram of liver tissue compared to saline and desferoxamine treated animals. These studies warrent the further development of short PS-ODNs for nontoxic chelation therapy.

1929 HPLC METHOD FOR THE DETERMINATION OF SELAMECTIN (REVOLUTION®), A NOVEL PET ENDECTOCIDE.

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Selamectin is a semi-synthetic avermectin indicated for prophylactic use against heartworm and for the control or treatment of endo- and ectoparasites in dogs and cats. To enable detection of very low levels of selamectin in biological fluids, an analytical method similar to that used for other avermectins (Demontigny et. al. 1990) in general was developed. The analyte was extracted from plasma matrix using methanol/acetonitrile mixture. The extracted analyte was then subjected to a series of derivatization steps which involved use of triethylamine, trifluoroacetic anhydride, and ammonia. The fluorescent derivative was identified as containing an N-trifluoroacetamide group. The fluorescent derivative in the extracted sample was separated by HPLC and detected using a fluorescence detector. Two dynamic ranges, 10 to 200 ng/ml and 25 to 800 ng/ml, were established for this assay. The recovery of the analyte from the plasma matrix ranged from 91-105%. The intra and inter-assay accuracy was determined by analyzing quality control standards at the low (20% above), mid, and high (20% below) concentrations of the standard curve

and was between 91-109% and 88-101%, respectively. The precision of the assay was between ±1 to ±10% over a plasma concentration range of 10 - 800 ng/mL. In summary, the validated assay proved to be sensitive and reproducible in quantitating plasma levels of the drug ranging from 10 to 800 ng/ml. Demontigny P, Shim JSK, Pivnivhny JV (1990). Liquid-chromatographic determination of ivermectin in animal plasma with trifluoroacetic-anhydride and N-methylimidazole as the derivatization reagent. Journal of Pharmaceutical and Biomedical Analysis, 8(6):507-511

1930 THE VALUE AND ETHICS OF USING HUMAN DATA FOR THE REGISTRATION OF PESTICIDES.

E. E. McConnell. *ToxPath, Inc., Raleigh, NC.*

A joint meeting of the EPA Science Advisory Board and FIFRA Science Advisory Panel was convened on December 10-11, 1998 to provide advice and comment to the EPA on issues related to data derived from testing on human subjects, particularly the use of human data for making pesticide registration decisions. Both scientific and ethical questions were raised about such data, the manner in which they are obtained, and how these data should be used in risk assessments. Proponents of using human data felt such data were of prime value for developing a risk assessment of a given pesticide, if the data were obtained in a scientifically credible and ethical manner, similar to what is expected in the field of pharmaceutical testing. In fact, it was felt that testing in human volunteers was particularly important in the case of pesticides because of their potential for contamination of food and water. Opponents felt it was unethical to test pesticides in human volunteers under most circumstances. They posed two basic arguments for supporting their case: 1) Pesticides are unique chemicals because they are designed to be "poisons," and 2) Many pesticides are neurotoxic and it is unethical to test neurotoxins in people. This is a particularly important issue because it impacts on some fundamental concepts in toxicology and is a basic policy decision for the EPA.

1931 METALLOTHIONEIN SUBCELLULAR LOCALIZATION AND REGULATION OF CELL CYCLE AND APOPTOSIS.

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Metallothionein (MT), a transition metal binding protein, has been implicated in a diversity of biologic functions. Recent work has provided new insights into the subcellular localization of MT and its role in regulation of cell cycle and apoptosis. A better understanding of cellular and molecular mechanisms for MT protection from oxidative tissue injury has also been achieved. These areas of high impact progress in MT research are the focus of this symposium. MT is well below the size exclusion limit for diffusion through the nuclear envelope. However, its nuclear localization involves the action of glycoproteins and is energy dependent. Translocation of MT into and out of nucleus is regulated by cell cycle progression and linked to cell differentiation. Cellular redox status also regulates the subcellular localization of MT. Cytoplasmic MT interferes with signaling pathways evoked by oxidative stress. For example, p38 mitogen-activated protein kinases (MAPKs) are activated in cardiac myocytes treated with doxorubicin (DOX), an antitumor agent causing cardiac apoptosis by producing reactive oxygen species. MT inhibits DOX-induced p38 MAPK activation and apoptosis. DOX also induces mitochondrial cytochrome c release, thereby causing activation of proteases, such as caspases 9 and 3, that are involved in apoptosis. MT inhibits this process as well. Nuclear MT protects against oxidative DNA damage and regulates activities of transcription factors. For example, MT prevents DNA damage induced by antitumor agents and regulates nuclear factor κB activity. Continued efforts on these fundamental investigations will help to further define these important roles of MT in regulation of cell cycle and apoptosis.

1932 REGULATION OF NUCLEAR AND CYTOPLASMIC LOCALIZATION OF METALLOTHIONEIN.

J. S. Lazo. *University of Pittsburgh, School of Medicine, Pittsburgh, PA.*
Sponsor: Y. J. Kang.

Spatial compartmentalization of intracellular macromolecules represents a potentially powerful yet poorly understood post-translational regulatory mechanism. Metallothionein (MT) represents a prototype low molecular weight, stress-activated protein with protective functions against exogenous toxins that is found partitioned in the nucleus or cytoplasm of cells even though it has a size well below the exclusion limit for diffusion through the nuclear envelope. Work from several laboratories indicate that the subcellular distribution of MT is cell type specific and inherited. Three general approaches have been used to detect MT subcellular localization: immunocytochemistry, fusion protein tagging and exogenous fluorescent labeling techniques. Nuclear sequestration appears to require cellular ATP, is saturable and involves one and more nuclear glycoproteins. Recent data also suggest nuclear entry is diffusion-based and nuclear retention is an active process that involves at least two biochemically distinct binding locales. These nuclear binding sites may have an important role in regulating the protective function of MT or other low molecular weight proteins against heavy metals and other toxins.

1933 INHIBITION OF METALLOTHIONEIN OF OXIDATIVE STRESS-INDUCED APOPTOSIS.

Y. J. Kang, G. W. Wang, Z. X. Zhou and J. B. Klein. *University of Louisville, Louisville, KY.*

Cardiac-specific metallothionein (MT) overexpressing transgenic mice are highly resistant to oxidative myocardial injury induced by doxorubicin (DOX), an anticancer agent. This resistance is associated with MT inhibition of lipid peroxidation. Most of the elevated MT is localized in cytosol. DOX induces myocardial apoptosis, which is highly responsible for the cardiomyopathy. MT significantly reduces the number of myocytes undergoing apoptosis. At least two distinct signaling pathways are involved in the DOX-induced myocyte apoptosis, as demonstrated by using primary cultures of neonatal mouse cardiomyocytes. First, DOX activates p38 mitogen-activated protein kinases (MAPK), which plays an important role in DOX-induced apoptosis, as revealed by a specific p38 MAPK inhibitor, SB203580, which partially inhibits DOX-induced apoptosis. Second, DOX causes mitochondria to release cytochrome c, which leads to activation of caspases 9 and 3, and subsequent apoptosis. MT inhibits DOX-induced p38 MAPK activation, which correlates with its inhibition of apoptosis in the myocyte cultures. MT also blocks DOX-induced cytochrome c release. This event is accompanied by inhibition of activation of caspases 9 and 3 and suppression of apoptosis. Two possible mechanisms are implicated for MT protection from oxidative stress-induced apoptosis *in vivo*: (1) zinc released from MT is an anti-apoptotic agent, and (2) MT reacts with oxidants to preserve cellular reductant potential.

1934 NUCLEAR TRANSLOCATION OF METALLOTHIONEIN DURING CELL CYCLE PROGRESSION AND DIFFERENTIATION.

M. G. Cherian and M. D. Apostolova. *University of Western Ontario, London, Ontario, Canada.*

Although metallothionein (MT) is localized mainly in the cytoplasm of hepatocytes in adult liver, its translocation to the nuclei has been observed during development in fetal liver, and after partial hepatectomy. The high levels of MT in the cell nucleus may be related to the increased requirement for Zn and Cu for several metallo-enzymes and transcription factors for gene expression. High levels of MT expression including its nuclear localization have been observed in proliferative epithelial cell tumors which are not well differentiated. The cellular signals responsible for nuclear localization of MT during proliferation and differentiation are not well defined. Studies in myoblast cell lines (L_6 and H9C2) showed that during early stage of differentiation with either insulin-like growth factor-1 or low serum concentration MT is translocated to the nucleus along with Zn. This was demonstrated by FITC labeling of MT antibody and TSQ binding to Zn using fluorescent confocal microscopy. When differentiation is completed as shown by morphology or creatinline kinase activity, both MT and Zn are found only in the cytoplasm or myotubes. The results demonstrate that nuclear localization of MT in early differentiation coincides with the increased requirement for Zn. In 3T3-L1 fibroblasts, when the growth is synchronized with low serum, the cells enter intoG_0/G_1 phase of cell cycle, and MT is detected in the nucleus. When cell cycle is blocked at S-phase with aphidicoline, an inhibitor of DNA polymerase-alpha, the nuclear localization MT persists. However, after removal of aphidicoline, the cells re-enter the cell cycle, and at G_2/M phase, MT is localized mainly in the cytoplasm. These results demonstrated that localization of MT in the nuclear occurs during G1/S phase transition of cell cycle when the requirement for Zn is highest.

1935 REGULATION OF NUCLEAR FACTOR-κB ACTIVITY BY METALLOTHIONEIN.

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Metallothionein (MT), a low molecular weight, cysteine-rich metal binding protein, has multiple functions such as detoxification of heavy metals and protection of cells against reactive oxygen species. As MT has an ability to scavenge hydroxyl radicals, MT may control intracellular redox status. Recently, several evidences suggest that some transcription factors such as nuclear factor κ-B (NF-κB) are regulated by intracellular redox status. One of the functions of MT may be regulation of the activity of such factors. We here examined whether or not MT regulates the activity of NF-κB using the MT null embryonic cell lines (MT-/- cells) established from MT null mice. We first found that tumor necrosis factor (TNF)-induced activation of the binding of NF-κB protein to DNA in wild type MT+/+ cells was lower than that in MT-/- cells by an electrophoretic mobility shift assay. The NF-κB activation in MT-expressing cells obtained from MT-/- cells by the transfection of mouse MT-I (mMTI) gene was also significantly lower than that in MT-/- cells. In addition, the transfection of MT gene suppressed NF-κB-dependent gene expression induced by TNF. These results demonstrate that MT may function as a negative regulator of NF-κB activity. To clarify the mechanism by which MT regulates NF-κB activity, we next examined the effects of transfection of mMT-I gene on TNF-induced degradation of inhibitory protein IκB. IκB was degraded after the treatment of TNF in mock-transfected MT-/- cells. In mMT-I-transfected cells, however, a lesser extent of IκB degradation was observed in response to TNF. These results suggest that MT may suppress TNF-induced NF-κB activation by inhibiting IκB degradation in the cytoplasm of cells, although the possibility still remains that MT may also regulate NF-κB activity in the nucleus.

1936 NEW HORIZONS IN METALLOTHIONEIN RESEARCH.

M. P. Waalkes and J. Liu. *NIEHS, Research Triangle Park, NC.*

Metallothionein (MT) is a remarkable, low-molecular weight protein with a variety of potential functions. However, none has been conclusively defined and, unfortunately, even the advent of MT over-expressing transgenic and MT knock out animals and cells has still not allowed for a conclusive assignment of functionality. MT, which normally binds zinc, is likely important in metal toxicity, essential metal metabolism, and resistance to oxidative injury, but beyond this precise roles are not well characterized. The intentional nuclear localization of MT presents several intriguing possibilities for control of cell cycle and gene regulation and probably impacts development and carcinogenesis. A model whereby nuclear MT provides zinc for enzymes during their synthesis or for transcription factors that could modify gene expression at critical times would likely include specific MT-binding proteins and a means for controlled zinc release form MT. However, very little is known about exactly what signals the nuclear accumulation of MT and how the zinc bound to MT is released and these should be fertile avenues for future studies. The release of zinc from MT may be important blocking apoptosis, as zinc can inhibit caspases, but MT also sequesters reactive intermediates and thereby inhibits oxidative stress-induced apoptosis. So defining the conditions under which MT acts as an proapoptotic or antiapoptotic agent is also another important future direction. It appears that MT may also effect transcription factor activity outside the nucleus through post-translational events, such as inhibition of degradation inhibitory factors. So the roles of MT in the control or modification of gene expression, cell cycle, or apoptosis need to be defined at many different levels and this creates a large number of fascinating research opportunities.

1937 TOXICOLOGICAL DATABASE MINING IN THE TWENTY-FIRST CENTURY.

M. J. Cunningham. *Incyte Pharmaceuticals, Palo Alto, CA.*

Genomic technologies are currently being used as an investigative tool in risk and safety assessment. Data obtained from gene expression assays using microarrays (10^5 to 10^6 data points/assay) can be overwhelming. Recent research into data mining and visualization may provide answers to this key issue. Initially, the raw data may need to be filtered for noise and threshholding parameters set for the differential expression values. Then, several methods such as subsetting and agglomerative clustering, multidimensional scaling, motif discovery, and prinicipal components analysis may be applied to numerically analyze the trends due to various compound treatments. Finally, the trends can be visualized using pattern recognition methods. Data analysis by these methods may help predict unknown or alternative modes of action, classification of unknown NCEs by their expression profiles and comparison to known compound profiles and allowing further analysis of dose-response relationships and chemical interactions. This symposium will start with an overview of the current database mining practices and then delve into each method available and its application to a gene expression data set (containing several time points) resulting from the treatment of rat livers with three different hepatotoxins.

1938 IDENTIFYING THERAPEUTIC PROTEINS AND TARGETS IN DNA DATABASES.

J. R. Greene. *Schering-Plough Research Institute, Kenilworth, NJ.* Sponsor: M. J. Cunningham.

The rapid and voluminous accumulation of genomic and cDNA sequence data as well as the rapid advance in genome-scale analysis technologies have presented opportunities and challenges in the area of new drug development. This onslaught of information has affected drug development efforts in virtually all therapeutic areas. Not only must the sequences be analyzed and evaluated for their potential as therapeutic proteins or targets, but the results of multiple analyses must be communicated to the appropriate laboratory scientists. In the environment of a large pharmaceutical company, several practical approaches must be applied to this problem given the diverse needs of the company's research community. This presentation will discuss some of the ways that genomic and post-genomic information can be integrated into the drug discovery process. Examples will be provided from pre-clinical drug development efforts in the areas of oncology and infectious diseases, two areas in which genomic sequence information and microarray gene expression analysis have driven drug development programs out of the databases and into the laboratories.

1939 FUNDAMENTAL GENE EXPRESSION ANALYSIS TO TOXICOLOGICAL PROFILING.

R. Somogyi. *Incyte Phamaceuticals, Palo Alto, CA.* Sponsor: M. J. Cunningham.

Through large-scale gene expression analysis we aim to increase the depth of toxicological and therapeutic drug-effect profiling, and accelerate the discovery of key molecular indicators and processses. Success hinges on the integration of computational tools for data analysis with measurements that reflect the breadth of the biological complexity, e.g. large numbers of genes surveyed on microarrays. Basic data analysis can be broken down into three consecutive stages: 1) filtering of noisy data, 2) thresholding for differential expression and subsetting, and 3) clustering of expression response profiles. These techniques allow us to classify drugs according to their gene expression profiles and identify typical pathways of drug action. We have applied this analysis to set of temporal expression profiles (6 time points; >7000 genes) of rat liver to three different drugs from different classes. Comparison of these results to existing knowledge allows for a critical evaluation of these new technologies in how much they reproduce previous conclusions and provide new insight.

1940 APPLYING VISUAL AND NUMERICAL PATTERN RECOGNITION TOOLS TO GENE EXPRESSION DATA.

T. J. Downey, D. J. Meyer and M. K. Greene. *Partek Incorporated, St. Peters, MO.* Sponsor: M. J. Cunningham.

Microarray technologies have created challenges for biologists and data analysts alike. Designing an experiment to achieve clearly defined research goals is difficult during the uncertainty surrounding the infancy of new and powerful technologies. We will look at visual and numerical techniques used in pattern recognition and statistical analysis to identify patterns in the genes. Two examples are a.) genes which exhibit significantly similar expression patterns within treatment groups but different patterns in different treatment groups and b.) genes whose expression patterns can be identified as statistically linked to a particular treatment. The analyzed data comes from a microarray experiment where rat liver genes are measured under toxic doses from three different compounds. The analysis methods will include Pincipal Components Analysis (PCA) and Multi-Dimensional Scaling (MDS) for visualization of expression patterns, and cluster analysis, analysis of variance (ANOVA) and t-tests to identify patterns of genes which display significant similarity and/or

dissimilarity in expression within and between the treatment groups. In short, we will identify which genes fall into particular groups, which genes are unlike all others, and which genes appear to be uninvolved in the treatment(s).

1941 COMBINING CHEMICAL STRUCTURE AND GENE EXPRESSION ANALYSES FOR TOXICOLOGICAL PROFILING AND PREDICTION.

E. W. Steeg. *Molecular Mining Corporation, Kingston, Ontario, Canada.* Sponsor: M. J. Cunningham.

As ever-larger databases arise and are linked over networks, knowledge discovery and data mining (KDD) methodologies are making an impact in toxicological analysis and prediction. In recent years two distinct streams of KDD application have emerged. One stream, the focus of this session, is characterized by clustering and pattern recognition in high-throughput gene expression data. The second, stream features motif discovery in 2D and 3D chemical structure databases: for example, the discovery of recurring substructures in compounds of different known toxicity classes, using inductive logic programming (ILP). By uniting the gene-based and structure-based approaches within one discovery methodology, it is possible to get more value from the available data. I will discuss how our combined approach contributes to the creation of systems for the classification and prediction of mode of action, dose-response relationships and chemical interactions with respect to toxicity data.

1942 TRICHLOROETHYLENE HEALTH ASSESSMENT: INTRODUCTION.

W. Farland. *USEPA, Washington, DC.*

USEPA's trichloroethylene health assessment is being shaped by several new developments in risk assessment. Foremost among these developments is revision of EPA's cancer guidelines which emphasize detailed characterization discussions; replace classification groups with a brief narrative description; and promote the use of mode-of-action information in evaluating relevance of experimental results, suggesting approaches for dose-response assessment, and identifying susceptible populations. Also evident is a trend toward harmonizing cancer and non-cancer assessment methods. Other influences come from recommendations for improving risk assessment by the National Research Council, Executive Order 13045 which focuses on risks that may disproportionately affect children, and on EPA guidance on cumulative risk assessment. The updated trichloroethylene assessment illustrates these concepts. The assessment takes steps toward integrating assessment of cancer and non-cancer effects, using common methods as it considers mode of action for both; uses pharmacokinetic models and uncertainty analysis of these models; and applies benchmark-dose models for some cancer and non-cancer effects utilizing similar dose-scaling procedures. Further, the assessment discusses intrinsic and acquired factors that affect susceptibility to TCE, and discusses joint effects of other chlorinated solvents and agents that have metabolic pathways, potential modes of action, and toxicity effects similar to TCE. This presentation provides a background as support for the new integrative approach to the trichloroethylene risk assessment and introduces the more detailed presentations that follow.

1943 WHAT DO THE HUMAN DATA TELL US?

C. Siegel Scott[1] and D. Wartenberg[2]. [1]*USEPA/NCEA, Washington, DC and* [2]*UMDNJ-RW Johnson Medical School, Piscataway, NJ.* Sponsor: W. H. Farland.

The updated trichloroethylene (TCE) health assessment utilizes mode of action data to understand the development of carcinogenic effects. Findings from a review of the epidemiologic body of literature are discussed from this perspective. The cancers that are most well studied and show the strongest evidence include kidney cancer, liver cancer, cervical cancer, and non-Hodgkin's lymphoma. Several of these findings are biologically plausible. Trichloroethylene exposure has demonstrated target organ toxicity to the kidney, liver, and immune system. The kidney cancer finding is strengthened by the observation of larger risks among those with genetic polymorphisms of the glutathione-S-transferase metabolic pathway and by recent molecular epidemiologic observations. The liver cancer observation suggests that humans may not be very different from rodents regarding susceptibility to TCE. TCE and metabolites affect carbohydrate handling. In support of the linkage between carbohydrate metabolic control and cancer is the association of glycogen storage disease in humans with early development of hepatic adenomas and the increased risk diabetics show for liver cancer. The remaining site-specific cancers have more limited direct mechanistic support. The review of the TCE epidemiologic studies reinforces the essentiality of information from humans when evaluating mode-of-action hypotheses.

1944 IS THE INDUCTION OF MUTATION A KEY EVENT IN THE ETIOLOGY OF TRICHLOROETHYLENE-INDUCED TUMORS?

M. Moore. *USEPA NHEERL, Research Triangle Park, NC.*

This presentation addresses the evidence that trichloroethylene (TCE) or its metabolites might mediate tumor formation via a mutagenic mode of action. We review and draw conclusions from the published mutagenicity and genotoxicity information for TCE and several of its metabolites. Consistent with the thinking outlined in the proposed Cancer Risk Assessment Guidelines, which provide for an assessment of the key events involved in the development of specific tumors, we provide a new and general strategy for interpreting genotoxicity data that goes beyond a simple determination that the chemical is or is not genotoxic. For TCE, we conclude that the weight of the evidence argues that chemically induced mutation is unlikely to be a key event in the induction of human tumors that might be caused by TCE. This conclusion derives primarily from the fact that TCE and those metabolites for which information is available require very high doses to be genotoxic. However, conclusions as to whether TCE will induce tumors in humans via a mutagenic mode of action cannot be drawn from the available information. More research, including the development and use of new techniques, is required before it is possible to make an assessment as to whether chemically induced mutation is a key event in any human tumors resulting from exposure to TCE.

1945 INTEGRATION OF MODE-OF-ACTION INFORMATION TO GUIDE DOSE-RESPONSE ANALYSES.

V. J. Cogliano. *USEPA NCEA, Washington, DC.* Sponsor: W. H. Farland.

New approaches are being used in the updated health risk assessment for trichloroethylene (TCE). Past TCE assessments emphasized linear extrapolation from bioassays in which TCE induced liver tumors and lung tumors in mice and kidney tumors in rats. The updated TCE assessment is an example of how EPA's new cancer guidelines encourage the use of mechanistic information in risk assessment. Pharmacokinetic and mechanistic studies on TCE are able to suggest better ways to use the high-dose animal results to make inferences about human environmental exposure and to guide approaches to dose-response modeling. Further, the revised cancer guidelines encourage the utilization of biologically based modeling and, as a default, establishes a two-step dose-response process that separates analysis of observed data from extrapolation to lower doses. Pharmacokinetic models describe how TCE's cancer response is mediated though several metabolites. Mechanistic information is evaluated to identify the key events involved in tumor formation and indicate whether linear or nonlinear extrapolation to lower doses is more appropriate.

1946 FUTURE DIRECTIONS IN THE USE OF MODE OF ACTION INFORMATION IN ASSESSING CANCER RISKS FROM TRICHLOROETHYLENE (TCE).

R. J. Bull. *Battell-Pacific Northwest National Laboratory, Richland, WA.*

TCE is an environmental chemical that has diverse data implicating it as a carcinogen. Its activity between species and strains is diverse with tumors appearing at different sites in experimental animals. In all cases very large doses have been required to induce tumors. Epidemiological studies provide limited support for TCE as a human carcinogen for a variety of reasons. Different metabolites and modes of action induce tumors in the different organs and species. Moreover, the type of tumor produced in each species relates largely to organs in which there is a high spontaneous rate. Assessing liver cancer induction was relatively straight forward because there was experimental evidence that certain metabolites (principally trichloroacetate and perhaps dichloroacetate) are both liver carcinogens and produced at concentrations in the metabolism of TCE to account for liver tumor induction.

The modes of action of both metabolites affect cell cycle kinetics and do not require direct interaction with cellular DNA, allowing a consideration of liver cancer risk being non-linear with dose. What remains to be pursued is a better understanding of the mechanisms that actually underlie the induction of these tumors. Kidney tumor induction remains unresolved. Pharmacokinetic modeling of the kidney needs to be better defined and key events in tumor induction identified. One metabolite, dichlorovinylcysteine (DCVC) is both mutagenic and cytotoxic. However, it is important to also recognize that the involvement of DCVC as the renal carcinogen remains a hypothesis, not a fact.

1947 TOXICOLOGICAL CONSIDERATIONS OF PHARMACEUTICALS FOR PEDIATRIC PATIENTS.

H. V. Sheevers[1] and M. S. Tassinari[2]. [1]*Milestone Biomedical Assoc., A Division of PAI-SAIC, Rockville, MD and* [2]*Pfizer Central Research, Groton, CT.*

The number of pharmaceuticals designed and tested clinically for use by pediatric patients is rapidly increasing. Additionally, it has long been recognized that important stages of development continues postnatally in both humans and animals. These two factors—more drugs developed for pediatric populations and the importance of needed evaluations of postnatal development—have led toxicologists to recognize and manage a rapidly increasing demand for juvenile animal studies. At the same time, however, it is increasingly recognized that the necessary scientific basis to design reasonable studies may not exist. This workshop will introduce some of the issues associated with testing in juvenile animals. The workshop will first focus on the development of several important organ systems that undergo postnatal development, including skeletal growth, the immune system, and the central nervous system. It is clear that some drugs, such as the corticosteroids, may affect development. It is not clear, however, whether all drugs will affect pediatric populations differently from adults during this postnatal developmental period. Available animal models will be described and evaluated, and regulatory expectations will be discussed. It is expected that this workshop will help define the questions that studies should answer, review current animal models, and identify research gaps that should be considered prior to additional regulation. While this workshop will be focussed primarily on recent testing requests by the US FDA, recent changes in EPA policy will be noted as well. The workshop will conclude with a presentation of several case studies of pharmaceuticals intended for pediatric patients.

1948 SKELETAL GROWTH AND GROWTH DYNAMICS: ASPECTS TO CONSIDER FOR THE DESIGN OF JUVENILE ANIMAL STUDIES.

M. S. Tassinari. *Pfizer Central Research, Groton, CT.*

How well a child or young animal grows is typically viewed as a measure of the health and nutritional status of that individual. Recent changes in the labeling for the use of corticosteroids in children have highlighted the need to carefully assess overall growth in drug safety testing in addition to the evaluation of specific organ systems. Growth is easily measured by following the incremental changes in height and weight. Growth velocity yields a useful measure of growth over time and is easily determined by frequent measures of weight and height (length) in most model systems. Catch-up growth provides periods of recovery after periods of growth inhibition. Recovery from growth failure is influenced by the age and stage of maturation of the young animal at the time of the growth impairment, as well as the duration and the severity of the insult. Postnatal skeletal growth is affected not only by nutrition but also by genetics, gender and hormonal status. Most maturational changes in the skeleton are closely linked to the maturation of the reproductive system. Sexual dimorphism evident at puberty is inherent in most species and affects both skeletal mass and size. In addition to discussion of the above elements influencing growth, approaches to the evaluation of skeletal growth and overall growth in available animal models will be discussed in the context of regulatory testing strategies.

1949 ANIMAL MODELS AND CONSIDERATION OF IMMUNE SYSTEM DEVELOPMENT.

K. L. Hastings. *FDA/CDER, Rockville, MD.*

The developing immune system is an important potential target of drug toxicity. Assessing immunotoxicologic effects in animals can provide clues that such effects could be of concern in pediatric patients, but as yet there appears to be a very small data base upon which to draw definitive conclusions. In some cases, *in utero* exposure (in reproductive toxicology studies) is used to model potential adverse effects of pediatric exposure. For example, *in utero* exposure to diazepam has been shown to impair host defense to challenge infections in rats and hamsters. Similarly, in Fisher 344 rats maternal exposure to lead has been shown to impair immune function in the offspring. In both cases it is not clear that these effects would also be seen in animals first exposed after birth. The timing of immune system development in animals relative to humans presents a further complication. The immune systems of rodents are immature relative to humans at birth: it is thus likely that rodents would be more sensitive to xenobiotic-induced immunotoxicity when exposed *in utero*. Cocaine and ethanol have been shown to be immunotoxic in Lewis rat pups: the implications for human immunotoxicity are unclear given the species differences in immune system development. Developmental immunotoxicology is an area where much research is needed and ideas concerning particular problems and approaches will be presented.

1950 CNS DEVELOPMENT: DEVELOPMENT OF THE AXIS AND CONSEQUENCES OF EARLY DRUG EXPOSURE.

M. T. Williams and C. V. Vorhees. *Children's Hospital Research Foundation, Cincinnati, OH.*

The development of the central nervous system (CNS) is dependent upon many different factors. The influence of steroid hormones on CNS development has been investigated extensively in recent years. The adrenal steroids, namely the glucocorticoids and mineralocorticoids, have been implicated in both cell death and cell survival, especially in the hippocampus, an area important in spatial learning and memory. Early environmental influences, such as drugs that cause release of glucocorticoids, may have deleterious effects on the ability of an animal to learn and recall memories or may result in physiological changes that cause an animal to behave abnormally when faced with a challenging situation. Behavioral testing, such as learning and memory paradigms, following drug exposure during development is a useful measure to detect functional changes in the CNS that may not be related to gross physical alterations. Animal models used to detect changes in learning and memory are invaluable, since controlled studies in humans presents many ethical concerns. This presentation will focus on the development of the central nervous system, age-related correlations between human and rat brain development, and the development of the HPA axis. Furthermore, animal models of learning and memory following exposure to substituted amphetamines during different periods of neonatal development will be discussed. We will show data demonstrating that exposure of neonatal animals to substituted amphetamines produces no alterations to neurotransmitter systems, pronounced elevations in pituitary and adrenal hormones during drug exposure, and deficits in learning and memory when tested as adults. The effects on learning and memory may be related to elevated levels of HPA axis hormones produced by drug exposure during development. Such effects may have implications for understanding potential risks associated with pediatric exposure to new drugs that cause HPA axis activation.

1951 INTERNATIONAL REGULATORY CONSIDERATIONS OF PHARMACEUTICALS FOR PEDIATRIC PATIENTS.

H. V. Sheevers. *Milestone Biomedical Assoc., A Division of PAI-SAIC, Rockville, MD.*

Developmental toxicologists have long recognized that postnatal development continues for years in humans, and that potent drugs may have deleterious effects on that development. Widely recognized examples include decreased skeletal growth caused by glucocorticosteroids and the effects on bone by fluoroquinolones. Pharmaceutical development has often included testing in juvenile animal models for drugs intended for very young patients. This testing has been requested by the Food and Drug Administration (FDA) on a case-by-case basis. Drug divisions within the FDA have had relative freedom to request whatever studies they thought appropriate, and there was an

expectation that the pharmaceutical industry would perform the tests requested. With the recent enactment of the Pediatric Rule (in the Food and Drug Administration Modernization Act), which focused primarily on clinical testing, the FDA as well as industry recognized that there was a need for a more standardized approach to juvenile animal testing to support the more standardized expectation of human clinical testing of children for pharmaceuticals. This presentation will focus on juvenile animal testing currently being performed to meet FDA requirements, as well as testing that has been found acceptable to meet international expectations. Examples of juvenile animal tests that have been requested by various FDA divisions will be discussed. For example, the FDA Pulmonary Division requests inhalation testing in animals "as young as possible" for drugs intended for children less than 2 years old. Other divisions, however, specify that rats be 4 days old or younger and specify additional needed brain histopathology. This presentation will focus on various suggested FDA approaches, and will focus on an update of FDA and worldwide guidance of juvenile animal testing.

1952 CASE STUDIES: JUVENILE ANIMAL TESTING FOR PHARMACEUTICAL PRODUCTS.

S. Morseth. *Quintiles Consulting, Rockville, MD.*

Case studies of ten serve as excellent examples of the challenges associated with real life experiences based on either theoretical considerations or the lack of appropriate testing. This presentation will cover several case studies that are intended to demonstrate and emphasize major points made in the target organ presentations, to suggest an appropriate approach for designing juvenile animal studies, as well as to identify and clarify areas of basic research needed to better design future studies. Case studies will include examples from drugs commonly used by children, such as antibiotics used for numerous childhood infections; and glucocorticosteroids currently used as first line therapy for asthmatics. Several studies in children have shown that even nasal glucocorticosteroids can decrease total height. The case studies will also include illustrative examples of drugs that effect the central nervous system, such as methamphetamine, and cardiovascular drugs, such as verapamil. The case study presentations will end with an overview of the lessons learned from the case studies in order to identify future research, testing, and regulatory decisions.

1953 CADMIUM UPTAKE KINETICS IN RAT HEPATOCYTES: CORRECTION FOR ALBUMIN BINDING.

N. DelRaso[1], B. Foy[2] and J. Frazier[1]. [1]*AFRL/HEST, Wright-Patterson AFB, OH and* [2]*Wright State University, Dayton, OH.*

Primary rat hepatocytes isolated from male Fischer 344 rats were exposed to cadmium concentrations ranging from 0.42 to 1000μM in protein-free buffer or 32 to 8000 μM in buffer containing physiological albumin (~ 600μM). Cadmium toxicity following a 1 h exposure was observed to occur at free cadmium concentrations exceeding 80μM (equivalent to 1,000μM Cd in buffer containing albumin). A Scatchard plot of cadmium binding to albumin indicated a single high affinity binding site. This was supported by the finding of a rapid rise in free-cadmium concentrations at total cadmium concentrations greater than 1000μM (in the presence of albumin). Comparison of cadmium uptake rates (15 to 60 min) between hepatocytes exposed in buffer containing albumin and buffer lacking albumin indicated that uptake rates, when related to free-cadmium concentrations, were faster when hepatocytes were exposed in buffer containing albumin. In the presence of albumin in the exposure buffer a complex, dose-dependent uptake process was observed. The observed anomalous behavior of cadmium uptake in the presence of albumin is hypothesized to result from diffusion limited non-equilibrium conditions occurring at the cell surface.

1954 DIURETICS ENHANCE CADMIUM ENTRY IN DISTAL NEPHRON CELLS.

F. A. Gesek and K. J. DiPetrillo. *Dartmouth Medical School, Hanover, NH.*
Sponsor: J. W. Hamilton.

Cadmium (Cd) is an environmental pollutant of increasing concern due to its widespread use in batteries and anticorrosive agents. The kidney is the main target organ of Cd toxicity. Significant toxic effects of Cd are postulated to occur in the distal tubule (DT). The DT is the site that critically regulates Na excretion and dissociates Na and Ca reabsorption. The mechanism for the enhanced retention of Na that occurs in Cd-induced hypertension is unknown but likely to occur in DT. To test this we treated DT cells with 10 μM Cd for 6 hours then measured uptake of 22Na. Cd-increased Na entry by 30% from a basal rate of 289±25 to 377±32 nmols/min/mg protein in Cd-treated DT cells. Thiazide diuretics inhibit NaCl cotransport and amiloride inhibits epithelial Na channels (ENaC) in DT. Diuretics are commonly used first line agents to treat hypertension. Both diuretics increase Ca entry through dihydropyridine-sensitive Ca channels. To test if diuretics increase Cd uptake we measured 109Cd entry in DT cells. The basal rate of Cd entry in DT cells is 0.47±.02 nmols/min/mg protein. Cells treated with 100 μM chlorothiazide or 1 μM amiloride increased Cd uptake by 40 and 46%, respectively. Diuretic-induced uptake was abolished with the Ca channel blocker nifedipine. These findings provide evidence that diuretics increase Cd entry in DT through a dihydropyridine-inhibitable Ca channel. We conclude that Cd enhances Na entry in DT cells and that the clinical use of the diuretics amiloride and thiazides as antihypertensive agents may not be an appropriate choice for increasing Na excretion since these agents increase Cd absorption in DT cells. As a result, the concomitant use of these diuretics in workers exposed to Cd may enhance Cd nephrotoxicity.

1955 TUMOR NECROSIS FACTOR INCREASES CADMIUM UPTAKE IN THE DISTAL NEPHRON.

K. J. DiPetrillo and F. A. Gesek. *Dartmouth Medical School, Hanover, NH.*
Sponsor: J. W. Hamilton.

Cd is reported to induce release of inflammatory cytokines in the kidney. One cytokine released is tumor necrosis factor alpha (TNF-α). Receptors for TNF-α are located in the distal tubule (DT). We previously showed that TNF-α increases Na absorption and decreases parathyroid hormone (PTH)-stimulated Ca absorption in DT cells. To understand the role of TNF-a in DT toxicity, we tested the hypothesis that TNF-α regulates Cd absorption in DT cells. As an index of Cd entry, we measured 109Cd uptake in immortalized distal convoluted tubule cells. The basal rate of Cd entry in these cells is 0.45±.02 nmols/min/mg protein. DT cells treated with TNF-α (1 ng/ml) for 5 min exhibited a rate of 0.74±.02 nmols/min/mg protein, an increase of 64%. PTH stimulates Cd entry by activating dihydropyridine-sensitive Ca channels. PTH-stimulated Cd entry is blocked by nifedipine. To test if this is the mechanism for TNF-α stimulated Cd entry in DT cells, uptake was performed in cells pretreated with 10 μM nifedipine. There was no discernible difference in the basal rate of entry in DT cells treated with nifedipine (0.43 nmols/min/mg protein). TNF-α stimulated Cd entry was not blocked by nifedipine (0.71 nmols/min/mg protein). We conclude that TNF-α increases Cd entry in DT cells. The mechanism of entry is unknown but does not occur through nifedipine-inhibitable Ca channels. In summary, Cd induces TNF-α release and in turn stimulates Cd uptake in DT cells. Agents that prevent TNF-α synthesis or binding may alleviate TNF-α stimulated Cd uptake and nephrotoxicity.

1956 CADMIUM-INDUCED UP-REGULATION IN EXPRESSION OF THE REGULATORY γ-GLUTAMYLCYSTEINE SYNTHETASE SUBUNIT IN RAT LUNG AND ALVEOLAR EPITHELIAL CELLS.

G. Shukla, J. F. Chiu and B. Hart. *University of Vermont College of Medicine, Burlington, VT.*

Inhalation of cadmium (Cd) has been implicated as a causative factor in the development of several pulmonary diseases, including emphysema, pulmonary fibrosis, and cancer. Glutathione (GSH) is thought to play a pivotal role in modulating Cd toxicity. The controlled step in *de novo* GSH synthesis is catalyzed by γ-glutamylcysteine synthetase (γ-GCS), a dimeric enzyme consisting of a heavy (~ 72 kDa) catalytic subunit (γ-GCS-HS) and a light (~ 28 kDa) regulatory subunit (γ-GCS-LS). We previously reported that Cd exposure induces pulmonary γ-GCS-HS mRNA and protein and that these alterations in the catalytic subunit are accompanied by increases in GSH. The current study was designed to test the hypothesis that Cd exposure also up-regulates the gene expression of the regulatory γ-GCS subunit. Using a Northern blotting procedure, we demonstrate that a single 3 hr (nose-only) Cd aerosol exposure of Lewis rats results in time- and dose-dependent increases in the pulmonary levels of γ-GCS-LS mRNA. Transcripts of γ-GCS-LS in rat lung are maximally elevated (8-fold) 2 hrs following Cd inhalation exposure and remain significantly higher than air controls at 24 hrs. This response is highly correlated with Cd dose, that ranged from 0.9 mg Cd/m^3 to 5 mg Cd/m^3, and with lung Cd burden. We also examined Cd-induced regulation of γ-GCS-LS expression *in vitro* using an alveolar epithelial cell (AEC) line. Treatment of AECs by the addition of 10 μM $CdCl_2$ to the culture medium also caused increases in γ-GCS-LS mRNA that achieved levels approximate-

ly 13-fold higher than in unexposed cells. Epithelial cells that were made resistant to Cd by repeated culturing in Cd-containing medium also had γ-GCS-LS mRNA levels about 4-fold higher than cells that were not adapted to Cd. The studies presented support the conclusion that coordinate up-regulation in the gene expression of the regulatory and the catalytic subunits of γ-GCS occurs in the lungs of rats following inhalation of Cd aerosols and in AECs exposed to Cd *in vitro*. These responses may contribute to pulmonary Cd resistance.

1957 INHIBITION OF DNA-(CYTOSINE-5) METHYLTRANSFERASE ACTIVITY BY CARCINOGENIC METALS.

M. Takiguchi and M. P. Waalkes. *Laboratory of Comparative Carcinogenesis, NCI at NIEHS, Research Triangle Park, NC.*

Several different metal compounds have been identified as human carcinogens. The methylation of cytosine by the DNA-(cytosine-5) methyltransferase (DNA-MeTase) is the predominant post-replication base modification and defects resulting in hypo- or hyper-methylation of particular DNA sequences have also been shown to be either the cause or consequence of malignant transformation. Therefore, we have carried out a study on the effects of a series of metals (As^{3+}, As^{5+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) on the DNA-MeTase activity. Both lysed rat liver epithelial cells (TRL 1215) or purified bacterial *M. SssI* were used as the source of DNA-MeTase. Regardless of source, the activity of DNA-MeTase was found to be strongly inhibited by a number of metal ions. Cd^{2+} (IC_{50} = 25 μM) was the most effective followed by Zn^{2+}, Pb^{2+}, and Ni^{2+}. Only in the case of mammalian cell lysis did As^{3+} inhibit the activity of DNA-MeTase. Kinetic analysis revealed Cd^{2+} to be a noncompetitive inhibitor with respect to DNA, and an uncompetitive inhibitor with respect to the methyl donating co-factor for DNA-MeTase, S-adenosylmethionine. These results indicate that Cd^{2+} interacts with DNA binding domain on DNA-MeTase. DNA-MeTase activity in intact TRL 1215 cells, when treated with 10 μM Cd for 24 hours, was depressed by up to 84%. During longer exposure experiments 0.5-2.5 μM Cd was used. After 1 week of Cd exposure, DNA-MeTase activity in cells was decreased in a concentration dependent fashion. Although Cd decreased DNA-MeTase activity, global DNA hypermethylation occurred after Cd exposure for 10 weeks. These results raise the possibility that one of the mechanisms of Cd^{2+} carcinogenicity could be an induction of abnormal DNA methylation. For other carcinogenic metals such as As^{3+}, Pb^{2+} or Ni^{2+} altering DNA methylation status may also contribute to their carcinogenic effects.

1958 ARSENIC-SELENIUM INTERACTIONS IN HUMAN AND RAT HEPATOCYTES.

M. Styblo[1], E. L. LeCluyse[2], G. A. Hamilton[2] and D. J. Thomas[3]. [1]*Department of Pediatrics, School of Medicine, University of North Carolina, Chapel Hill, NC,* [2]*Division of Drug Delivery and Disposition, School of Pharmacy, University of North Carolina, Chapel Hill, NC and* [3]*Experimental Toxicology Division, NHEERL, USEPA, Research Triangle Park, NC.*

Arsenic (As) and selenium (Se) are metalloids that share chemical properties and metabolic fates. The metabolism of either metalloid involves glutathione (GSH)-dependent reduction and enzymatic methylation to mono-, di- and trimethylated metabolites. Each metalloid modifies the metabolism and toxicity of the other in laboratory animals. It has been suggested that Se might be used as an antidote to mollify the adverse effects associated with exposure to As in humans. The present work examines the effects of Se on the metabolism and toxicity of As in primary cultures of human and rat hepatocytes. The simultaneous addition of 2 μM sodium selenite (Se^{IV}) to cultures significantly increased cellular retention and inhibited methylation of 0.1 μM sodium arsenite (iAs^{III}) in both cell types. The ratio of the methylated metabolites, dimethylarsenic (DMAs): monomethylarsenic (MAs), decreased markedly in the presence of Se^{IV}, suggesting that synthesis of DMAs from MAs may be more susceptible to inhibition by Se^{IV} than is the production of MAs from iAs^{III}. The inhibitory effect of Se^{IV} was concentration-dependent and enhanced by concurrent addition of 2.5 mM GSH. The addition of 2 μM Se^{IV} into the culture 24 hours before addition of 0.1 or 1 μM iAs^{III} increased cellular retention of iAs^{III} but did not alter the rate and yield of the methylation reactions. 24-hour exposure to 2 μM Se^{IV} had no effect on the viability of cultured hepatocytes. Concurrent addition of 2 μM Se^{IV} increased the cytotoxicity of iAs^{III} and its trivalent metabolites, MAs^{III} and $DMAs^{III}$. GSH effectively protected cells against toxicity of arsenicals. However, the protective effect of GSH was significantly less in the presence of Se^{IV}. These data suggest that treatment with Se may in fact enhance the toxic effects of As, increasing its retention in tissues and reducing its methylation which may be pathway for the detoxification of As. (This abstract does not necessarily reflect EPA policy.)

1959 DMPS MODULATION OF ARSENIC SPECIES, INCLUDING MONOMETHYLARSONOUS ACID (MMAIII), IN HUMAN URINE.

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The administration of sodium 2,3-dimercapto-1-propane sulfonate (DMPS) to humans chronically exposed to inorganic arsenic in their drinking water resulted in the increased urinary excretion of arsenic, the appearance and identification of monomethylrsonous acid (MMAIII) in their urine and a large decrease in the urinary excretion of dimethylarsinic acid (DMA). Experiments were designed to understand the appearance of MMAIII and decrease of DMA in the urine. DMPS, *in vitro*, inhibited rabbit liver MMAIII methyltransferase. This and other evidence supports the hypothesis that DMPS competes with endogenous ligands for MMAIII, forming a complex that is readily excreted in the urine and points out the need for studying the toxicology of MMAIII. The results of these studies raise many questions about the potential central role of MMAIII in the toxicity of inorganic arsenic and to the potential involvement of MMAIII in the little understood etiology of hyperkeratosis, hyperpigmentation and cancer that can result from chronic inorganic arsenic exposure. (Supported in part by Superfund Basic Research Program NIEHS Grant ES-04940 and Southwest Environmental Health Sciences Center Grant P30-ES-06694.)

1960 MEDICAL MONITORING SURVEY RESULTS AND BERYLLIUM EXPOSURE AT A BERYLLIUM MINE AND EXTRACTION FACILITY.

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A survey to evaluate employees for beryllium sensitization and chronic beryllium disease (CBD) was conducted at the Brush Wellman Inc. mine and beryllium extraction mill in Delta, Utah, using a medical history and the beryllium blood lymphocyte proliferation test (BLPT). Historical industrial hygiene data, including general area, breathing zone, and limited personal lapel measurements were used to assess the levels of ambient beryllium exposures at this worksite. Beryllium exposures at this facility occur as a result of the presence of dust from bertranite (hydrated beryllium silicate) and beryl (beryllium aluminum silicate) ores, mists containing soluble beryllium salts (beryllium sulfate, beryllium ammonium carbonate and beryllium carbonate), and the precipitation and packaging of the plant's end product, hydrated beryllium hydroxide. 76 of 85 current employees agreed to participate. The rates of beryllium sensitization (4%) and CBD (1.4%) at Delta were intermediate compared to rates expected in the general population (1% and 0% respectively) and those found in other company beryllium surveillance programs (6% and 4% respectively), but were not significantly different from either reference point (P values > 5%). Furthermore, the one case of CBD detected was in the only individual who had spent significant time working elsewhere with beryllium (10 years) where clinical and subclinical CBE is more prevalent. Excluding this individual, the plant has never detected a case of CBD in current or former workers, which yields a rate of CBD significantly lower than that observed in persons ever employed at either the Elmore and Tucson facilities. Former and current levels of occupational exposure to airborne beryllium at the three plants are comparable. We hypothesize that differences in rates of sensitization and CBD result from the a) low bioavailability of beryllium in ore dusts, b) low potency of soluble beryllium salts, and c) localized presence of beryllium hydroxide and/or d) differences in work practices and personal protection controls.

1961 LUMINAL TRANSPORT OF DI-N ACETYLCYSTEINYLMERCURY ($(NAC)_2$ -HG) IN THE RABBIT PROXIMAL TUBULE.

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Previous animal experiments indicate that inorganic mercury is not taken up or accumulated significantly when it is in the proximal tubular lumen in the form of a di-*N*-acetylcysteinylmercury ($(NAC)_2$-Hg) complex. These studies do, however, indicate that $(NAC)_2$-Hg is taken up by cortical epithelial cells via transport mechanism(s) in the basolateral membrane. To extend these findings further we studied the luminal disappearance rate and cellular uptake of $(NAC)_2$-Hg in isolated perfused S_2 segments of the rabbit proximal tubule. The perfusion fluid was a balanced saline solution that contained 20μM Hg^{++} and 80μM *N*-acetylcysteine. Under these conditions the disappearance rate of $(NAC)_2$-Hg from the luminal fluid was 9.6 fmol min^{-1} (mm tubule length)$^{-1}$ while cellular accumulation was 103.6 fmol (mm tubular length)$^{-1}$. In pervious studies with dicysteinylmercury these values were 102 and 169 respectively. This is in contrast to the luminal disappearance rate of125.5 fmol min^{-1} (mm tubular length)$^{-1}$ and cellular accumulation of 1147.4 fmol (mm tubular length)$^{-1}$ when tubules were perfused with the balance saline solution containing 20μM Hg^{2+} only. The presence of NAC in the lumen eliminated the acute toxicity that is present in tubules perfused with Hg^{2+} alone. No evidence of cellular blebbing, tubular swelling or cellular uptake of the vital dye were detected. When NAC (200μM) was present in the bathing solution and 20μM Hg^{2+} in the perfusate only, the disappearance rate of Hg^{2+} from the lumen was reduced to 77.4 fmol min^{-1}(mm tubular length)$^{-1}$, while cellular accumulation was reduced to 368 fmol (mm tubular length)$^{-1}$. The acute toxicity in these tubules was reduced but not eliminated. We conclude that $(NAC)_2$-Hg is not transported well at the luminal membrane of the S_2 segment of the proximal tubule. This confirms our findings from intact animals. Also, when NAC is present in the bathing solution, it provides protection to perfused proximal tubular segments from toxic effects of luminal Hg^{2+}.

1962 WATER LEAD EXPOSURE IN BRUSSELS CHILDREN.

A. Y. Steenhout. *Free University of Brussels, Brussels, Belgium.* Sponsor: G. J. Pounds.

Water lead exposure has been studied in houses inhabited by young children in Brussels, with 3 types of sampling: (1) overnight stagnation, (2) running water at the end of a volume used for preparing meal (3) stagnation daytime til return from work. Values higher than WHO 10μg/l are encountered even in flushed water. 37% of the houses display water Pb content higher than 10μg/l at least in one sample. Whereas Pb pipes or mixed (i.e., Pb and Cu) material account for more than 3/4 of the cases >10μg/l, 1/4 of the excessive amounts occur within pipes in other material than Pb (Cu, galva, PVC). Concern about drinking water quality is increased in lower socio-economic status (SES) families: poorer calcium intake, a factor of enhanced Pb ingestion, is more frequent in children in ancient housing with lead piping. WHO guideline has just been included in the new drinking water European directive, but for up to 15 yrs, the intermediate 25μgPb/l upper value will be accepted. More than 17% of the houses show water lead higher than this upper value. By reference to our risk assessment model ECOTOX (presented in 1998 SOT meeting), the increment in blood lead resulting from various sources can be evaluated, as well as the prevalence of blood lead higher than 10μg/dl. Even in houses without lead paints in Brussels, a level of 25μg/l is not be considered as safe, or the acceptable uptake from other sources should be reduced as well.

1963 DISTINCT EFFECTS OF LEAD ON INDIVIDUAL PROTEIN KINASE C ISOFORMS.

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Lead is an important toxicant that can affect the function of several different organ systems over a range of exposures and blood levels. Protein kinase C (PKC) has been identified as an extremely high affinity target of lead in several cells and tissues, with lead inducing activation of this kinase at concentrations less than 10^{-9} M. PKC is a family of ser/thr kinases with some isoforms that normally require calcium for activation and others that do not. In this study, the ability of lead to activate individual calcium-dependent and calcium-independent isoforms of PKC was examined. PKC isoforms were made from human cDNAs expressed in a baculovirus sf9 system. Free divalent lead and calcium concentrations were set using 5F-BAPTA as a buffer and free metal concentrations were confirmed using ^{19}F NMR. Of the calcium-dependent isoforms, PKC alpha was most sensitive to activation. Lead activated PKC alpha to the same extent as calcium but at lead concentrations from 10^{-11} to 10^{-7} M compared to calcium concentrations from 10^{-7} to 10^{-4} M. PKCs beta and gamma were not as potently activated by lead. PKC gamma was most sensitive to inhibition, with inhibition by lead occurring at concentrations greater than 10^{-9} M. Neither PKC delta nor epsilon, calcium-independent isoforms, was activated by lead or by calcium. PKC epsilon was the most sensitive to inhibition, with complete inhibition at 10^{-9} M lead. These data demonstrate that only calcium dependent isoforms of PKC are activated by lead suggesting that lead simply replaces calcium in the activation of the enzyme. However, lead does not replace calcium with the same effectiveness in all calcium dependent isoforms. Furthermore, the sensitively to inhibition by lead differs greatly across this family of kinases. Altogether, these findings indicate that while low levels of lead may activate the overall PKC activity in cells and tissues, the specific effects of lead on the PKC signal transduction may depend on both the specific isoform composition of the cells and the specific free lead concentration achieved upon exposure.

1964 FERRITIN IS A NUCLEAR PROTEIN THAT RESPONDS TO CELL STRESS AND PROTECTS DNA.

K. Thompson, Z. Ye and J. R. Connor. *Pennsylvania State University, Hershey, PA.* Sponsor: E. Malecki.

The cytoplasmic iron storage protein ferritin has been recently described as a transiently expressed protein in select cell nuclei. Within nuclei, ferritin's function may involve both DNA protection and DNA damage. We found ferritin is present in neuronal nuclei in both humans and rodents at early postnatal periods but is predominantly cytoplasmic in the adult. In adult mice, ferritin can be induced to return to neuronal nuclei by hypoxic ischemic insult indicating selective cell expression and suggesting a protective role. We established a cell culture model to determine the mechanism and consequence of ferritin nuclear translocation using human astrocytoma cells. Ferritin's presence in the nucleus increases by increasing cellular iron levels and through exposure to hydrogen peroxide and cytokines indicating cell stress as a factor in inducing ferritin movement into the nucleus. In vitro extracellular uptake studies reveal that H-ferritin is selectively taken up into the nucleus, whereas extracellular L-ferritin remains cytoplasmic. Nuclear translocation of ferritin is blocked by wheat germ agglutinin, ATP depletion, and incubation at 4°C indicating entry through an active nuclear pore. Cycloheximide treatment decreased ferritin nuclear levels indicating that the presence of ferritin in the nucleus is post-transcriptionally regulated and requires de novo synthesis. Cells in which ferritin is removed from the nucleus and translocation blocked with WGA are susceptible to hydrogen peroxide induced DNA damage. Supercoil relaxation assays found H-ferritin protects DNA better than other weak iron binding proteins. In conclusion, this study presents evidence for the novel findings that localization of ferritin to cell nuclei is ferritin subunit specific, post-transcriptionally regulated, actively transported into the nucleus through nuclear pore complexes *in vitro*, and protects DNA. These data expand the function of ferritin and suggest ferritin nuclear localization is a well-regulated cellular event that responds to cell stresses and protects DNA from oxidative damage.

1965 PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA: SPECIES DIFFERENCES IN QUALITY AND QUANTITY.

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Peroxisome proliferators (PPs) are nongenotoxic rodent hepatocarcinogens that cause liver enlargement and hepatocarcinogenesis associated with peroxisome proliferation, induction of hepatocyte DNA synthesis and suppression of apoptosis. Acyl CoA oxidase (ACO) is a key enzyme of peroxisomal β-oxidation and its transcriptional activation by PPs is often used as marker for the rodent response. PPs activate the peroxisome proliferator activated receptor-alpha, PPARα. There are marked species differences in response to nongenotoxic carcinogens and the data available support the position that human and guinea pigs are resistant to the adverse effects of PPs seen in rodents. Species differences could be attributed both to quantity of PPARα and the quality of the PPARα-mediated response. Human and guinea pig liver has a reduced quantity of full length functional PPARα; we and others have found hPPARα8/14, a truncated receptor that results from aberrant splicing of the PPARα mRNA, in all samples at levels of between 10-40% relative to the full length hPPARα. To determine if species differences in response to PPs are due to the low levels of PPARα in nonresponsive species, we over-expressed PPARα in guinea pig hepatocytes and monitored PCO as a measure of perox-

isome proliferation. As expected, control guinea pig hepatocytes failed to respond to PPs but the overexpression of mouse, human or guinea pig PPARα conferred responsiveness. This induction of PCO in guinea pig hepatocytes over-expressing PPARα demonstrates that species differences in quantity of PPARα is at least in part responsible for species differences in response. In addition, species differences could be attributed to qualitative differences in the PPARα-mediated response since we have shown recently that the promoter for human ACO differs in sequence and activity from the rat equivalent. Additionally, back mutagenesis of the rat ACO PPRE to the human sequence resulted in a loss of activity at the rat promoter. These data contribute to our understanding of how chemicals may cause tumours in rodents and how this response may differ in humans.

1966 USE OF MICROARRAY EXPRESSION PROFILING IN PPARα NULL AND WILD-TYPE MICE TO INVESTIGATE THE MECHANISM OF HEPATOCARCINOGENESIS INDUCED BY THE PEROXISOME PROLIFERATOR DIETHYLHEXYLPHTHALATE (DEHP).

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Peroxisome proliferators (PPs) are a diverse group of chemicals that cause non-genotoxic hepatocarcinogenesis in rodents. Administration of PPs to rodents results in peroxisome proliferation, suppression of apoptosis, hepatocellular proliferation and, ultimately, hepatocarcinogenesis. The adverse effects of these compounds are mediated by the PPARα receptor (Peroxisome Proliferator Activated receptor α). Mice lacking functional PPARα do not exhibit the characteristic response to PPs and do not develop liver tumours. In addition, hepatocytes isolated from PPARα null mice are refractory to the effects of PPs, including induction of fatty acid oxidising enzymes and suppression of apoptosis. We have used microarray gene expression profiling to examine in greater detail alterations in mouse hepatocyte gene expression associated with activation of PPARα. PPARα null and wild-type mice received diethylhexylpthalate (DEHP; 1150 mg/Kg in corn oil) daily for two days by gavage and their livers isolated at 48 hrs after the first administration for extraction of mRNA. Probes generated from these mRNAs were hybridised to custom-made cDNA microarrays containing approximately 1200 cDNA sequences representing 600 genes relevant to mechanisms of toxicity. Using this approach, we have identified genes regulated by PPARα in response to DEHP which may be implicated in cellular proliferation and hepatocarcinogenesis. These include genes involved in lipid metabolism in the liver and several genes not previously known to be regulated by PPs.

1967 ROLE OF PPAR ALPHA IN HEPATIC RESPONSES TO DIISONONYL PHTHALATE (DINP).

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Diisononyl phthalate (DINP) is a compound widely used as a plasticizer in the production of polyvinyl chloride (PVC) products. Many phthalates are considered to be relatively weak peroxisome proliferators (PP), a group of rodent hepatocarcinogens that cause a variety of adaptive responses in liver through the PP-activated receptor alpha (PPAR). The objective of this study was to determine if DINP-induced effects in the liver are mediated by PPAR. Male and female SV129 PPAR-null (-/-), SV129 PPAR (+/+) and B6C3F1 mice were fed a control diet or a diet containing 8000 ppm of DINP for 1 or 3 weeks. There was an increase in relative liver weight in male and female PPAR (+/+) and B6C3F1 mice exposed for 1 week. The increase in liver weights was abolished in the 1 week treated male and female PPAR (-/-) mice. Western blot analysis of liver protein extracts demonstrated a strong induction of acyl-CoA oxidase and Cyp4a proteins in PPAR (+/+) but not PPAR (-/-) female mice after a 3 week exposure. Preliminary experiments using arrays containing 144 genes showed that 6 genes were altered in livers after a 3 week exposure in female PPAR (+/+) but not in PPAR (-/-) mice. These genes include those involved in drug metabolism and protein trafficking. These data support the hypothesis that DINP, like other PP induces liver effects through a PPAR alpha-dependent mechanism.

1968 SUPPRESSION OF APOPTOSIS AND INDUCTION OF DNA SYNTHESIS BY THE PEROXISOME PROLIFERATORS MONOETHYLHEXYLPHTHALATE (MEHP) AND DIISONONYLPHTHALATE (DINP): A COMPARISON OF RAT AND HUMAN HEPATOCYTES *IN VITRO.*

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Diethylhexylphthalate (DEHP) and diisononylphthalate (DINP) are plasticizers with many important commercial, industrial and medical applications. However, both DEHP and DINP are rodent peroxisome proliferators, a class of compounds that cause rodent liver tumours associated with peroxisome proliferation, induction of hepatic DNA synthesis and the suppression of apoptosis. Despite these effects in the rodent, humans appear to be nonresponsive to the adverse effects of PPs. Previously, we have shown that the fibrate hypolipidaemic peroxisome proliferator, nafenopin, induced DNA synthesis and suppressed apoptosis in rat but not in human hepatocytes. Here, we have examined species differences in the response of rat and human hepatocytes to DEHP and DINP *in vitro*. In rat hepatocytes *in vitro*, both DINP and MEHP (a principle metabolite of DEHP and the proximal peroxisome proliferator) caused a concentration-dependent induction of DNA synthesis and suppression of both spontaneous and TGFβ1-induced apoptosis. Similarly, both MEHP and DINP caused a concentration-dependent induction of peroxisomal β-oxidation although the response to DINP was less robust. In contrast to the pleiotropic response noted in rat hepatocytes, neither DINP nor MEHP caused an induction of β-oxidation, stimulation of DNA synthesis or suppression of apoptosis in human hepatocytes cultured from three separate donors. These data provide evidence for species differences in the hepatic response to the phthalates DEHP and DINP, suggesting that human hepatocytes are refractory to the hepatocarcinogenic effects of PPs noted in rodents.

1969 THE USE OF PROTEOMIC TECHNOLOGY AND AN EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITOR TO COMPARE PEROXISOME PROLIFERATOR AND GROWTH FACTOR - INDUCED S PHASE.

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Rodent hepatocytes undergo rapid proliferation in response to peroxisome proliferators, a diverse group of chemicals of therapeutic, industrial and environmental significance. It has been suggested that peroxisome proliferators may cause tumors by altering the epidermal growth factor (EGF) signalling pathway and therefore inducing DNA synthesis and cell proliferation in rodents. In rat primary hepatocyte cultures, EGF (1 ng/ml) induced a 3.27-fold increase, HGF (0.5 ng/ml) a 2.84-fold increase, and the peroxisome proliferator nafenopin a 1.92 fold increase in S phase. A specific EGF-Receptor tyrosine kinase inhibitor (4-anilinoquinazoline; EGFR-TKI) efficiently inhibited EGF -induced S phase but did not prevent HGF-induced S phase. Nafenopin-induced S phase remained at 1.91 fold in the presence of the EGFR-TKI, indicating that signalling through EGF-R is not required for nafenopin to induce DNA replication. Cyclin-dependent kinases (CDKs) and cyclins allow cells to progress from G1 to S phase in response to growth factors. Cyclin E, cyclin D1, CDK4 and CDK2 accumulated upon stimulation with nafenopin although their expression was delayed by 24 hours compared to EGF stimulation. Overall, there was no gross dysregulation of the CDK and cyclin protein expression profile upon stimulation with nafenopin. Using proteomic technology, we compared the molecular mechanisms that induce hepatocyte proliferation in response to peroxisome proliferators or EGF. BioImage analysis of highly reproducible 2 dimensional-gels have indicated that the stimulation of S phase with nafenopin produces a protein profile different to its vehicle control (DMF) and to EGF. Stimulus dependent differences in protein expression have been analysed, and individual proteins with altered expression have been identified by mass spectrometry.

1970 HEPATIC EXPRESSION OF DNA POL BETA, REF-1, BCL-2 AND BAX PROTEINS IN PEROXISOME PROLIFERATOR(PP)-TREATED RATS AND HAMSTERS.

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The mechanism of PP-induced hepatocarcinogenesis and the basis for species differences in the tumorigenic potential of the PP's in mammals have yet to be fully elucidated. We investigated the hepatic expression of oxidant-sensitive gene products in male SD rats(R) (which are relatively susceptible to PP-induced tumors) and male Syrian hamsters(H) (which are relatively resistant) after 6 days of ad lib administration of feed containing one of the following PP's at the level indicated: WY-14,643 (WY): 500 ppm; gemfibrozil (G):16,000ppm(R) or 24,000ppm(H); 2,4-dichloro phenoxyacetic acid (24D): 2,500ppm(R) or 5,000ppm(H); dibutyl phthalate(DBP): 20,000 ppm. Proteins in liver supernatants from treated animals and matched controls were separated by one-dimensional SDS-PAGE and transferred to PVDF membranes. Relative levels of protein expression were estimated by immunoblotting, scanning densitometry and image analysis. Evaluation of constitutive protein expression in untreated R and H showed: a 6-fold increase in 38 kD Pol Beta in R as compared to H; a 20-fold increase in 35 kD Ref-1 and a 1.5-fold increase in 19 kD Bax in H as compared to R; expression of 26kD Bcl-2 in H but not in R. PP treatment was associated with the induction of a novel 47 kD isoform of Pol Beta in R but not in H. This isoform was only slightly induced by 24D but markedly induced by the other 3 PP's. The expression of 35 kD Ref-1 was increased 6-fold in WY treated R but decreased to 30% of control levels in treated H. PP treatment did not significantly affect the expression of 19 kD Bax or 26 kD Bcl-2. These results suggest that increased basal and PP-induced expression of Pol Beta, which has poor fidelity compared to other DNA polymerases, may lead to cancer predisposition and tumor progression in R. The WY-related increase in Ref-1 expression in R is consistent with an increase in oxidative stress to the liver. A decreased constitutive Ref-1 expression in R as compared to H suggests a significant species difference in the capacity for DNA repair. (Supported by 1 R03 ES09783-01 to EWH.)

1971 GLUTATHIONE RELATED ENZYME ACTIVITIES IN RATS AND HAMSTERS ADMINISTERED THREE PEROXISOME PROLIFERATORS: DIBUTYL PHTHALATE, GEMFIBROZIL, AND WY-14,643.

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Peroxisome proliferators (PPs) are a diverse group of compounds that cause hepatomegaly and peroxisome proliferation, ultimately resulting in hepatocarcinogenesis, in rats and mice. Conversely, species such as humans and hamsters are non-responsive to these compounds. PPs are non-genotoxic carcinogens that activate the peroxisome proliferator activated receptor (PPAR), resulting in increased transcription of genes involved in peroxisomal beta-oxidation. This increased peroxisomal beta-oxidation has been hypothesized to result in oxidative stress. We hypothesized that differential modulation of antioxidant enzymes and/or cofactors could account for the resulting difference in species susceptibility following PP administration. Accordingly, we have measured glutathione transferase (GST), Se and non-Se dependent glutathione peroxidase (GPx), and glutathione reductase (GR) activities as well as total glutathione (GSH) in male Sprague Dawley rats and Syrian hamsters fed two doses of three known peroxisome proliferators [dibutyl phthalate (DB), gemfibrozil and Wy-14,643] for 6, 34, or 90 days. In rats, a decrease in GR, GST, and Se-GPx was observed following the administration of Wy-14,643 and DB; the effect following DB treatment was less than that seen with the more potent PP Wy-14,643. A decrease in total GSH was also observed with both compounds. Rats treated with gemfibrozil had elevated GR and total GSH; however, Se-GPx was decreased in the 90-day treatment group. In hamsters, we observed a higher basal level of activities for GR, GST, and Se-GST as compared to rats. In addition, hamsters also showed a decrease in GR and GST activities but had increased total GSH following Wy-14, 643 administration. Gemfibrozil treatment in hamsters increased GR but decreased GST activity. Interestingly, Se-GPx activity was increased in hamsters at various time points in DB and gemfibrozil treatment groups and in all three-time points of the Wy-14, 643 group. Furthermore, the decrease in GPx1 in rats was not at the mRNA level, while the increase in hamsters correlated with an increase in mRNA. This divergence in the hydrogen peroxide detoxification ability between rats and hamsters could be a contributing factor in the proposed oxidative stress mechanism of PP's observed in responsive and non-responsive species. (Supported by ES 09771.)

1972 EFFECTS OF 9-CIS, 11-TRANS CONJUGATED LINOLEIC ACID (CLA) ON HUMAN HT-29 COLON CARCINOMA CELLS.

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Epidemiological studies have demonstrated a strong correlation between dietary fat intake and the development of colon cancer. Interestingly, conjugated linoleic acid (CLA), an animal-derived dietary fat consisting of a group of linoleic acid isomers, inhibits several animal models of chemical carcinogenesis including colon cancer. The anti-cancer mechanism of CLA remains unclear. Our data for the first time indicate that 9-cis, 11-trans CLA significantly inhibits cellular proliferation of the colon epithelial cell line HT-29 following a twenty four hour treatment; the effect was similar to 15-deoxy-Δ12, 14- PGJ2, a natural PPAR gamma ligand. Equimolar concentration of linoleic acid however, failed to produce any effect on cell growth. Lactate dehydrogenase activity measured in the growth media was found not to be different from the untreated cells suggesting absence of toxic effects produced by the compound per se. Induction of apoptosis may not be an anti-cancer mechanism of 9-cis, 11-trans CLA as it did not increase caspase-3, a marker of apoptotic cell death. Since PPAR gamma has been previously reported to be associated with cellular differentiation and CLA has been shown to activate this steroid hormone receptor, we propose that CLA's anti-cancer effect may be via enhanced cellular differentiation. We are testing this hypothesis by examining CEA (carcinoembryonic antigen) gene in 9-cis, 11-trans CLA-treated colon cancer cells utilizing RT-PCR techniques. (Supported by PDA grant ME 448435.)

1973 NADPH OXIDASE IS THE SOURCE OF OXIDANTS FOR ACTIVATION OF NF-κB BY THE PEROXISOME PROLIFERATOR WY-14,643.

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The role of oxidants in the mechanism of tumor promotion by peroxisome proliferators (PPs) remains controversial. The idea that induction of acyl-CoA oxidase leads to increased production of H_2O_2 which damages DNA appears unlikely; still, radicals might be important in signaling in specialized cell types such as Kupffer cells, which produce mitogens (*e.g.*, TNFα) and increase proliferation of hepatocytes. In fact, it was shown that superoxide production by isolated Kupffer cells is stimulated by PPs (*e.g.*, WY-14,643) and that activation of transcription factor NF-κB by PPs occurs in Kupffer cells and is oxidant dependent. Since NADPH oxidase is the major source of radicals in Kupffer cells, it was hypothesized that this enzyme is involved in production of oxidants *in vivo* after treatment with PPs. Indeed, both the activation of NF-κB and an increase in cell proliferation due to a single dose of WY-14,643 (WY, 100 mg/kg, *i.g.*) were prevented completely when rats were pretreated with the NADPH oxidase inhibitor diphenyleneiodonium (1 mg/kg, *s.c.*) for 4 days. Further, NADPH oxidase deficient ($p47^{phox}$-/-) mice were used to specifically address the hypothesis of NADPH oxidase involvement. When mice were fed WY-containing (0.1% w/w) or control (NIH-07) diets for 7 days, the increase in liver weight and cell proliferation caused by WY in +/+ mice was blocked in $p47^{phox}$ -/- mice. Moreover, in +/+ mice, WY (100 mg/kg, *i.g.*) caused a 2.5-fold activation of NF-κB in liver 5-8 hrs after treatment. This effect was followed by an increase in mRNA levels for mitogenic cytokines (*i.e.*, TNFα, IL-6). Importantly, neither activation of NF-κB nor increase in cytokine transcription occurred in $p47^{phox}$-/- mice. It is concluded that PPs activate NADPH oxidase to produce superoxide which increases NF-κB. This triggers production of mitogens and causes hepatocellular proliferation characteristic of this class of carcinogens (ES 04325).

1974 EFFECT OF WY-14,643 ON THE METHYLATION AND EXPRESSION OF C-*MYC* AND CONNEXINS-26 AND -32 IN THE LIVER OF B6C3F1 MICE AND SPRAGUE-DAWLEY RATS.

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Peroxisome proliferators including Wy-14,643 are carcino-genic in rodent liver. Increased expression of protooncogenes and decreased expression of

connexin have been associated with non-genotoxic carcinogens. One mechanism controlling expression of these genes is the presence of 5-methylcytosine in their promoter regions resulting in decreased expression. Male $B6C3F_1$ mice and male Sprague-Dawley rats were administered Wy-14,643 (0,10, 50, 100 and 500 mg/kg diet) for 6, 34, or 90 days. Exposure of mice to Wy-14,643 resulted in the liver in decreased methylation in the promoter region of the C-*myc* gene as determined by *Hpa* II restriction endonucle-ase digestion and Southern blot analysis. However, expression of the mRNA for C-*myc* was not altered. In rat liver, Western blot analysis revealed that connexins-26 and -32 levels were decreased at 6, 34, and 90 days. Using *Hpa* II and Southern analysis, no evidence was found for methylation of the promoter region of the gene for connexin-32. Thus, Wy-14,643 decreased both the methylation of the C-*myc* gene in mouse liver and the expression of connexins-26 and -32 proteins in rat liver, two activities associated with hepatocarcinogenesis. (Supported in part by a NIEHS grant #1R03 ES09772-01.)

1975 TUMOR NECROSIS FACTOR α (TNFα) IS NOT REQUIRED FOR WY-14,643 INDUCED CELL PROLIFERATION IN MICE.

B. L. Givler, D. W. Alberts, G. Wollenberg, M. H. Pitzenberger, J. G. Deluca and J. W. Lawrence. *Merck & Co Inc., West Point, PA*. Sponsor: R. Dixit.

It has been proposed that peroxisome proliferators induce hepatic cell proliferation through the induction of the cytokine TNFα. To test this hypothesis, peroxisome proliferation and cell proliferation induction was compared in wild-type C57Bl/6 and TNFα knockout mice. Animals were dosed for with either vehicle or 100 mg/kg/day WY14,643 by oral gavage for 4 days. Liver to body weight ratios increased in both wild-type and TNFα knockout animals after WY14,643 administration. In addition, WY14,643 treated wild-type C57Bl/6 and TNFα knockout mice displayed marked induction of fatty acyl-CoA oxidase activity (approximately 8-fold) and mRNA content (approximately 5-fold). Electron microscopic examination confirmed hepatocellular proliferation of peroxisomes in both mouse models. Moreover, WY14,643 markedly induced hepatic cell proliferation (approximately 20-fold) in both wild-type and C57BL/6 TNFα knockout mice as measured by BrdU incorporation into hepatocyte nuclei. In addition, a 50% decrease in TNFα mRNA was observed in wild-type mice after treatment with WY14,643. These results suggest that the hepatocellular proliferation induced after peroxisome proliferator treatment can occur independently of TNFα signaling.

1976 HEPATIC MITOGENESIS AND CARCINOGENESIS INDUCED BY PEROXISOME PROLIFERATORS IS ASSOCIATED WITH ALTERATIONS IN IL-1β SIGNALING PATHWAYS.

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Peroxisome proliferator exposure is associated with hepatocellular mitogenesis, carcinogenesis, and altered synthesis of acute-phase proteins in the livers of rats and mice. Several of the cytokine signaling pathways that regulate acute-phase gene expression have also been implicated as mediators of hepatic regeneration following partial hepatectomy or exposure to certain hepatotoxins. We investigated the mRNA expression of select cytokine signaling pathways during hepatic mitogenesis and carcinogenesis in mice treated with the potent peroxisome proliferator and experimental hypolipidemic drug, Wy-14,643 (WY). Hepatic mitogenesis was associated with increased expression of IL-1β, IL-1 receptor 1, and the Stat-3, and Hnf-4 transcription factors. Normal and adenomatous liver from mice chronically treated with Wy-14,643 had increased expression of IL-1β, IL-1 receptor 1, and PPARΓ, as well as increases in the cell-cycle regulatory genes p21, c-Myc, and Cyclin D1. p21, c-Myc, Cyclin D1, and Bcl-xL, which we previously found altered in the majority of WY-induced hepatic adenomas, are all downstream targets of Stat-3. Recently, Stat-3 was identified as an oncogene and a downstream target of IL-1β. These results suggest that the hepatic mitogenesis and carcinogenesis induced by treating rodents with peroxisome proliferators may be mediated in part via a pathway involving IL-1β and Stat-3. Preliminary expression data from cDNA arrays suggest that additional, related genes involved in cytokine signaling may be altered as well. (Supported in part by NIEHS grant ES09775-01.)

1977 DOSE-RELATED CHANGES IN THE PHARMACOKINETICS OF TRICHLOROETHYLENE IN RAT.

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Trichloroethylene (TCE), a widely used organic solvent, and degreasing agent, is regarded as a hepatotoxicant. The overall objective of these studies were to investigate the role of parent compound as well as its metabolites in eliciting injury as well as tissue repair and to evaluate the potential for predictive metabolite dosimetry using a physiologically based pharmacokinetic (PB PK) model. Male S-D rats were injected with a ten-fold dose range of TCE (250, 500, 1250 and 2500 mg/kg) and hepatotoxicity, tissue repair and bioactivation of TCE were studied during a time course of 0 to 96 hr. Results on hepatotoxicity and tissue repair were presented earlier. In the present study the plasma levels of TCE, trichloroacetic acid (TCA) and trichloroethanol (TCOH) were measured at different time points. Peak levels of TCE were reached between 1 and 3 h after TCE administration and by 24 to 36 h TCE levels in plasma declined to very low levels. By 1 h TCOH levels were significantly increased and peak levels were noted between 3 to 12 hr. After 12 h TCOH levels started declining and by 36 to 72 h almost all TCOH was eliminated from plasma. TCA, an important metabolite of TCE, appeared by 1 h and peaked between 12 to 24 h. Plasma TCA levels were dose-dependent. TCA declined to very low levels by 48 to 72 h suggesting rapid metabolic and excretory elimination. A PBPK model was utilized to compare the model prediction from this study with predictions from previously published studies. Model predictions of TCE, TCA and TCOH concentrations in rats by intraperitoneal route are in good agreement with previously published experimental data by oral route. (Supported by ATSDR U61/ATD 681482.)

1978 PHYSIOLOGICALLY BASED ESTIMATES OF THE HETEROGENEITY IN CANCER RISK VALUES FOR TETRACHLOROETHYLENE.

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In 1996, the US Environmental Protection Agency (EPA) released for public comment a revised draft guidelines for carcinogen risk assessment. This prompted us to test how the ranges of cancer risk values, estimated by following these new guidelines, compare to the values obtained by the classical EPA-prescribed 1986 method. Tetrachloroethylene (PERC) was selected as a test compound for this comparison (q1*=2.373E-2 ppm-1). The purpose of this study was to provide estimates of the cancer risk values for PERC, based on heterogeneity of cytochrome P450 (CYP) activity in humans. This was accomplished by the use of a physiologically based pharmacokinetic (PBPK) model coupled with a Monte Carlo (MC) analysis and mouse bioassay data. Based on these simulations the lower 95% confidence limit on a dose associated with a 10% extra tumor probability in mice was determined. The distribution of CYP activities in humans was estimated from trichloroethylene experimental data and converted to the equivalent PERC metabolism rates. Because liver is the site of tumors in mice, it was assumed that the same amounts metabolized per kg of liver tissue over the lifetime of mice and humans give the same probabilities of causing tumors. Based on these PBPK/MC simulations a carcinogenicity slope factor for PERC in humans was derived (geomean q1*=2.073E-2 ppm-1; mean q1*=2.99E-2 ppm-1), along with its probability distribution (5th and 95th percentile: 7.3E-3 and 8.0E-2 ppm-1) and confidence interval for a simulated human population (95% CLs on arithmetic mean=2.59E-2 to 3.39E-2 ppm-1).

1979 DERIVATION OF A HUMAN HEALTH-BASED GROUNDWATER CRITERION FOR *TERTIARY*-BUTYL ALCOHOL.

T. Ledoux and G. Post. *New Jersey Department of Environmental Protection, Trenton, NJ.*

A human health-based groundwater criterion for tertiary- butyl alcohol (TBA) was derived, based on lifetime exposure to TBA in drinking water. TBA contamination of groundwater may arise from its uses in chemical processes and as a gasoline additive, and because TBA is an environmental degradation product of methyl tertiary-butyl ether. TBA was classified as a possible human carcinogen based on renal tumors in male rats and thyroid tumors in male and female mice in a bioassay conducted by the National Toxicology Program. A Reference Dose of 0.0175 mg/kg/day was developed based on

increased severity of nephropathy in female rats at a dose of 175 mg/kg/day, with an additional uncertainty factor of 10 for possible carcinogenicity. The application of exposure assumptions appropriate for drinking water resulted in a criterion of 122.5 μg/L, which was rounded to 100 μg/L. The TBA criterion is currently applied on an interim basis, and proposal as part of an upcoming regulation is anticipated.

1980 UNIFIED PROBABILISTIC APPROACH TO CHARACTERIZE CANCER AND NONCANCER RISKS: THE CASE OF TRICHLOROETHYLENE IN RESIDENTIAL WATER.

K. T. Bogen. *Lawrence Livermore National Laboratory, University California, Livermore, CA.* Sponsor: H. Witschi.

Traditional risk estimates may be inflated, particularly if cancer is the dominant endpoint and there is fundamental uncertainty as to mechanism(s) of action. Risk is more realistically characterized if it accounts for joint uncertainty and interindividual variability (JUV) after applying a unified probabilistic approach to the distributed parameters of all (linear as well as nonlinear) risk-extrapolation models involved. Such an approach was applied to characterize lifetime risk of incurring either cancer or a noncancer endpoint due to hypothetical residential exposure to trichloroethylene (TCE) from ground water at an inactive landfill site on a U.S. Air Force Base in California. Variability and uncertainty were addressed in exposure-route-specific estimates of applied dose, in pharmacokinetically based estimates of route-specific metabolized fractions of absorbed TCE, and in corresponding biologically effective doses estimated under a genotoxic/linear (MAG) vs. a cytotoxic/nonlinear (MAC) mechanistic assumption for TCE-induced cancer. Increased risk conditional on effective dose was estimated under MAG based on seven rodent-bioassay data sets, and under MAC from data on acute hepatocellular lipoperoxidation in mice exposed to the reactive TCE metabolite, trichloroacetic acid. Mean and upper-bound risk estimates of were estimated to be <2 and <50 per million, respectively; estimates based on traditional deterministic methods were >100 per million. Based on JUV analysis, the potential for any site-induced harm was estimated to be very unlikely. The approach used is well suited to characterize risks involving uncertain and/or diverse mechanisms of action. [This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract W-7405-Eng-48.]

1981 DERIVATION OF A DRINKING WATER ACTION LEVEL FOR 1-HYDROXYETHYLENE DIPHOSPHONIC ACID (HEDP).

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1-Hydroxyethylene diphosphonic acid (HEDP) is a chelating agent that has secondary direct and indirect food additive approvals from U.S. FDA. It has been granted an exemption from a pesticide tolerance by U.S. EPA. HEDP is also used in potable water as a scale control chemical. Its disodium salt, ethane-1-hydroxy-1,1-diphosphonate (EHDP) is used pharmacologically to treat bone mineralization disorders such as Paget's disease. In order to derive a maximum drinking water level (MDWL) for HEDP, clinical, toxicological, and pharmacokinetic data on HEDP and EHDP were examined. Both compounds are biphosphonates, which inhibit bone and cartilage mineralization. Sufficient chronic animal data, as well as clinical data, exist to characterize the potential for HEDP and EHDP to adversely affect the bone matrix. This critical effect was used to evaluate HEDP toxicity because effects on the bone matrix were observed at much lower concentrations than other effects, such as kidney damage. The MDWL was calculated using a NOAEL of 1 mg/kg/day EHDP reported from a six-month oral study in humans. This study was selected over other HEDP and EHDP studies because of its use of multiple dose groups, a clear NOAEL, and an evaluation of EHDP's effect on the bone matrix. Because humans have safely ingested EHDP at concentrations up to 5 mg/kg/day for a six-month time period, followed by a six-month recovery period, the 1 mg/kg/day NOAEL was multiplied by 0.5 (180 days/365 days). This adjustment results in a NOAEL of 0.5 mg/kg/day. As part of the MDWL derivation, safety factors totaling 100 were used (10 to account for sensitive human subpopulations (particularly individuals with increased bone turnover, such as growing children) and 10 to account for less-than-chronic exposures).

1982 RISK ASSESSMENT OF ALUMINUM, BENZENE, CARBOFURAN, CARBON TETRACHLORIDE, DICHLOROMETHANE, DIQUAT, NICKEL, SIMAZINE, TETRACHLOROETHYLENE, THIOBENCARB, URANIUM AND VINYL CHLORIDE IN DRINKING WATER.

A. Fan, J. Brown, T. McDonald, T. Parker, Y. Wang, D. Ting, A. Salmon, D. Morry, M. DiBartolomeis, G. Alexeeff, H. Russell and E. Hernandez. *California Environmental Protection Agency, Oakland, CA.*

Risk assessments were performed to develop health protective concentrations of chemicals in drinking water, along with an analysis of the toxicological basis for differences between these concentrations and the corresponding federal maximum contaminant levels, or MCLs. The concentrations (mg/L) are provided below for non-cancer endpoint and cancer endpoint, and federal MCL (NA means not applicable), respectively, for each chemical: Aluminum, 0.06 - increased blood level, NA, NA; benzene, 0.026 - hematological effects, 0.00014, NA; carbofuran, 0.0017 - testes changes, NA, 0.04 - cholinesterase inhibition, testicular toxicity, clinical signs; carbon tetrachloride, 0.007 - liver lesion, 0.00012, 0.005 - cancer; dichloromethane, 0.133 - liver toxicity, 0.00013, 0.005 - cancer; diquat, 0.015 - lens opacity, NA, 0.02 - lens opacity and cataracts; nickel, 0.001 - reproductive toxicity, NA, NA; simazine, 0.004 - reduced body weight, 0.0004, 0.004 - cancer; tetrachloroethylene, 0.011 - neurobehavioral effect, 0.000056, NA; thiobencarb, 0.07 - decreased body weight gain, NA, NA; uranium, 0.0002/0.2 pCi/L - kidney function, NA, NA; and vinyl chloride, 0.004 - liver toxicity, 0.00005, 0.002 - cancer. The concentrations derived (the more protective one when two values exist for a single chemical) are proposed as the public health goals (PHGs) which are to be used for derivation of California MCLs.

1983 RISK ASSESSMENT OF TETRACHLOROETHYLENE (PCE) FOR CALIFORNIA DRINKING WATER.

A. G. Salmon[1], E. W. Fanning[2], J. P. Brown[1] and A. M. Fan[1]. [1]*Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA and* [2]*University of California, Berkeley, CA.*

As part of California's update of drinking water standards, a Public Health Goal (PHG) of 0.056 μg/L for PCE in drinking water is proposed. The PHG is based on carcinogenic effects observed in animals, and a *de minimis* theoretical excess individual cancer risk of 10^{-6}. Exposure to PCE induced hepatocellular adenoma or carcinoma in mice by inhalation (NTP, 1986) or ingestion (NCI, 1977), and mononuclear cell leukemia in rats by inhalation (NTP, 1986). Increases in tumor incidence at several sites have been observed in studies of workers in the dry-cleaning industry (Blair et al., 1990; Ruder et al., 1994). For the proposed PHG, cancer potency was estimated by fitting a time-dependent polynomial model to the experimental data in animals to establish the lower 95% confidence bound on the dose associated with a 10% tumor incidence (LED_{10}). Oral and inhalation human potency estimates, estimated from data in mice and (for inhalation) rats were 0.42 $(mg/kg\text{-}day)^{-1}$ (oral) and 0.26 $(mg/kg\text{-}day)^{-1}$ (inhalation). Dosimetry for oral and inhalation exposures in animals was based on a steady-state pharmacokinetic calculation. For human potency estimates, the fractions of PCE metabolized during low dose inhalation and oral exposures predicted by Bois et al. (1996) were used. The epidemiological studies of PCE-exposed humans are subject to many uncertainties, and are not useful as the primary basis of a cancer risk assessment. However, the observed outcomes appear to be consistent with the potency estimates based on animal studies. The concentration of PCE in drinking water protective against non-cancer chronic toxicity is estimated to be 11 ppb, based on neurobehavioral effects observed in three epidemiological studies of humans inhaling PCE.

1984 REASSESSMENT OF THE CANCER RISKS OF BENZENE FOR CALIFORNIA'S DRINKING WATER PROGRAM.

T. A. McDonald, E. W. Fanning and L. Zeise. *State of California, EPA, Oakland, CA.*

A Public Health Goal (PHG) of 0.00014 mg/L (0.14 ppb) is proposed for benzene in drinking water, and assumes a de minimis cancer risk level of 10^{-6} from lifetime exposure to benzene. The PHG is based on observations of increased rates of leukemia among two cohorts of workers exposed to benzene: rubber hydrochloride workers in the U.S. (Paxton et al., 1994) and benzene-exposed workers from various industries in China (Hayes et al., 1997). After evaluating the evidence regarding the expected shape of the dose-response curve at low doses, cancer potency estimates were calculated from

the cohort data using Poisson regression and linear relative risk models. Estimates of lifetime risk to the general population from constant exposure to benzene were calculated using life table analyses. The pattern of leukemia risk following exposure to benzene was investigated and observed to be similar to the pattern of risk observed for radiation and chemotherapeutic agents. Accordingly, lifetime risk estimates were calculated such that exposures greater than 30 years in the past would not contributed significantly to leukemia risk. The best upper-bound estimates of lifetime leukemia risk resulting from air exposures of the general population to benzene were similar for the U.S. rubber workers (0.044 ppm^{-1}) and the Chinese workers (0.056 ppm^{-1}). The two risk estimates were combined and converted to a population-based cancer potency of 0.055 $(mg/kg\text{-}d)^{-1}$ for inhalation exposures, which was scaled to 0.11 $(mg/kg\text{-}d)^{-1}$ for oral exposures. Due to the shape of the dose-response curves and other considerations, the best estimates were calculated from workers in the lowest exposure groups only. Additional estimates, based on the use of linear extrapolation of all dose groups, the absolute risk model, and U.S. EPA proposed methodology, were also calculated and ranged from 0.19 to0.0014 $(mg/kg\text{-}d)^{-1}$.

1985 RISK ASSESSMENT OF DRINKING WATER DISINFECTION BY- PRODUCTS (DBPs): USE OF STRUCTURE-ACTIVITY RELATIONSHIPS (SAR) ANALYSIS IN RANKING OF CARCINOGENIC POTENTIAL AND PRIORITIZATION FOR TESTING.

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Disinfection by-products (DBPs) are formed when disinfectants such as chlorine, chloramine and ozone react with inorganic and organic matters in the water. The observations that some DBPs such as trihalomethanes (THMs), haloacetic acids, and halo-furanones (e.g., MX) are carcinogenic in animal studies have raised public concern over the possible adverse health effects of DBPs. Several hundreds DBPs have been identified. An in-depth mechanism-based SAR analysis, supplemented by extensive literature search for genotoxicity and other data, was used for ranking of the carcinogenic potential of DBPs that met the following criteria: 1) detected in actual drinking water samples; 2) have insufficient cancer bioassay data for risk assessment; and 3) genotoxicity and physicochemical properties indicate possible concern. Of the 215 DBPs analyzed, only 14 were found to be of a moderate or moderate-high concern level for carcinogenic potential. Of these, four were structurally related to MX. Five other are halogenated alkanes which presumably will be controlled by existing and future THM regulations. The halogenated nitriles, propanones, and nitrobromomethane in the moderate or moderate-high concern categories may be suitable candidates for testing. (The scientific views expressed are solely those of the authors and do not necessarily reflect those of the USEPA.)

1986 DAILY WATER INGESTION IN THE U.S. - AN UPDATE.

J. T. Du. *USEPA, Washington, DC.*

Water ingestion rate is used in risk assessments. To obtain up-dated estimates of water ingestion, we analyzed data from USDA's 1994 - 1996 Continuing Survey of Food Intake by Individuals. Mean and percentile distributions of water ingestion for community, bottled, and other water were estimated by age, gender, race/ethnicity, socioeconomic status and geographic region. Water ingestion refers both to the drinking water (direct) and water added in final preparation of beverages and foods at home or in a restaurant (indirect). Intrinsic water (water contained in foods and beverages at times of market purchase) is not included in our estimate. Since we presented our preliminary results in SOT last year, we have checked thousands of food recipes to refine the estimates of indirect water. Current results show that the estimated mean, 90th and 95th percentile of community water ingestion are 0.9, 2 and 2.5 L/day, respectively for the general population; and 0.3, 0.9 and 1 L/day, respectively for a child less than one-year old. These values are equivalent to 0.02, 0.03 and 0.04 L/kg body weight/day, respectively for the general population; and 0.05, 0.13 and 0.16 L/kg bw/day, respectively for a child less than one-year old. (The opinions expressed are those of the authors and do not necessarily reflect U.S. EPA policy.)

1987 ACCEPTABLE CONCENTRATIONS FOR CHLOROFORM (TCM) IN DRINKING WATER ON THE INTERNATIONAL SPACE STATION.

H. D. Garcia. *Wyle Laboratories Life Sciences Systems and Services, Houston, TX.* Sponsor: C. Lam.

Drinking water on the International Space Station (ISS), and possibly on Mars missions, will be generated from recycled hygiene water, urine, and humidity condensate. On ISS, this will be supplemented by water from the U.S. Shuttle or Russian Progress resupply vehicles. Since trace levels of volatile chlorinated organic compounds are often found in spacecraft air, traces of TCM are expected occasionally in spacecraft drinking water under normal conditions. Astronauts will ingest this water for 6 months (ISS), 2 years (Mars) or longer. In collaboration with the National Research Council's Committee on Toxicology, NASA's Toxicology Group has calculated acceptable concentrations (ACs) of TCM in spacecraft water, based on a critical review of the scientific literature on the toxicity of ingested TCM. All major toxic effects were evaluated; hepatotoxicity and taste aversion (reduced water consumption) were the most sensitive indicators of TCM ingestion. Central nervous system depression, a major effect of inhaled TCM vapors, has not been reported for TCM ingested in drinking water, even at high concentrations, probably due to a "first pass" effect of metabolism by the liver before reaching the blood and brain. The weight of evidence from many studies indicates that TCM exposures that do not produce cytotoxicity and cell regeneration will not result in tumorigenesis. In humans, the liver is the organ most sensitive to TCM toxicity. Thus, ACs that protect against hepatotoxicity will also protect against nephro-toxicity, thyroid toxicity, carcinogenicity, reproductive and developmental toxicity. AC calculations assumed a 70 kg healthy adult astronaut consuming 2.8 liters of water per day (including 0.8 L for food preparation). Safety factors of 1 to 10 were applied for inter-species extrapolation, inter-individual variations, and exposure duration. ACs were calculated for both hepatotoxicity and taste aversion for exposure durations of 1 day to 1000 days. The lower of the ACs for each duration was selected as the Spacecraft Water Exposure Guideline (SWEG): 1-d: 60 mg/L, 10-d: 60 mg/L, 100-d: 18 mg/L, and 1000-d: 6.4 mg/L. Due to differences in the safety factors applied and toxic effects considered, these SWEGs are substantially higher than limits set by government agencies for public drinking water.

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