1 APPLICATION OF STEM CELLS IN BIOMEDICAL RESEARCH.

C. N. Svendsen. *University of Wisconsin, Madison, WI.* Sponsor: J. Davila.

In recent years stem cells are a subject of increasing scientific interest because of their potential utility in numerous biomedical applications. Stem cell technology has provided unprecedented opportunities not only for studying and understanding human development but also for changing the way we potentially will discover and develop new drugs, as well as test them for safety. This presentation will provide an overview on the evolving concept of applying stem cell technology to biomedical research, derivation of diverse tissue-specific cell types, and specific example of stem cell applications in biomedical research. This mini-course will be of interest to all toxicologists and related scientists from industry, academia, and government who are interested in the application of *in vitro* approaches using stem cells to predict the impact of drug exposure in humans as well as the generation of cells and tissues that could be used as therapies.

2 ESSENTIAL INFORMATICS FOR TOXICOLOGISTS.

J. Fostel[4], R. Thomas[3], L. Burgoon[2] and W. B. Mattes[1]. *[1]Pharmacia Corporation, Kalamazoo, MI, [2]Michigan State University, East Lansing, MI, [3]Kalypsys, Inc., La Jolla, CA and [4]Pharmacia Corporation, Kalamazoo, MI.*

The combination of the Internet, automated data acquisition, and genomic information has transformed the role of the computer in the modern scientist's life. A familiarity with word processing and simple spreadsheets is simply not adequate preparation for dealing with large datasets such as those generated by toxicogenomics or high-throughput screens. Increasingly, the software tools used to deal with such data require an understanding of basic concepts in computer science, database design, bioinformatics and statistics. This basic level course hopes to provide the beginnings of such an understanding. Thus the first lecture will cover some of the essential concepts of operating systems, file and data concepts and programming concepts. This will be followed by a talk discussing the essentials of database design and use, contrasting flat-file and relational databases. A third lecture will provide an overview of how to work with protein and nucleic acid sequences: homology searching and sequence alignment. The final lecture will cover concepts of visual analysis of large data sets, and contrast some of the various approaches used. Hopefully after this course the student will be conversant in informatics to the level of effectively interacting with computer scientists, as well as collecting and manipulating datasets with reasonable skill. Exercises will be included that the attendee can review and practice on their own computer in order to better understand the principles discussed.

3 UNFOLDING THE SECRETS IN CULTURING BRAIN CELLS: THEORY, TECHNIQUES, AND BEYOND.

M. Noble[5], W. Zheng[4], M. Aschner[3], W. R. Mundy[2] and T. J. Shafer[1]. *[1]USEPA, Research Trangle Park, NC, [2]USEPA, Research Trangle Park, NC, [3]Wake Forest University School of Medicine, Winston-Salem, NC, [4]Columbia University, New York, NY and [5]University of Rochester Mecial Center, Rochester, NY.*

In vitro culture of selected cell types of the CNS is an indispensable tool in modern neuroscience research. Rapid development in theories and techniques has revolutionized the traditional way in culturing neurons, neuroglia, and brain barrier cells. For example, co-culture techniques make it possible to investigate molecular mechanisms underlying regeneration of neurons, interaction between neurons and neuroglia, and induction of brain barriers. Recent advancement in stem cell research provides an additional dimension toward the reproduction of nearly all brain cell types. The fast-paced progress in *in vitro* culture of brain cells has presented itself as the most dynamic and rapidly advancing field in neuroscience. It is for that matter that molecular mechanisms are rarely elucidated without evidence obtained from studies using cell cultures. This course will provide comprehensive reviews of cutting edge technology in culturing brain cells beginning with a brief introduction to tissue culture theory and practice. Subsequent lectures will focus on three major cell types of the CNS, *i.e.*, neurons, astrocytes, and barrier cells, and discuss in detail the techniques to establish a primary culture, to maintain an established cell line, and to create an immortalized cell line. The theory and practice of stem cell cultures in neurotoxicological studies will also be addressed. Application of these techniques in basic research, regulatory monitoring, and industrial R&D will be discussed. This course serves as an ideal introduction to students, postdoctoral fellows, and industrial researchers beginning in their culture research, and also is suitable to those who want to improve *in vitro* culture techniques in their own laboratories. For experienced researchers, the course will provide a comprehensive review of the most recent progress in cell culture techniques.

4 THE NUTS AND BOLTS OF GENETICALLY ENGINEERED MICE IN TOXICOLOGY.

B. Bolon[4], C. A. Montgomery[3], L. K. Riley[2] and N. W. Fox[1]. *[1]Lilly Research Laboratories, Indianapolis, IN, [2]University of Missouri, Columbia, MO, [3]ComPath, Conroe, TX and [4]Amgen, Inc., Thousand Oaks, CA.*

The recent advent of genetic engineering techniques has allowed intentional engineering of novel animal models for human diseases. This has had a profound impact on basic biomedical research and has accelerated the pace at which pharmaceutical and biotechnology firms can discover new targets and innovative drug candidates. In addition it has greatly enhanced the study of mechanisms of toxicity. This course will provide an overview of how genetic engineering is used to create such models, specific issues related to management of such animals, the identification of phenotypic alterations and their evaluation in the development of models, and the use of these models in the pharmaceutical industry for discovering new targets for therapy interventions and developing new drug candidates. The course will be presented at the basic level and will provide information for scientists of all toxicology disciplines who are considering the use genetically engineered models in their research or need to understand the information provided by the use of such models. It will especially be of use to those who are not currently working with whole animals or such genetically engineered models.

5 FUNDAMENTALS OF RISK ASSESSMENT AND APPLICATIONS OF RECENT METHODOLOGIES TO DIFFICULT PROBLEMS.

G. P. Broby[6], E. C. Hubal[5], J. S. Tsuji[4], T. E. Widner[3], D. J. Paustenbach[2] and P. Williams[1]. *[1]Exponent, Inc., Boulder, CO, [2]Exponent, Inc., Menlo Park, CA, [3]ENSR International, Alameda, CA, [4]Exponent, Inc., Bellevue, WA, [5]National Exposure Research Laboratory, Research Triangle Park, NC and [6]Exponent, Inc., Oakland, CA.*

The field of risk assessment has evolved at a fairly rapid pace over the past 4-5 years. Fifteen years ago, it was not uncommon for risk assessments to be conservative descriptions of the plausible risks posed by chemicals; often the approach was dictated by regulatory guidance or criteria. Today, the approach to characterizing risks is more flexible than in years past. This course will include an introductory lecture on the fundamentals of risk assessment with an emphasis on the changes in risk assessment procedures that have occurred over the past five years (*e.g.*, new EPA cancer guidelines, children's health guidelines, monte carlo techniques, aggregate and cumulative risk, etc.). The four basic parts of a risk assessment will be described (hazard identification, dose-response assessment, exposure assessment, and risk characterization) and state-of-the-art approaches to each will be presented. This lecture is followed by three case studies. The first will discuss one of the most complex risk assessments ever conducted. It involves a former government facility which used and emitted both chemical and radiological agents. The second will present an analysis of the risks to children posed by CCA treated wood. The last case study will present several examples of how to evaluate some of the hazards posed by consumer products. Applicable regulatory guidance (both domestic and international) will be cited in the various talks.

6 CUTANEOUS TOXICITY-CURRENT METHODS AND CONCEPTS IN SAFETY EVALUATION AND RELEVANCE TO HUMAN EXPOSURE.

A. C. Jacobs[5], R. C. Wester[4], C. S. Auletta[3], C. A. Ryan[2] and N. A. Monteiro-Riviere[1]. *[1]North Carolina State University, Raleigh, NC, [2]Procter & Gamble Company, Cincinnati, OH, [3]Huntingdon Life Sciences, East Millstone, NJ, [4]Universtity of California at San Francisco, San Francisco, CA and [5]USFDA, Rockville, MD.*

Humans are exposed to a large number of potentially toxic substances through the skin. Cutaneous exposure may occur intentionally, as with use of pharmaceuticals and consumer products, or accidentally, as a result of industrial or environmental exposure. Evaluation of the safety of these substances is an important function of toxicologists. It has been several years since SOT presented a continuing education course on this topic. The course will provide presentations and discussions of the current status of cutaneous toxicity safety evaluation, using *in vitro* and *in vivo* (animal model) systems as well as clinical evaluations in humans. Areas covered will include irritation, toxicity and phototoxicity. Regulatory aspects, current protocols, guidance on study design and interpretation of results will be discussed as will the relevance to human exposure. This would be a basic course of interest to toxicologists who may work with materials with a potential for cutaneous exposure (pharmaceuticals, consumer products, chemicals). It will provide an overview of the topic for those with little or no experience in this area and will provide updates, with information on current ideas and methods in the field, for those currently working in dermal toxicology. The presentation on *in vitro* assays will be of special

interest to scientists specializing in this area. Presentations on human testing and CDER/U.S. FDA practice will be of interest to those with concerns in the areas of drug development and regulatory affairs.

7 MEDICINAL HERBALS AND DIETARY SUPPLEMENTS.

G. N. Scott[4], J. Betz[3], E. A. Yetley[2] and C. S. Smith[1]. [1]*National Institutes of Environmental Health, Research Triangle Park, NC,* [2]*USFDA, College Park, MD,* [3]*NIH, Bethesda, MD and* [4]*Eastern Virginia Medical, Norfolk, VA.* Sponsor: A. Fuciarelli.

Medicinal herbals and other dietary supplements are consumed by an estimated one-third of the US population. Over 1500 botanicals are sold as dietary supplements or ethnic traditional medicines. Their use has increased substantially since passage of the 1994 Dietary Supplement Health and Education Act. Herbal formulations are not subjected to FDA pre-market toxicity testing to assure their safety or efficacy. However, there is an increased public awareness of the need to conduct toxicity studies on herbs and herbal ingredients and many government and private laboratories are contributing to this effort. Perhaps the largest single effort in this area is being conducted by the National Toxicology Program where studies are being conducted on the following medicinal herbals and compounds found in herbs: aloe vera gel, black cohash, comfrey, ginseng and ginsenosides, goldenseal, kava kava, pulegone, thujone, and extracts of grape seed, pine bark, black walnut, *Echinacea purpurea, Ginkgo biloba* and milk thistle. In this course, speakers will present information relevant to toxicity testing for safety and efficacy. Presentations describe the on-going efforts in chemical analyses and dosing/formulation issues; safety assessment including carcinogenicity, reproductive toxicity, immunotoxicity, neurotoxicity, and effects associated with acute exposure to high doses and chronic exposures to low doses; adverse human health effects with an emphasis on circumstances under which the adverse reactions may occur; interactions with pharmaceutical products; and perspectives on research needs and priorities for safety assessment of herbal medicines and dietary supplements.

8 GENOMIC AND PROTEOMIC ARRAY FORMATS ON THE CUTTING-EDGE.

S. Mousses[4], P. Bertone[3], M. Madou[2] and D. Walt[1]. [1]*Tufts University, Medford, MA,* [2]*University of California, Irvine, CA,* [3] *Yale University, New Javen, CT and* [4]*NIH, Bethesda, MD.* Sponsor: M. Cunningham.

Microarrays are now being used to explore gene expression from a variety of tissues and cells on the scale of tens of thousands of genes. They have been used in toxicology over the past three to four years to investigate and predict toxic effects and explore the mechanisms of action by which compounds cause these effects. Several past courses and seminars have detailed the most common formats: oligonucleotide and cDNA arrays. In this course, new formats which are on the cutting-edge and in some cases, in development, will be explored. These formats include optic fiber, electronic, tissue and protein microarrays. Each format presented has its own advantages and limitations but can offer a more defined look at gene and protein expression compared to the current common formats. Optic fiber and electronic arrays offer instantaneous hybridization and detection. Protein arrays offer a quicker scan of protein activity with better resolution than the more traditional method of performing two-dimensional electrophoresis and annotating by mass spectroscopy. Tissue arrays are essentially a microarray of histology and can be used to detect DNA, RNA or protein. Each format will be presented in detail with how it is applied to current genomic and proteomic issues. Specific applications or perceived applications to issues in toxicology will be presented

9 INTEGRATING TOXICOLOGIC PATHOLOGY INTO COMPOUND EVALUATION AND RISK ASSESSMENT II.

R. L. Peiffer[4], A. Fix[3], W. Kerns[2] and P. Mann[1]. [1]*Experimental Pathology Laboratory Northeast, Galena, MD,* [2]*Pharmacology Consulting Inc., Harvard, MA,* [3]*Procter & Gamble Company, Cincinnati, OH and* [4]*Merck Research Laboratories, West Point, PA.*

The contribution of pathology assessment to toxicity assessment is invaluable but often not clearly understood. Pathology endpoints are the central response around which human health risk assessment is determined. Therefore, it is important that the general toxicology community understands current concepts and nomenclature of toxicologic pathology. Toxicologic pathology is a discipline that changes and adapts over time including methods of analysis and nomenclature of lesions. As risk assessments are reevaluated and updated on commodity chemicals, frequently the older literature must be evaluated. This course will present ideas on how to evaluate terminology and diagnoses in light of current standards, diagnostic drift, and changed interpretation. Then a continuation of a systems approach to toxicologic pathology assessment will continue with a review of the cardiovascular system, neuropathology, and the eye. Lectures will cover normal structure, function, diagnostic terminology, and specific case examples.

10 CHOICE AND APPLICATION OF CLASSICAL, POPULATION OR PHYSIOLOGICALLY-BASED PK FOR CHEMICAL ASSESSMENT AND PHARMACEUTICAL DEVELOPMENT.

D. R. Plowchalk[5], H. A. Barton[4], S. Gupta[3], R. Dixit[2] and J. C. Lipscomb[1]. [1]*USEPA, Cincinnati, OH,* [2]*Merck Research Laboratories, West Point, PA,* [3]*Schering-Plough Research Institute, Kenilworth, NJ,* [4]*USEPA, Research Triangle Park, NC and* [5]*Pfizer Inc., Groton, CT.*

Both toxicity and therapeutic effectiveness are dictated by the delivery of a chemical to its target (pharmacokinetics, PK) and the response (pharmacodynamics, PD) which follows the molecular interaction between xenobiotic and target molecule. This session will instruct participants in proper methods to conduct investigations and interpret PK findings. It will contain an overview of PK in risk and safety assessment and four lectures on topics critical to the identification of PK models and their proper application to derive toxicologically, risk and safety-relevant measures. Lecture content will be aimed at instructing the selection of therapeutic doses; identification of margins of safety; methods applicable to the extrapolation of doses; the separate benefits of classical versus physiologically-based PK models; and proper methodology to collect, interpret and employ measures of population PK variance. PK is a critical modulator of toxicity, and is a fundamental component in risk assessments, safety assessment and drug development. This advanced level course is intended to educate professionals who are considering or have recently begun to increase their studies of PK; it will guide the development of toxicity studies which can also collect useful PK information. The course has been designed to address the interests of scientists involved in the design of basic toxicity and pharmacokinetic studies, preclinical and clinical studies, and those scientists conducting safety and/or risk assessments.

11 EVALUATION OF IMMUNOMODULATION IN SAFETY ASSESSMENT.

K. Hastings[4], J. Bussiere[3], R. V. House[2] and J. Dean[1]. [1]*Sanofi-Synthelabo, Malvern, PA,* [2]*Dynport Vaccine Company, Frederick, MD,* [3]*Immunex Corporation, Seattle, WA and* [4]*USFDA, Rockville, MD.*

Assessment of adverse effects on the immune system is of considerable importance in the safety evaluation of investigational new drugs. This course will cover the practical aspects of immunotoxicology for pharmaceutical development and is targeted to toxicologists in the industry. Although it will focus on safety assessment of therapeutics, the concepts discussed are also applicable to a variety of test materials. The course will be introduced with a session on the historical role that immunotoxicology has played in drug development, the types of adverse events that have been reported in both clinical trials and non-clinical toxicology studies and the growing importance of this field of study in safety assessment. The second speaker will discuss basic methods for assessing immune function in rodents, including which tests may be combined with standard 28-day toxicity studies, assays to evaluate specific immune targets, as well as special studies that may be informative or necessary to conduct when immune effects are observed. The third speaker will cover immune assessment in non-human primates, and will focus on species selection, testing strategies, and technical issues specific to primates. The final speaker will discuss the current international regulatory requirements for immunotoxicology in submission of new drugs. Although this is a basic course, it is assumed that the participants will have a basic knowledge of immunology.

12 THE EFFECTS OF NON-REPRODUCTIVE HORMONES ON THE REPRODUCTIVE SYSTEM AND THE IMPLICATIONS FOR TOXICOLOGY.

R. J. Witorsch[4], P. Cooke[3], P. Morris[2] and J. Meredith[1]. [1]*Schering-Plough Research Institute, Lafayette, NJ,* [2]*The Population Council, New York City, NY,* [3]*University of Illinios, Urbana, IL and* [4]*Medical College of Virginia, Richmond, VA.*

More and more, we are coming to understand the degree of interconnectedness that ties together organ and hormone systems that were previously thought to be separate. This interconnectedness is important in both human health and in animal toxicology studies. The mammalian reproductive system undergoes basic activation events during development, and then constant fine-tuning as adults. This activa-

tion and fine-tuning rely on hormones and growth factors which can have effects both subtle and profound. This course is designed to give both the general and specialist audience a better appreciation of the impact of several different hormonal systems on male and female reproduction, and how we can use these to explain mechanisms of toxicity in the reproductive systems. John Meredith will provide a background understanding of the signaling events in reproductive tissues by reviewing normal mechanisms of endocrine interaction and signaling. Patricia Morris will then describe the complex and intriguing world of cytokines, with their pleiotropic effects on all aspects of mammalian reproduction. Paul Cooke will show how the thyroid hormones significantly impact the reproductive system both developmentally and in adults. Finally, Raphael Witorsch will explore what is known about the effects of glucocorticoids on various stages of reproduction. At the end of the course, the student will have an improved understanding of how these other hormone systems impinge on reproduction, and will be better able to determine their involvement in a lesion when puzzling out mechanisms of toxicity.

13 EPIGENETICS OF CANCER.

R. Roberts[4], J. Moggs[3], J. Goodman[2] and J. Trosko[1]. *[1]Michigan State University, East Lansing, MI, [2]Michigan State University, East Lansing, MI, [3]Syngenta Central Toxicology Laboratory, Macclesfield, United Kingdom and [4]Aventis Pharmacology, Vitry sur Seine, France.*

For the past few decades, research has focused on understanding the mechanisms of genotoxic or nongenotoxic carcinogenesis. However, recent evidence suggests that gene expression can be markedly altered *via* several epigenetic mechanisms that can lead to permanent or reversible changes in cellular behavior. Thus, cancer may develop as a result of interplay between genetic alteration and epigenetic changes. Developing an understanding of the role played by epigenetic modulation of gene expression is a key element in understanding the response of cells and organisms to toxicants, in particular carcinogens. This course will bring together several leading speakers in this area to address the different aspects of epigenetics ranging from methylation through to proposed non-genomic modes of action for transcription factors such as ER and PPAR. This is of interest to all toxicologists, particularly those interested in the mechanisms of rodent and human cancer. This basic course is intended to assist investigators who may want to incorporate a consideration of epigenetics into their research and/or teaching. Through the use of appropriate examples, emphasis will be placed upon the conceptual and theoretical basis of epigenetics. Furthermore, there will be a focus on practical aspects concerning safety assessment, *e.g.*, how an epigenetic mechanism of action might provide information concerning extrapolation from species to species and the shape of the dose-response curve at low doses.

14 UNDERSTANDING MECHANISMS OF TOXICITY OF IMMUNOSUPPRESSIVE DRUGS TO IMPROVE THEIR SAFETY PROFILES AND BROADEN THE SCOPE OF USE.

U. Christians. *Anesthesiology, University of Colorado Health Sciences Center, Denver, CO.* Sponsor: T. Kawabata.

Organ transplantation requires life-long prophylaxis with immunosuppressants to avoid rejection of the transplant organ. Today, calcineurin inhibitors are still the cornerstone of most immunosuppressive protocols. The propensity of these agents to ultimately damage the very organs they were intended to protect, especially the kidney, was always recognized, but largely tolerated due to the impressive ability to improve short-term outcomes. With mycophenolic acid and the TOR (target of rapamycin) inhibitor sirolimus, equally potent immunosuppressants that themselves are lacking the most important side effects of calcineurin inhibitors, such as nephrotoxicity and neurotoxicity, have become available. Although devoid of nephrotoxicity when administered alone, TOR inhibitors unexpectedly enhanced cyclosporine nephrotoxicity in clinical studies. Until recently, the biochemical mechanisms underlying immunosuppressant toxicity alone and in combination were poorly understood. Immunosuppressive drugs used in the prevention of transplant rejection have limited application in other fields such as autoimmune diseases due to the severe toxicities produced. Progress in pharmacogenomics, proteomics, and analytical technology such as magnetic resonance spectroscopy has led to a better understanding of the mechanisms of immunosuppressant toxicity and variability of their pharmacokinetics. The cyclosporine derivative ISATX247 is a good example for how the understanding of molecular toxicity mechanisms can result in the design of a calcineurin inhibitor with a significantly improved therapeutic index. Better knowledge of immunosuppressant pharmacodynamics will lead to (a) more effective clinical long-term management strategies of toxicity in transplant patients, (b) the development of potent immunosuppressants with better safety profiles, and (c) broadening of the scope of use of immunosuppressants used mainly in transplantation.

15 PHARMACODYNAMIC, PHARMACOKINETIC AND PHARMACOGENOMIC INVESTIGATIONS OF IMMUNOSUPPRESSANTS PROVIDE THE BASIS FOR SAFER AND MORE EFFECTIVE REJECTION PROPHYLAXIS.

L. M. Shaw. *Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA.* Sponsor: T. Kawabata.

The efficacious and safe use of the "critical-dose" immunosuppressive drugs (ISDs) cyclosporine, tacrolimus, mycophenolic acid and rapamycin requires drug concentration-guided dosing in the individual transplant recipient. Retrospective and prospective investigations of the relationship between active immunosuppressive drug concentration and clinical effects are the basis for current protocols used in transplant centers worldwide. Recognition of significant clinical pharmacodynamic variables or risk factors and adjustment of the immunosuppressive regimen, both the agents selected and the target concentration range used, is essential in order to obtain the best possible outcome. Among the most important immunologic and non-immunologic pharmacodynamic variables are: the occurrence of acute rejection, inadequate immunosuppression, degree of HLA mismatch, previous transplant, poor early graft function, infection, chronic nephrotoxicity, cold ischemia time, donor age and ethnicity. Thus the selection of a particular combination of ISDs and the corresponding target concentration range is based in part on the presence of particular clinical pharmacodynamic variables. The pharmacokinetic parameter that best reflects drug exposure is not necessarily the trough, Co value, in every case. The preferred pharmacokinetic parameter and the experimental basis for each of the ISDs will be described. The identification and use of cellular pharmacodynamic factors, illustrated by calcineurin phosphatase, the enzyme inhibited by the complex of cyclosporine or tacrolimus with either cyclophilin or FKBP12, respectively, and inosine monophosphate dehydrogenase, the enzyme inhibited by mycophenolic acid and the current status of incorporation of pharmacogenomics in clinical trials will be critically reviewed. The experimental basis for current "best practice" control of pharmacodynamic and pharmacokinetic variability of ISDs will be presented.

16 GENOTYPIC AND PHENOTYPIC EVALUATIONS IN CONNECTION WITH AZATHIOPRINE TOXICITY.

M. Oellerich, N. von Ahsen, M. Shipkova and V. W. Armstrong. *Department of Clinical Chemistry, George-August-University, Göttingen, Germany.* Sponsor: U. Christians.

Azathioprine is a pro-drug that is converted *in vivo* to 6-mercaptopurine (6-MP), which is subsequently metabolised to the pharmacologically active 6-thioguanine nucleotides (6-TGN). The latter are also responsible for the toxic side effects associated with this drug. Thiopurine-S-methyltransferase (TPMT) plays an important role in the catabolism of 6-MP, thereby opposing its transformation to 6-TGN. Patients with TPMT deficiency are at high risk for thiopurine drug-induced toxicity. TPMT is subject to a genetic polymorphism that leads to a heterozygous deficiency of this enzyme in 11 % of the Caucasian population and a homozygous deficiency in 0.3 %. If patients with a homozygous deficiency of TPMT are given thiopurine derivatives at a standard therapeutic dosage 6-TGN accumulate resulting in severe myelosuppression and possible life-threatening pancytopenia. Identification of such individuals through measurement of TPMT phenotype is essential. TPMT activity can be determined in a cytosolic preparation of erythrocytes. Genotyping is a further option. Out the 10 TPMT gene mutations TPMT *2, *3A, *3C account for > 90 % of cases with TPMT deficiency. Genotyping with conventional methods is hampered by the fact that not all TPMT mutations are known. There is also the possibility of a pseudo-heterozygosity, in which TPMT*3B / *3C cannot be discriminated from TPMT*3A / *1, the former bearing the consequence of complete TPMT deficiency. Such a patient has a high risk of developing toxicity but would be falsely categorized by routine genotyping as having a moderate or low risk. Novel haplotyping methods can overcome this problem. Genotyping is mandatory in patients who have recently received blood transfusions. In most other clinical situations phenotyping is the superior approach to exclude homozygous deficiency. In addition, monitoring the pharmacologically active 6-TGN metabolites in a cytosolic preparation of erythrocytes is recommended to optimise therapy during treatment with azathioprine.

17 MAGNETIC RESONANCE SPECTROSCOPY AS A TOOL TO IDENTIFY MECHANISMS OF IMMUNOSUPPRESSANT TOXICITY.

N. J. Serkova and U. Christians. *Anesthesiology, University of Colorado Health Sciences Center, Denver, CO.* Sponsor: K. Thomas.

The clinical use of the immunosuppressant cyclosporine is limited by its toxicity, mainly nephrotoxicity, and narrow therapeutic index. Although cyclosporine has been the basis for most immunosuppressive protocols in transplantation for two

decades, the exact molecular mechanisms of its toxicity remain unknown. The reason was limitations of the existing experimental methods to the evaluation of only selected biochemical pathways. In the last decade, MRS (magnetic resonance spectroscopy) has shown great promise in toxicology, drug discovery, pre-clinical drug-development and in hypothesis-driven biomedical research. MRS allows for the simultaneous assessment of all important metabolic pathways in cells including lipid (1H-MRS), glucose (13C-MRS) and high-energy phosphate metabolism (31P-MRS). We applied MRS to systematically evaluate the effects of immunosuppressants and their combinations on cell and organ metabolism *in vitro* and *in vivo* and used a novel dual lipid/ perchloric acid (PCA) extraction procedure. In rats treated with 10 mg/kg/d PO for 6 days, cyclosporine significantly inhibited high-energy metabolism: ATP: 57% of control (kidney, P<0.001), 62% (liver, P<0.01), 83% (heart, P<0.01). Also, concentrations of glutamate (mitochondrial Krebs cycle: 78% in kidneys, P<0.02) and NAD+ (mitochondrial oxidative phosphorylation: 52% in kidneys, P<0.0003) were significantly reduced. Our results showed that cyclosporine inhibits the Krebs cycle and oxidative phosphorylation in the mitochondria. As a compensatory effect, ATP production by anaerobic glycolysis was increased (elevated lactate concentrations) in all tissues. The negative effects of cyclosporine on cell metabolism were antagonized by oxygen radical scavengers indicating that oxygen radical formation plays a key role in cyclosporine-induced mitochondrial dysfunction. Our studies demonstrate that MRS is a powerful tool for pre-clinical toxicology screening of immunosuppressants and their combinations.

18 DEVELOPMENT OF THE NOVEL IMMUNOSUPPRESSIVE AGENT ISATX247 USING A PHARMACODYNAMIC APPROACH.

D. G. Freitag[1], M. D. Abel[1], L. J. Aspeslet[1], D. J. Trepanier[1], P. R. Mayo[1], P. F. Halloran[2], N. T. Kneteman[2], R. E. Morris[3], C. R. Gregory[4], R. T. Foster[1] and R. W. Yatscoff[1]. *[1]Isotechnika, Edmonton, AB, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]Stanford University, Stanford, CA and [4]University of California, Davis, CA.* Sponsor: U. Christians.

As the mechanism of action for various immunosuppressive drugs becomes understood, it is possible to utilize pharmacodynamic (PD) data with toxicokinetics to discover new drug candidates. This approach was used by Isotechnika to develop the novel immunosuppressant ISATX247 (ISA) which was found to be a more potent inhibitor of calcineurin (CN) in whole blood than cyclosporine A (CsA). Initially, several compounds were evaluated *in vitro* using the CN assay. Candidates with increased activity were tested further *in vivo* using a rat heart transplant model. The final candidate, ISA, was found to be 2.5 fold more potent than CsA *in vitro* and 3-fold more effective in promoting graft survival. Flow cytometric studies on T cell markers further characterized the immunosuppressant effects in non-human primates. These investigations determined that ISA was up to 6 times more potent at inhibiting immune functions than CsA. Based on this data a study was conducted to compare the survival times of renal allografts in non-human primates treated with either ISA or CsA. The results showed that the group receiving ISA survived significantly longer (p = 0.0036). An increase in potency could be associated with increased toxicity. Therefore, renal toxicity was evaluated in a rabbit model. The CsA treated animals had a 33 % increase in serum creatinine concentration over the treatment period. In Phase I trials, pharmacokinetic/PD correlation allowed for the determination of dose and a therapeutic window much earlier in the drug development process. Based on these correlations, ISA was used in Phase II trials, in stable renal transplant patients, at 1/3 trough concentration to CsA because the increased potency was demonstrated. Therefore, PD data may greatly accelerate the development of novel compounds.

19 USE AND APPLICATION OF STEM CELLS IN TOXICOLOGY.

G. N. Cosma[1], M. A. Thiede[2], S. Strom[3], J. A. Johnson[4] and J. E. Trosko[5]. *[1]Investigative Toxicology, Pharmacia Corporation, Kalamazoo, MI, [2]Discovery, Pharmacia Corporation, St. Louis, MO, [3]Pathology, University of Pittsburgh, Pittsburgh, PA, [4]Pharmacy School, University of Wisconsin, Madison, WI and [5]Pediatrics and Human Development, Michigan State University, East Lansing, MI.*

Recent published reports on the isolation and culturing of stem cells have created a great deal of interest and excitement in both the scientific and the public community. Stem cell technology holds great promise for advances in biomedical research, and will allow us to understand the complex events that occur during human development, provide new platforms for drug discovery and development, and replace organ-specific cell populations damaged by diseases. Embryonic stem cells are pluripotent cell populations with the ability to give rise to differentiated cells of the human body such as brain, heart, liver, bone and blood cells. Other stem cell types have been derived from fetal tissues, blood and bone marrow. Most isolated stem cells are capable of limitless division and undergo self-renewal. Thus, the cells can be maintained for extended periods of time in tissue culture making them a vital resource for biomedical research. The objective of this symposium is to provide information on the current status of efforts focused on derivation of stem cells and the application of tissue-specific stem cell types in toxicology. This symposium will be of interest to all toxicologists and related scientists from industry, academia, and government who are interested in the application of *in vitro* approaches using stem cells to predict the impact of drug exposure in humans as well as the generation of cells and tissues that could be used as therapies.

20 EXPLOITING MARROW-DERIVED ADULT STEM CELLS FOR PRE-CLINICAL SAFETY EVALUATION.

M. A. Thiede. *Discovery, Pharmacia Corporation, St. Louis, MO.* Sponsor: J. Davila.

The preclinical safety evaluation of drug candidates in cultures of human primary cells is limited by the availability of healthy donor tissue and the difficulties of maintaining the human cell isolates in culture. While Primary human hepatocyte or cardiomyocyte cultures provide an important surrogate for preclinical evaluation of liver and cardio-toxicities these cell types are particularly difficult to propagate in culture and therefore their use is greatly limited. Recent advances in stem cell biology offer the possibility of creating unlimited supplies of cell types, which can be used for research and even therapeutic purposes. While embryonic cells isolated from the pre-implantation blastocyst perhaps offer the greatest potential for producing every cell type found in the adult body, their general use for research purposes remains controversial. Alternatively, adult stem cells present in many adult tissues and which are critical to tissue repair and homeostasis, possess the unique capacity for self-renewal and differentiation into one or more specialized cell types. Furthermore, methods have been developed for isolating adult stem cells from tissue sources such as bone marrow aspirates adipose tissue recovered during liposuction procedures. Early stem cell research demonstrated that adult stem cells cultured under specific conditions could differentiate into several lineages including cells that produce bone, cartilage and fat. Recent advances in stem cell research have revealed an even greater differentiation potential for adult stem cells isolated from bone marrow. Culture conditions have now been developed to induce these adult stem cells into a vast spectrum of differentiated cell types including neurons, endothelial cells, hepatocytes and cardiomyocytes. The potential use of adult stem cells a as an alternative source of critical cells required to perform needed safety evaluation in human cells will be discussed.

21 PRODUCTION OF HEPATOCYTES FROM HUMAN AMNIOTIC STEM CELLS FOR DRUG METABOLISM AND TOXICITY STUDIES.

S. Strom, T. Miki and H. Cai. *Pathology, University of Pittsburgh, Pittsburgh, PA.* Sponsor: J. Davila.

Isolated human hepatocytes have been shown to be useful for both basic and clinical science. Hepatocyte transplantation has been shown to be an effective treatment for liver disease, and isolated human hepatocytes have been shown to be a useful model for drug metabolism, CYP450 induction or toxicology assays. The availability of sufficient numbers of hepatocytes with which to conduct the basic research or the clinical studies limits the numbers of studies which can be conducted. Stem cells could provide a new source of liver cells for cell transplantation studies or basic research. Human amniotic epithelial (AE) cells were isolated from term placenta following live birth. By immunohistochemical analysis amniotic tissue was found to react with cytokeratin antibodies AE1/AE3 and with specific antibodies to cytokeratins (CK) 18 and 19 and the hepatocyte marker Alpha 1-antitrypsin (A1AT). In addition, AE tissue showed significant reaction with antibodies to c-kit. Epithelial cells isolated from the amniotic tissue can be propagated in culture and react positively with the pan-cytokeratin AE1/AE3 and specific antibodies to CK 18, CK19, Thy-1, c-kit, A1AT and Alb. Albumin mRNA and protein were detected in extracts from cultured AE cells by RT-PCR and Western blot, respectively and A1AT protein was detected in cells by Western blot. The liver enriched transcription factor, HNF4, was detected in cultured AE cells by immunohistochemical analysis and by RT-PCR and HNF4 expression is increased under conditions which favor differentiation. Taken together these data indicate that AE tissue contains stem cells which under appropriate conditions are able to differentiate into cells with characteristics of hepatocytes. Amniotic stem cells may be useful for clinical hepatocyte transplantation and for the production of human hepatocytes for basic research such as drug metabolism and toxicology.

22 ANTIOXIDANT RESPONSE ELEMENT DRIVEN GENE EXPRESSION IN MULTIPOTENT HUMAN NEURAL PROGENITORS AND THE DIFFERENTIATED CELLS ARISING FROM THESE PROGENITORS.

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Multipotent neural progenitors can be obtained from the developing human brain and expanded *in vitro* as neuroshperes. To determine the changes in human antioxidant response element (ARE)-driven gene expression, neurospheres were treated with tert-butylhydroquinone (tBHQ), a potent activator of the ARE. Microarray analysis revealed that tBHQ could significantly upregulate ARE-driven genes such as NAD(P)H:quinone oxidoreductase (NQO1), glutathione reductase, heme oxygenase 1(HO1), thioredoxin reductase (TR), ferritin heavy and light chains, and gamma-glutamylcysteine ligase regulatory (GCLR) and catalytic (GCLC) subunits. Addition of LY294002, a selective inhibitor of phosphatidylinositol 3-kinase (PI3K), 30 min prior to tBHQ treatment, inhibited this induction by tBHQ and reduced basal levels of gene expression for most genes, except TR, GCLR, and GCLC. After neural progenitors were dissociated and plated down to promote differentiation, dynamic changes in gene expression of NQO1 were observed. NQO1 histochemistry demonstrated that NQO1 activity in well-differentiated neurons had been decreased dramatically relative to their progenitors (neuroblasts). Whereas NQO1 activity in well-differentiated glia cells was consistent with their progenitors. tBHQ also increased ARE-driven gene expression in well-differentiated cells. The extent to which was dependent on the expression of Nrf2. These data are the first to show the dynamic changes in ARE-driven gene expression during development of human neural progenitor cells. Since increased expression of these genes has been shown to protect cells from oxidative stress-induced apoptosis, it will be of great interest to correlate the dynamic changes described here with differential sensitivities to stress. *Supported by NIEHS, the BWF New Investigator Award, and the University of Wisconsin Foundation*

23 USE OF HUMAN ADULT PLURIPOTENT STEM CELLS TO SCREEN FOR GENOTOXIC/EPIGENETIC TOXICANTS.

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Understanding the mechanisms by which physical/chemical mutagenic, cytotoxic or epigenetic toxicants lead to death, teratogenesis, carcinogenesis, atherogenesis, cataractogenesis, immuno-, reproductive-, and neuro-toxicities, as well as premature aging and other diseases, requires knowledge of the homeostatic regulation of cell proliferation, differentiation, apoptosis, and senescence of cells. In the developing, mature and aging organisms, pluripotent stem cells, by symmetrical (self-renewal) and asymmetric (differentiation) processes, provide cells for growth, cell replacement and wound repair. The micro-environment (niche) of the pluri-potent stem cells in each organ, influenced by cell-matrix, cell to cell adhesion and extra- and inter-cellular communication, the net affect which determines intra-cellular signaling of the stem cell, determines whether the cell stays quiescent, apoptosis, divides asymmetrically or symmetrically. Upsetting this niche-micro-environment can alter homeostatic regulation, adaptively or mal-adaptively, the quality and quantity of the stem cell pool, their progenitor daughter cells or the terminally differentiated cells in any tissue. Mono-clonally-derived abnormal tissues found in organs, such as tumors and atherosclerotic plaques, are hypothesized to arise from single stem cells whose critical genes have been altered by either mutagenic or epigenetic mechanisms. Reduction of the stem cell pool might be responsible for the aging process of any organ, hence ultimately the organism. With the ability to isolate the few stem cells in any tissue and the availability of modern molecular biological tools such as DNA micro-array analyses, the goal of toxicology will be to characterize these stem cells and compare their sensitivity/resistance to physical/chemical toxicants to those of their progenitor and differentiated daughters.

24 BIOTERRORISM AND ITS TOXICOLOGICAL EFFECTS.

D. B. Warheit. *DuPont Haskell Laboratories, Newark, DE.*

The workshop is designed to present a basic primer on the timely topic of biological warfare agents. The first presentation will provide an overview of the major aspects of biological terror/warfare agents. A Center for Disease Control's classification of biological warfare agents will be presented, followed by a brief listing of 1) biological agents - including bacterial agents such as anthrax, cholera toxin, pneumonic plague and brucellosis; 2) viruses- such as smallpox, ebola, VEE, and VHF; 3) biological toxins - such as botulinum, Staph entero-B, ricin, and T-2 mycotoxins. In the second talk, Dr. Aileen Marty, a long-standing pathology expert (pre 9/11) on bioterrorism/ infectious disease will discuss the development of plans and methods for defending against the deliberate use of biological agents. Dr. Marty has been presenting a course on bioterrorism at her institution for several years and, in addition to her expertise on infectious diseases, is an expert on forensic and legal issues related to bioterror agents. In the 3rd presentation, Dr. Elliott Kagan, will discuss bioregulators as naturally occurring compounds that regulate physiological processes and their potential for misuse in bioterrorism - included in this category are cytokines, eicosanoids, neurotransmitters, hormones, and plasma proteases. The final presentation will focus on current concepts related to the pathogenesis of infectious agents such as inhalation anthrax and smallpox disease. For example, the talk will discuss inhalation anthrax, a deadly disease in which spores attack the lung macrophage *via* a number of novel proteins, including protective antigen (misnamed), lethal factor and edema factor, which hijack the macrophage's defensive functions and direct the cellular machinery to secrete destructive levels of cytokines. This Workshop should provide basic information on the infectious agents that pose a potential threat in a bioterrorist attack.

25 BIOTERRORISM: AN OVERVIEW.

D. K. Bhalla. *Wayne State University, Detroit, MI.*

This presentation provides an introductory primer on the classification of biological agents associated with potential uses in bioterrorism. The Federal Centers for Disease Control has recently evaluated some bioactive agents for potential bioterror attack and has classified many of these agents into priority categories (e.g., A, B, C) in order to forecast public health preparedness requirements. Agents assigned to the A Category are regarded as having the greatest potential for adverse public health impact with significant numbers of casualties. Included in this category are bacterial-derived products of anthrax, plague, botulism and tularemia. Viral agents in this category include viral hemorrhagic fevers (e.g. Ebola and Marburg viruses) and the smallpox virus. Anthrax is an acute infectious disease caused by the spores of *Bacillus anthracis* and is manifested in oral, cutaneous and inhalation forms of the disease. The inhalation form of anthrax is known to be lethal if antibiotic therapy is not initiated in a timely fashion. Plague is a historical disease caused by the bacteria *Yersinia pestis*. In 541 AD, the first plague pandemic occurred in Egypt and spread throughout the world for the next 4 years, resulting in 50-60% fatalities. Inhalation of aerosolized plague particles leads to pneumonic plague, a highly lethal and potentially contagious form. Botulinum toxin is derived from the genus of anaerobic bacteria named *Clostridia*. The toxin binds irreversibly to peripheral cholinergic synapses, inhibiting the release of the neurotransmitter acetylcholine from motor neurons and results in muscle paralysis. Smallpox is considered to be one of the most serious bioterrorist threats to the civilian population. Smallpox spreads directly from person to person, primarily as droplet nuclei expelled from the oropharynx of the infected person or by aerosol. Viral hemorrhagic fever viruses are a diverse group of viruses that cause clinical disease associated with fever and bleeding disorders. Most Category B agents also have potential for large-scale dissemination but generally have a reduced likelihood for causing illness and death.

26 RECENT CHALLENGES IN INFECTIOUS DISEASE: BIOLOGICAL PATHOGENS AS WEAPONS AND EMERGING ENDEMIC THREATS.

A. M. Marty. *Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD.*

This presentation will put in perspective emerging infections with the biological agents whose characteristics make them likely potential threat agents. It introduces the pathobiological, biochemical, molecular, and medical laboratory aspects of living agents or organic products of potential use in warfare, terrorism, or criminal activities. The talk then provides an understanding of the clinicopathologic features of potential threat agents and their toxins. Routes of host entry, the pathophysiology of host reactions, the specific cellular, biochemical, and molecular pathology in target organs and diagnostic techniques for living agents or organic products are discussed. Environmental & agricultural issues of biological agents and toxins are included. Mechanisms for providing a rapid clinicopathologic diagnosis of animal and zoonotic diseases used by terrorist are addressed. New equipment for rapid detection, including molecular techniques, and hand-held equipment are introduced. Mention of techniques to distinguish innocent from questionable use of dual-use high tech equipment will be discussed. Forensic investigation and the function of the pathologist, microbiologist, and toxicologist in chain of custody, legal, and political issues is explained. An understanding of the policy & decision-making issues will be addressed.

27 BIOREGULATORS AS INSTRUMENTS OF TERROR.

E. Kagan. *Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD.*

Bioregulators are naturally occurring organic compounds that regulate diverse cellular processes, such as bronchial and vascular tone, muscle contraction, blood pressure, heart rate, temperature and immune responses. They are produced in very small quantities in a variety of living organisms and are essential for normal homeostasis. These substances can be harmful, however, in large concentrations or if modifications to them bring about changes in the nature and duration of their action. Unlike traditional disease-causing bioterrorism agents that take hours or days to act, many bioregulators act within minutes of administration. Depending on factors such as dosage and route of administration, the action of several bioregulators may be lethal. If exploited for the purpose of bioterrorism, they could potentially cause profound physiological effects such as rapid loss of consciousness, fever, dyspnea, hypoglycemia, disseminated intravascular coagulation, heart failure, paralysis, shock, or hypertension. Other effects may be more subtle (e.g., exaggerated fear and pain). The main groups of bioregulators to be discussed comprise cytokines (IL-1, IL-6, TNF-alpha), eicosanoids (PGD2, LTC4), proteolytic enzymes (kallikrein), neurotransmitters and hormones (catecholamines). As advances continually are being made in their development, bioregulators should be considered as weapons with increasing bioterrorism potential.

28 CURRENT CONCEPTS ON THE PATHOGENESIS OF SELECTED INFECTIOUS AGENTS.

D. B. Warheit. *Pulmonary Toxicology, DuPont Haskell Lab., Newark, DE.*

Four biological agents of greatest concern are smallpox virus, botulinum toxin, and anthrax and plague bacteria. This presentation focuses on current concepts on the pathogenesis related to two of the infectious agents, namely smallpox and anthrax. Smallpox has traditionally been feared as the most devastating of all the infectious diseases. An aerosol release of smallpox virus is easily disseminated and spreads directly from person to person, primarily by droplet nuclei expelled from the oropharynx of the infected person or by aerosol. Natural infection occurs following implantation of the virus on the oropharyngeal or respiratory mucosa. The smallpox virus enters the respiratory tract, infecting the mucous membranes and transmigrating rapidly into the local lymph nodes. After a brief period of viremia, there is a latent period of 4 to 14 days, during which the virus multiplies in the reticuloendothelial system. Inhalation anthrax is initiated by endospores of *B anthracis*, a gram-positive soil organism. The major target of the anthrax spores is the alveolar macrophage. After phagocytosis, a number of proteins including protective antigen, lethal factor and edema factor cause the macrophages to significantly increase secretion of TNF-α and IL-1β, resulting in immunopathological effects and subsequent death. Specifically, anthrax bacilli secrete two exotoxins that are active in host cells. Edema toxin is a calmodulin-dependent adenylate cyclase that increases intracellular levels of cAMP on entry into most types of cells and alters water homeostasis, resulting in massive edema. Lethal toxin is a zinc metalloprotease that causes a substantial inflammatory response by activating the oxidative pathways leading to massive production of TNF-α and IL-1β. Macrophages containing anthrax migrate to the regional lymph nodes. Vegetative anthrax bacilli grow in the lymph node, creating regional hemorrhagic lymphadenitis. Bacteria spread and proliferate through the blood and lymph, causing severe septicemia. High levels of endotoxins are produced that are responsible for overt symptoms and death.

29 CUMULATIVE RISK ASSESSMENT: GETTING FROM TOXICOLOGY TO QUANTITATIVE ANALYSIS.

H. A. Barton[1] and C. N. Pope[2]. *[1]ORD/NHEERL/ETD/PKB, US Environmental Protection Agency, Research Triangle Park, NC and [2]Department of Physiological Sciences, Oklahoma State University, Stillwater, OK.*

Assessment of the cumulative risk posed by exposure to multiple chemicals is a problem the USEPA's Program and Regional Offices confront regularly. This session will focus on the interplay of toxicology studies and quantitative analysis to assess cumulative risk using a variety of case studies. The Food Quality Protection Act of 1996 directs the Office of Pesticide Programs to include in its assessments the risk associated with the cumulative effects of pesticides that have a common mode of action. Organophosphorus pesticides (OPs) have been the first class addressed, based on the common mechanism of acetylcholinesterase inhibition. The toxicology and mode of action of these compounds will be described, noting factors that may confound the assessment of cumulative effects. The quantitative cumulative risk analysis of OPs based on anticholinesterase relative potency will then be presented. The Air Office is concerned with mixtures of criteria air pollutants and volatile organic compounds. Physiologically based pharmacokinetic modeling of multiple volatile chemicals provides an example of a biologically-based approach to cumulative analysis. The Office of Water is concerned with risk from mixtures of disinfectant byproducts and balancing that risk, either as single chemicals or mixtures, against the risk associated with microbial agents in water. Development of toxicity study designs to address interactions of chemicals in mixtures of increasing complexity is a critical part of developing improved evaluations of cumulative risk, so recent developments will be described. This workshop provides an opportunity for toxicologists and others involved in experimental studies and the development and application of quantitative analytical methods to discuss recent and ongoing efforts in this important and challenging area. (This abstract does not reflect EPA policy.)

30 TOXICITY STUDIES OF MIXTURES IMPACTING MULTIPLE TARGET ORGANS.

J. P. Groten, R. Stierum, D. Jonker and B. van Ommen. *Department of Biomolecular Sciences, TNO Nutrition and Food Research, Zeist, Netherlands.*
Sponsor: H. Barton.

Toxicity studies focussing on component-based analysis of mixtures indicated that for chemicals not sharing the same target and mechanism, the type of combined action or interaction found when each chemical in the mixture was present at clearly-toxic-effect levels did not predict the response when each chemical was present at or below the lowest observed adverse effect level (LOAEL). Moreover, mixtures of chemicals produced toxicity only for those chemicals that showed the same toxic effect on the same target organ when given singly at doses above the LOAEL values (simple similar action or similar joint action). However, end-points chosen in these (routine) toxicity studies do not offer a wide dynamic range of testable, significant responses around and above the NOAEL. Therefore, small effects that might have indicated similar joint action instead of independent joint action have not been detected, a problem inherent in toxicity testing. Sensitive methods that detect low level changes have not been employed in mixtures testing protocols. Also, interactions at the mechanistic level are not studied because, until recently, information on the mechanism of action of a single chemical, let alone a mixture, could only be obtained by studying the response of genes or proteins a priori suspected to be involved in a particular toxic response. Technologies based on the progress in functional genomics now enable the determination of the expression of thousands of genes (transcriptomics) and proteins (proteomics) in a single experiment. Toxicogenomics integrates functional genomics with classical toxicology and we believe that it has great potential to revolutionize mixtures research, since it will generate new hypotheses on combined mode(s) of action within one study. Studies applying transcriptomics are carried out at TNO to obtain new mechanism-derived biomarkers to assess e.g. hepatoxicity of combined exposure to food additives or environmental toxicants. Mechanism-based analysis, including principal component analysis, are discussed for its applicability in mixtures research.

31 INTERACTIVE TOXICITY OF ORGANOPHOSPHORUS INSECTICIDES.

C. N. Pope. *Physiological Sciences, Oklahoma State University, Stillwater, OK.*

The Food Quality Protection Act requires consideration of risk from cumulative effects of multiple pesticides acting through a common mechanism. The organophosphorus insecticides initiate toxicity through a common mechanism by inhibiting the enzyme acetylcholinesterase. Extensive inhibition of this enzyme leads to elevated acetylcholine levels in synapses of the central and peripheral nervous systems, with consequent signs of cholinergic toxicity including autonomic dysfunction, involuntary movements, cardiovascular alterations, dyspnea and in severe cases respiratory depression. Some OPs have additional sites of action, however, that can modify cholinergic toxicity or affect processes outside the cholinergic synapse. It has been known for decades that some OPs can markedly enhance the toxicity of others by blockade of detoxification pathways. Maturational expression of some of these same pathways appears critical in the higher sensitivity of younger individuals to some OPs. Most OPs in use today are phosphorothioates requiring metabolic activation by the cyp450s, thus concurrent exposures can lead to competition for activating pathways. Recent studies suggest that anticholinesterases may induce the synthesis of acetylcholinesterase, potentially altering the availability of target enzymes for subsequent OP exposures. Some OPs have selective, differential actions on presynaptic cholinergic processes, i.e., acetylcholine synthesis and release, that could alter toxic responses to other OPs. In some cases, these toxicokinetic/toxicodynamic interactions may only occur with combined high dose exposures. The determination of relative potencies of individual OPs may not, however, provide sufficient information to adequately estimate risk from cumulative exposures. While acetylcholinesterase inhibition represents the common mechanism of toxicity and thus the basis for the cumulative risk assessment, consideration of these other sites of action for some OPs, where such information is available, may strengthen the predictability of cumulative risk models.

32 CUMULATIVE RISK ANALYSIS FOR ORGANOPHOSPHORUS PESTICIDES.

R. W. Setzer. *NHEERL MD-74, USEPA, Research Triangle Park, NC.* Sponsor: H. Barton.

The USEPA has recently completed a risk assessment of the effects of exposure to 33 organophosphorous pesticides (OPs) through the diet, water, and residential exposures. Oral, dermal, and inhalation routes were considered, but the oral route has by far the richest data set, and is the route discussed in detail here. The risk assessment combined a relative potency factor (RPF) approach to dose-response assessment with a probabilistic exposure assessment to estimate distributions of margins of exposure in the US population. RPFs for the oral route of exposure were based on benchmark doses (BMDs) calculated as the expected daily dose that would result in a 10% inhibition of brain acetylcholinesterase (AChE) in rats. For almost all of the 33 OPs considered, multiple dose-response data sets were available. The data sets formed a nested hierarchy: major studies conducted by different laboratories or the same laboratory at different times, and observations from serial sacrifices within major studies. Dose-response models were fit to all the data for each OP using a hierarchical model to account for variation in both levels of the hierarchy. Two interesting aspects of the dose-response shape complicated the modeling. The dose-responses for about half of the chemicals had a "shoulder" at the low-dose end: the steepness of the response increased with increasing dose. At the other extreme of dose, for many OPs, as dose increased, AChE activity decreased asymptotically to a non-zero level that varied among OPs. The RPF approach applies strictly only when dose-additivity applies, and dose-additivity applies strictly only when dose-responses can be superimposed by rescaling dose. Thus, these observations question the quality of the approximation to risk that the RPF approach provides for these data. Reliable pharmacokinetic and pharmacodynamic models of the distribution and metabolism of the OPs and of their effects on AChE would have facilitated the interpretation and use of the data base by providing an estimate of the precision of the RPF approach. [This is an abstract of a presentation and does not necessarily reflect EPA policy.]

33 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING FOR MIXTURES.

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The available data on pharmacokinetic and pharmacodynamic interactions are not used in the context of cumulative risk assessments. Physiologically-based models represent an unique framework for integrating data on binary interactions, on the basis of quantitative nature of interaction mechanisms. Physiologically-based modeling also facilitates the prediction of the consequences of interactions in more complex mixtures on the basis of mechanistic considerations. The state-of-the art of this methodology consists of: (i) developing physiologically-based pharmacokinetic (PBPK) models for individual chemicals, (ii) interconnecting the individual chemical PBPK models at the level of binary chemical interaction mechanisms (e.g., competitive, uncompetitive, or noncompetitive metabolic inhibition in liver), and (iii) comparing a priori predictions of the interaction-based model to corresponding experimental data. This methodology was validated using quaternary mixtures of aromatic hydrocarbons [benzene (B), toluene (T), ethylbenzene (E), and m-xylene (X)] and a quiternary mixture [BTEX + dichloromethane]. The binary interaction-based mixture PBPK model adequately predicted the kinetics of all componenets of the mixtures in rats and humans. The results of these modeling studies suggest that data on interactions at the binary level alone are required for predicting the kinetics and tissue dose of components of complex mixtures. By taking into account the change in tissue dose of toxic moieties of mixture components during combined exposures (i.e., the output of the mixture PBPK model), the cumulative risk associated with multiple chemicals can be assessed. These methodological approaches, together, facilitate the consideration of pharmacokinetic interactions at a quantitative level for purpose of cumulative risk analysis.

34 DESIGNING STUDIES AND COLLECTING DATA USEFUL FOR CUMULATIVE RISK ASSESSMENT.

J. Simmons[1], C. Gennings[2], M. Casey[2], M. J. Plewa[3], E. D. Wagner[3], W. H. Carter[2], A. McDonald[1], Y. M. Sey[1] and L. K. Teuschler[4]. *[1]NHEERL/ORD, USEPA, Research Triangle Park, NC, [2]VCU, Richmond, VA, [3]University Illinois, Urbana, IL and [4]NCEA/ORD, USEPA, Cincinnati, OH.*

Humans are exposed to very complex environmental mixtures. In contrast to this reality, most laboratory investigations of mixtures, with the exception of mutagenicity assays, have focused on mixtures comprised of a few chemicals. Over the past decade, great advances have been made in component-based assessment of simple mixtures with development of appropriate, efficient and novel experimental designs, statistical methods and risk assessment techniques. These gains have increased greatly our ability to collect data useful for risk assessment. Additionally, these methods and approaches have been used to assess experimentally the validity of risk assessment assumptions. This is illustrated by experiments with the four trihalomethanes - chloroform, bromoform, bromodichloromethane and chlorodibromomethane - disinfection byproducts (DBPs) formed during chemical disinfection of drinking water. In low-dose regions, dose-additive hepatotoxicity was observed in mice; in high-dose regions, the mixtures were additive or antagonistic, but not synergistic. Similar gains need to be made for complex mixtures. Component-based approaches that use information on the individual chemicals contained in the mixture are not by themselves sufficient for complex, environmental mixtures because significant portions of the mixture mass are unidentified. Joint theoretical and experimental work to extend these methods is needed and can only be successful by the combined collaborative efforts of toxicologists, statisticians and risk assessors. An effort is in progress for a more complex chemical mixture, including power and sample-size estimates, and methods to determine the portion of any observed toxicity attributable to the unidentified fraction of the mixture. In summary, experimental methods and techniques are described and illustrated for collection of data useful for risk assessment. (This abstract does not reflect EPA policy.)

35 BIPHASIC INFLUENCE OF ALCOHOL ON ESTROGEN-MEDIATED PERTURBATION OF THE CELL CYCLE IN BREAST CANCER CELLS.

A. Sharga, Q. Felty, J. DuMond and D. Roy. *Environmental Health Sciences, UAB, Birmingham, AL.*

Alcohol consumption has been implicated to increase the risk of breast cancer. Experimental results indicate that ethanol is a tumor promoter, and chronic ethanol exposure enhances metastasis and growth of breast cancer. The exact mechanisms by which chronic alcohol intake supports the development of cancer are not clear. The metabolism of ethanol leads to the generation of acetaldehyde and free radicals, which may lead to instability in the genome. The present study used an *in vitro* model to investigate the mechanism(s) underlying tumor promoting effects of ethanol. Low doses of alcohol (1-3 ul/ml) stimulated the growth of MCF7 cells and increased S phase while high doses of alcohol (5-10 ul/ml) significantly inhibited the growth of MCF7 cells. Low doses of alcohol when cotreated with 17-beta-estradiol or diethylstilbestrol increased the growth stimulatory effect of estrogen in these cells. In addition, the growth inhibitory effects of high alcohol doses (5-10 ul/ml) were reduced by the cotreatment of 17-beta-estradiol or diethylstilbestrol (1-100 ng/ml). Our results suggest a biphasic influence of alcohol on the growth of breast cancer cells. Alcohol induced perturbation of cell cycle and genetic instability may add to estrogen-induced genotoxic as well as proliferative stresses and thus mediate the promoting effects of alcohol on breast cancer development.

36 ROLE OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA IN MODULATING THE EFFECTS OF FUMONISIN B1 IN MOUSE LIVER.

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Fumonisins are mycotoxins produced by Fusarium verticillioides that induce a broad spectrum of responses in animals and are suspected human esophageal carcinogens. Exposure of rodents to fumonisins causes liver and kidney toxicity and cancer. These effects are likely triggered through inhibition of ceramide synthase by fumonisins resulting in disrupted sphingolipid metabolism and accumulations of the lipids sphingosine and sphinganine in tissues. As fumonisin B1 can act as a weak peroxisome proliferator (PP), we hypothesized that fumonisin toxicity may be partly mediated through the PP-activated receptor alpha (PPAR), an important regulator of lipid metabolism in the liver. Wild-type and PPAR-null mice were fed the PPAR agonist WY-14, 643 (WY) (0.05% w/w in the diet), fumonisin-containing (300 ppm fumonisin B1) culture material (CM), or purified fumonisin B1 (300 ppm) in the diet for 8 days. Wild-type and PPAR-null mice responded similarly to the CM and fumonisin B1 diets, exhibiting almost identical hepatic pathology and increases in liver weights, hepatocellular apoptosis, hepatocellular mitoses, sphinganine to sphingosine ratios and tumor necrosis alpha mRNA levels. PPAR-null mice lacked WY-induced liver weight increases and cell proliferation as expected. Using Affymetrix gene chips containing ~9000 mouse genes, transcript profiles of liver gene expression in the wild-type mice showed that the CM and fumonisin B1 treatments exhibited similar profiles that were different than the profile altered by WY and did not alter genes typically associated with peroxisome proliferation. These results demonstrate that PPAR alpha does not mediate the effects of fumonisin.

37 ABERRANT DNA METHYLATION IS A MECHANISM INVOLVED IN TUMOR PROMOTION.

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DNA methylation (ME) is an epigenetic mechanism regulating normal gene expression, and aberrant ME is causally involved in carcinogenesis. Hypermethylation can silence tumor suppressor genes, while hypomethylation may facilitate expression of oncogenes and/or transposons normally silenced by ME. The objective of this study was to test the hypothesis that aberrant ME is a mechanism underlying the promotion stage of tumorigenesis. Hallmarks of promotion include clear dose-response relationships, existence of thresholds and reversibility. We used a SENCAR mouse skin initiation/promotion model of tumorigenesis that permits clear demarcation of these stages. Animals were initiated with one dermal application of 75 [mu]g dimethylbenzanthracene (DMBA) and promoted with 9, 18, 27, or 36 mg cigarette smoke condensate (CSC) thrice weekly for various time periods, up to 29 weeks. Global ME and ME of GC-rich regions were assessed using SssI methylase and arbitrarily primed PCR procedures, respectively. We report that GC-specific ME increases in a dose- and time-dependent manner. CSC doses necessary to detect these changes were between 18 and 27 mg at 6 wks, and between 9 and 18 mg and at 9 wks. Further examination of animals promoted for 9 wks indicated similar changes in the absence of initiation, and ME changes were reversible following cessation of promotion. Administration of 27 mg CSC for 29 wks led to increased ME of GC-rich regions for tumor vs. non-tumor tissue, with changes in global ME observed only in tumors. Compared to non-initiated counterparts, initiated animals promoted with 27 mg CSC for 29 wks exhibited a very substantial increase in tumor number. Summary: multiple changes in ME are observed during CSC tumor promotion; increased ME of GC-rich regions precedes global decreased ME. Significance: CSC acts as a classic promoter, and ME changes in a threshold-exhibiting, progressive and reversible manner, as expected for a mechanism underlying tumor promotion.

38 GLOBAL GENE EXPRESSION COMPARISONS AND PATHWAY ANALYSIS OF NONGENOTOXIC CARCINOGENS WITH DIFFERENT MECHANISMS OF ACTION.

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Nongenotoxic chemical carcinogens are those chemicals that induce tumor formation by mechanisms that do not directly change cellular DNA. Therefore, changes in the regulation of cell growth and gene expression are important factors in tumor formation. The goal of this study was to compare gene expression profiles of two nongenotoxic chemicals, WY-14643 and thioacetamide (TAA), that differ in their mechanism of carcinogenicity. TAA induces hepatocellular neoplasms in male rats due to chronic cellular injury. WY-14643 is a peroxisome proliferator (PP) that causes hepatocellular carcinoma in susceptible species such as rodents. TAA and WY-14643 were delivered orally on a daily basis at a dose previously shown to result in tumor formation (19.2mg/kg/d for TAA and 60mg/kg/d for WY-14643) as well as a 1/10 low dose for 14 days. Five male rats/dose/time were randomly assigned to sacrifice on days 1, 3, 7, and 14. Gene expression analysis was achieved with an open analysis platform (GeneCalling ®) on all rat livers. Gene dysregulation was observed in a number of biological pathways including lipid and protein metabolism, xenobiotic metabolism, and cellular signaling and proliferation. The use of global gene expression methodology enables researchers to compare and contrast the entire transcriptome at one time. This analysis permits us to delineate genes and pathways that appear to be common to nongenotoxic carcinogens and some that are specific to classes of nongenotoxic carcinogens.

39 TOXICOGENOMICS OF HUMAN CARCINOGENS.

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The objective of this study was to profile gene expression changes in response to carcinogen exposure using cDNA microarray technology, and correlate these with mutagenesis and toxicity. We used the human lymphoblast cell line TK6 as an *in vitro* model system, and reactive metabolites of human environmental and food carcinogens, benzo(a)pyrene (BP) and 4-aminobiphenyl (ABP) as model compounds, We first, measured the mutagenicity and toxicity of the model compounds N-hydroxy-4-acetylaminobiphenyl (N-OH-AABP) and Benzo(a)pyrene diol epoxide (BPDE) in TK6. We then chose three doses that resulted in low, medium and high toxicity to treat the cells and harvested cells at different time points. RNA extracted from treated cells or untreated cells was then labeled by reverse transcription in the presence of either fluorescence-labeled Cy5-dUTP or Cy3-dUTP. The labeled cDNAs were then co-hybridized human cDNA microarrays comprised of ~18, 000 genes/ESTs. Experiments were performed in triplicate, including one dye flip. The fold change in expression for each probe was determined as the mean ratio of fluorescence intensities relative to the reference sample (i.e. Cye5/Cye3). The normalized data were then analyzed using various clustering algorithms, The resulting analyses indicated a correlation between gene expression profiles and mechanisms of toxicity and mutagenicity. We found a subset of genes with decreased expression after exposure to N-OH-AABP or BPDE at all concentrations, which may indicate general response to toxicity. In contract, we also discovered a subset of genes that were highly decreased in BPDE treated cells, but not in N-OH-AABP treated cells. The latter group included DNA repair and replication genes, transcription factors, etc., which may help to explain why OH-AABP is about 1000 times less toxic and mutagenic than BPDE in the cell line TK6. Microarray technology is a power technology to provide the insight into the mechanism of toxicity and mutagenesity.

40 MICROARRAY ANALYSIS OF COMMON GENE EXPRESSION CHANGES IN THE HUMAN KERATINOCYTE CELL LINE RHEK-1 MALIGNANTLY TRANSFORMED BY MULTIPLE CHEMICAL AGENTS.

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We are interested in developing a predictive resource for hazard identification of carcinogenic chemicals and chemical mixtures. To this end, we are utilizing microarray analysis of chemically-induced gene expression alterations; genes commonly regulated by multiple carcinogenic agents may be useful predictive markers for detection of new and novel chemicals with transforming potential. RHEK-1, the human keratinocyte cell line used in our studies, was previously transformed with MNNG (at 0.01 and 0.1 mg/ml), TCDD, and 4-nitroquinoline oxide to a fully tumorigenic phenotype. Analysis of global gene expression alterations in the four malignant lines was carried out using the Clontech 3.8 II Human Microarray. Expression data was analyzed with ScanAlyze and Cluster. We applied stringent conditions that required alteration of the gene in triplicate samples from each cell line and a cut-off of 2-fold change in expression between the chemically-treated line and its control. Results of this analysis showed the following: 1) among genes with a known or putative function in the 3.8 II array, 11 were commonly up-regulated in all four transformed lines; 2) included in the genes commonly up-regulated are a member of the ras family, p21/cdc42, RNB6, a low fidelity DNA polymerase (k), and several genes involved in growth and differentiation; 3) a total of 23 genes showed decreased expression in all transformed lines versus control cells; 4) commonly down regulated genes include those encoding 5 membrane transporters, 2 Ser/Thr protein kinases involved in apoptosis, cadherin 12, and several novel proteins. Additionally, we were able to identify genes altered in their expression in a chemical-specific manner. These latter genes may give mechanistic clues as to chemical-class-specific toxicity in this cell type. This study was supported by ATSDR (Cooperative Agreement U61/ATU881475).

40a RECENT ADVANCEMENT IN *IN VITRO* METAL TOXICOLOGY FOR REGULATORY PURPOSE.

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The European Centre for the Validation of Alternative Methods has been set up in order to coordinate, at European level, activities to promote the scientific and regulatory acceptance of alternative toxicity testing which are important to biosciences. In this context, advanced approaches that have less or no dependence on animal experimentation (cell cultures) represent the basis of *in house* projects concerning development and validation of mechanistically-based *in vitro* toxicity testing. One of these projects is IMETOX (In vitro MEtal TOXicology) that aims at integrating aspects of metal toxicity in the field of metabolism, immunotoxicity, neurotoxicity, nephrotoxicity, reproductive toxicity, carcinogenicity in relation to environmental and occupational exposure, drug therapy, and the use of foods, cosmetics and biomaterials for hard tissue substitute. This work reviews the current IMETOX research activities concerning metabolism, toxicological effects and carcinogenic potential of metal compounds as investigated by cell cultures (e.g. mouse fibroblasts BALB/3T3, immortalised human keratinocytes (HaCaT), rat pheochromocytoma (PC12) cell lines) in combination with radioanalytical, spectrochemical and bioanalytical techniques. Examples concerning arsenic, chromium, platinum and vanadium compounds are reported in relation to low dose exposure, metal uptake and intracellular distribution, the influence of the different chemical forms of metals and their cellular biotransformation on cytotoxicity.

41 NOVEL PHYSIOLOGICAL AND TOXICOLOGICAL ROLES FOR ENDOPLASMIC RETICULUM BOUND CA^{2+}-INDEPENDENT PHOSPHOLIPASE A_2 (ER-IPLA$_2$) IN THE KIDNEY.

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We recently identified an 85 kDa, plasmalogen selective, Ca^{2+}-independent phospholipase A_2 (ER-iPLA$_2$) in rabbit renal proximal tubule cells (RPTC). ER-iPLA$_2$ inhibition with bromoenol lactone (BEL) potentiated oxidant-induced RPTC oncosis and lipid peroxidation suggesting that ER-iPLA$_2$ protects against oxidant-induced lipid dysfunction. To test this hypothesis RPTC phospholipid content was determined after *tert*-butylhydroperoxide (TBHP) exposure in the presence and absence of BEL. Twenty five individual phospholipid molecular species, differing in chain length and number of double bonds in the *sn-1* and *sn-2* fatty acids on the glycerol backbone, were identified and quantified in choline (phosphatidylcholine, PC and plasmenylcholine, PlasC) and ethanolamine (phosphatidylethanolamine, PE and plasmenylethanolamine PlasE) phospholipids. Treatment of RPTC with BEL (5 μM) for 1 hr caused 45% decreases in (18:0, 18:1)- and (18:2, 20:4)-PlasC compared to controls. Treatment of RPTC with TBHP (200 μM) for 1 hr resulted in 35% decreases in (16:0, 20:4)- and (18:0, 18:1)-PlasE, a 30% decrease in (16:0, 18:3)-PlasC, and a 25% decrease in (18:0, 20:4)-PE. In contrast, treatment of RPTC with BEL prior to TBHP exposure decreased the content of 10 phospholipids, decreasing all PlasE phospholipids an average of 60%, and 5 of the 8 PlasC phospholipids 40%, while PC and PE were essentially unaffected compared to TBHP. These data suggest a physiological role for ER-iPLA$_2$ in the maintenance of PlasC phospholipids. These data also demonstrate that ER-iPLA$_2$ inhibition increases oxidant-induced RPTC phospholipid loss, suggesting that ER-iPLA$_2$ has a role in the protection of RPTC against oxidant-induced toxicity.

42 INTERACTIVE TOXICITY BETWEEN TRICHLOROETHYLENE AND INORGANIC MERCURY IN RAT AND HUMAN KIDNEY PROXIMAL TUBULE.

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To test the hypothesis that prior exposure of rat or human kidney to subtoxic concentrations of either trichloroethylene (TRI), its metabolite *S*-(1, 2-dichlorovinyl)-L-cysteine (DCVC), or inorganic mercury (Hg) alters the toxic response to subsequent exposures, male Fischer 344 rats were given i.p. injections of either saline (= Control) or a subtoxic dose of $HgCl_2$ (0.5 μmol/kg) and 24 and 48 hr later, activities of several glutathione (GSH)-related enzymes and protein expression of GSH *S*-transferase alpha (GSTα) were determined. Pretreatment with Hg increased activities of GSH-related enzymes by 35-90% and increased expression of GSTα1 by 33%. Similar experiments in primary cultures of rat proximal tubular (rPT) cells showed preincubation with 0.5 or 1 μM $HgCl_2$ increased activities of GSH-related enzymes. *In vivo* treatments of rats with 0.5 μmol $HgCl_2$/kg increased rates of GSH conjugation of TRI, showing that these effects on GSH status influence TRI bioactivation. Pretreatment of primary cultures of human proximal tubular (hPT) cells for 24 hr with 0.25-1 μM $HgCl_2$ markedly enhanced acute cytotoxicity (lactate dehydrogenase release or inactivation) of 1 mM TRI and 50 or 200 μM DCVC. In contrast, pretreatment of hPT cells with 0.25 μM $HgCl_2$ markedly decreased apoptosis induced by either 1 mM TRI or 50 μM DCVC. This suggests that prior exposure of hPT cells to Hg shifts the cytotoxic response from apoptosis to necrosis. Pretreatment of hPT cells for 24 hr with either 1 mM TRI or 50 μM DCVC markedly diminished acute cytotoxicity due to 0.25-5 μM $HgCl_2$, decreased apoptosis due to Hg at 1 hr, but increased apoptosis by as much as 5-fold after 2- and 4-hr incubations. Pretreatment of hPT cells for 24 hr with 50 μM DCVC increased intracellular GSH content by more than 100%. These results show that prior exposure of either rat or human PT cells with low or subtoxic doses of TRI, DCVC, or Hg markedly alters cellular susceptibility to injury, in part due to changes in cellular GSH status. (Supported by NIH Grant ES08828.)

43 DICHLOROVINYL-L-CYSTEINE (DCVC) CAUSES G2/M CELL CYCLE ARREST IN RENAL PROXIMAL TUBULAR CELLS.

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We have demonstrated that primary cultures of renal proximal tubular cells (RPTC) proliferate following sub-lethal injury induced by the model oxidant t-butylhydroperoxide (TBHP), but proliferate poorly following sub-lethal injury produced by the nephrotoxicant DCVC. In the current study we used both model compounds to investigate the mechanisms of the differential proliferative responses. Treatment of RPTC with DCVC (200 μM, 90 min) or TBHP (250 μM, 30 min) decreased monolayer protein and DNA contents by 40% after 48 hr. TBHP injured monolayers increased their DNA content by 67 ± 14 % on day 6, while DCVC injured cells only increased their DNA content by 31 ± 7 % on day 6. Cell cycle status was determined at various times following toxicant exposure using flow cytometry. Cell cycle analysis revealed that 88% of control RPTC were in G0/G1, 6% in S and 5% in G2/M phase and that this ratio did not vary over time. In TBHP treated RPTC, 75% were in G0/G1, 15% in S and 10% in G2/M phase, and these ratios were maintained over 48 hr. Twenty-four hr after DCVC treatment, 71% of DCVC treated cells were in G0/G1, 23% in S and 6% in G2/M phase. Forty-eight hr after DCVC treatment, 43% of the cells were in the G0/G1, 23% were in the S and 34% were in the G2/M phase. These results demonstrate that RPTC monolayers regenerate following TBHP exposure through proliferation. In contrast, RPTC regenerate poorly following DCVC exposure due to cell cycle arrest in the G2/M phase.

44 DOSE RESPONSE EFFECTS OF EICOSAPENTAENOIC ACID ON EXPERIMENTAL IGA NEPHROPATHY INDUCED BY THE TRICHOTHECENE DEOXYNIVALENOL.

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Immunoglobulin A nephropathy (IgAN), characterized by diffused kidney mesangial deposition of IgA, is now recognized as the most common form of primary glomerulonephritis in many parts of the world. However the pathogenesis of this disease remains obscure and there exists no effective treatment for it. A murine IgA nephropathy model induced by the trichothecene mycotoxin deoxynivalenol (DON) has been established in our lab. Fish oil has been demonstrated to attenuate the elevation of serum IgA and IgA deposition in the kidney mesangium in this model. In this study, we evaluated the dose-response effects of eicosapentaenoic acid (EPA), one of the major components of omega-3 polyunsaturated fatty acids in fish oil, on serum IgA and IgA-immune complexes, mesangial IgA deposition and other pathological changes. B6C3F1 mice were fed for 12 wk with AIN-93G diets containing 1g/100 g corn oil and 6g/100g oleic acid (CONTROL group), 1g/100g corn oil and 6g/100g oleic acid and 20ppm DON (DON group), 1g/100g corn oil and 0.2g/100 g EPA and 5.8g/100g oleic acid and 20ppm DON (0.2%EPA+DON group), 1g/100g corn oil and 1g/100g EPA and 5g/100g oleic acid and 20ppm DON (1%EPA+DON group) and 1g/100g corn oil and 6g/100g EPA and 20ppm DON (6%EPA+DON group). After 8 wk, DON significantly increased serum IgA (6 times higher than CONTROL group) ($P<0.05$). Mice fed with the 6%EPA+DON diet exhibited a significantly increased IgA concentration (3 fold) ($P<0.05$) compared to CONTROL group, however this IgA level was significantly lower than that of DON group($P<0.05$). Serum IgA concentrations in mice fed with 0.2%EPA+DON and 1%EPA+DON were not significantly different from that of DON group ($P>0.05$). We got similar results after 12 wk. The results suggest that there is a threshold of EPA consumption required for ameliorative effects on DON-induced IgAN. (Supported by NIH grant DK58833)

45 MECHANISMS OF STIMULATED TISSUE REPAIR IN SURVIVAL FROM ACUTE RENAL TUBULAR NECROSIS: ROLE OF MAPK PATHWAY.

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Our earlier studies with S-(1, 2-Dichlorovinyl)-$_L$-cysteine (DCVC) showed that a priming/low dose (15 mg DCVC/kg, ip) given 72 h before administration of a normally lethal dose of DCVC (75 mg/kg, ip) to mice led to massive injury, but sustained renal tubular repair allowed recovery and survival. The objective of present study was to investigate whether this autoprotection was associated with upregulation of the mitogen activated protein kinase pathway (MAPK). Following the priming dose of DCVC, renal IL-6 protein (ELISA) and mRNA (RTPCR) levels increased as early as 1 h after dosing and declined by 24 h. EGFr {by immunohistochemistry (IHC)}, mainly localized in renal cortex and collecting ducts, peaked at 3 h returning to control by 9 h whereas the expression of its ligands, HB-EGF (IHC) and TGF-α (IHC) remained elevated from 3 to 36 h. Significantly higher expression of Grb-2 {IHC, (at 3, 9 and 36 h)} and ERK1/2 {IHC, (at 3, 9, 12, 24 and 36 h)} was observed selectively in the outer stripe of outer medulla (OSOM) and the papilla coincident with maximal S-phase DNA synthesis as assessed by proliferating cell nuclear antigen (PCNA) IHC. In contrast, increasing the dose to 75 mg DCVC/kg produced an increase in renal IL-6, HB-EGF and EGFr at earlier time points (3 to 12 h)-which then declined at 24 and 36

h along with Grb-2 and ERK1/2. Renal S-phase DNA synthesis in these mice was minimal and most of the mice died within 48 h of dosing. Prior treatment with a low dose of DCVC not only stimulated the renal MAPK pathway but also increased the expression of HB-EGF, TGF-α, EGFr, Grb-2 and ERK1/2 which was sustained even after administration of the normally lethal dose of DCVC, leading to augmented tissue repair and recovery from acute renal failure. These results suggest that the sustained activation of MAPK pathway may underlie protection from DCVC-induced acute renal tubular necrosis in the mouse.

46 EFFICACY AND TOXICITY STUDY OF VANADIUM NICOTINATE IN ALLOXAN INDUCED DIABETIC RATS.

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Vanadium compounds regulate blood glucose (BG) in Type 1 and 2 diabetes mellitus. Animal data indicate that high dose of vanadium can be harmful to renal tubules. Vanadium compounds are used as nutraceuticals in health food formulation. The objective of the present study was to evaluate whether vanadium nicotinate (VN) administration may be used to control BG in diabetic rats and to evaluate its potential toxicity. VN was synthesized by combining vanadium and niacin. Male Wistar rats (150 to 200 g) were made Type 1 diabetic by a single i.v injection of alloxan (70 mg/kg in saline). Diabetes was confirmed after 48 h later by estimating BG (303± 56 mg/dl). The diabetic rats received either VN (100 mg/kg/day, p.o as a suspension in 7% gum acacia solution, n=7) or gum acacia (vehicle, n=7) for 21 days. Rats were maintained for additional 7 days without administration of VN. On day 28 rats were sacrificed and hepatic as well as renal injury was examined in H & E stained sections under a light microscope. Blood samples were withdrawn retro orbitally and were analyzed for glucose on days 0 (314 ± 62 vs 377 ± 36), 7, 14, 21 and 28. VN was very effective in decreasing BG on day 21 of therapy (140 ± 30 vs 385 ± 43) and the hypoglycemic effect of VN continued even after cessation of treatment for 7 days (148 ± 54 vs 380 ± 34). Maximum reduction in BG (55.21%) was observed on 21st day. The drug showed late onset and prolonged duration of hypoglycemic effect. No abnormality was detected in liver while focal tubular necrosis with severe cloudy changes were seen in kidney in VN treated groups as compared to the vehicle treated group after 21 days administration of VN. The mechanisms of VN's hypoglycemic and nephrotoxic effects are worthy of further investigation. It is concluded that VN (100 mg/kg) has significant hypoglycemic activity. However, this hypoglycemic effect was not without the liability of nephrotoxic injury. (Supported by Indus Biotech Pvt. Ltd., India)

47 MITOCHONDRIAL OXIDATIVE STRESS IS CAUSED BY NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI) STAVUDINE (D4T) IN HEPG2 CELLS.

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The toxicity of NRTI is linked to altered mitochondrial DNA (mtDNA) replication and resultant mitochondrial dysfunction. Clinically this is manifested by elevated plasma lactate, mitochondrial myopathy, and cardiomyopathy. However, the subcellular mechanisms that relate changes in mtDNA replication to mitochondrial dysfunction remain elusive. We hypothesize that mitochondrial oxidative stress (MOS) links NRTI-induced changes in mtDNA replication to mitochondrial dysfunction. To test the hypothesis, cultured human hepatoma cells (HepG2) were treated for 48 hours with stavudine (d4T). Changes in mitochondrial function (using lactate/pyruvate ratio in medium), mtDNA amplification efficiency (using PCR method), and oxidative stress to mtDNA (using 8-hydroxy-2-deoxyguanosine (8-OHdG) abundance in mtDNA) were determined. Results showed d4T up to 500 uM did not produce cell death in HepG2 cells over 48 hours. However, lactate/pyruvate ratios from medium were doubled compared to control values after 48 hours. d4T produced a dose-dependent decrease in mtDNA amplification efficiency and this inversely correlated with a doubling in the levels of 8-OHdG in mtDNA samples. All of d4T deleterious effects were ameliorated by concomitant treatment with the catalytic antioxidant, MnTBAP (100 uM). Data from these studies suggest that NRTI treatment elevates mitochondrial reactive oxygen species resulting in MOS and this contributes to NRTI-induced toxicity. In turn, MOS from NRTIs may be amplified by aging and alcohol in which MOS has also been invoked mechanistically. In patients, it may be possible to ameliorate MOS from NRTIs with catalytic antioxidants. (supported in part by NIH grants HL59602(BJD), HL31992(BJD), HL63666(WL) and AA13551(WL)).

48 ROLE OF LIPID PEROXIDATION AS MECHANISM OF LIVER INJURY AFTER ACETAMINOPHEN OVERDOSE IN MICE.

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Acetaminophen (AAP) causes mitochondrial reactive oxygen (ROS) and peroxynitrite formation (Knight et al., Toxicol Sciences 62: 212-220, 2001). Thus, we tested the hypothesis that ROS-induced lipid peroxidation (LPO) is an important component of the injury mechanism by comparing AAP-induced injury in mice fed a high vitamin E-diet (1 g dl-alpha tocopheryl acetate (TA)/kg diet) compared to a control diet (35 mg TA/kg diet). Results: Male C3Heb/FeJ mice fed the vitamin E-diet for 1 week had higher tocopherol levels in the liver (28 ± 6 nmol/g liver) than animals on the control diet (8.2 ± 0.1 nmol/g). AAP (300 mg/kg) caused centrilobular necrosis with high plasma alanine aminotransferase (ALT) activities at 6 h (3280 ± 570 University/L). Liver malondialdehyde (MDA) content as indicator of LPO did not increase compared to untreated animals (9.9 ± 0.9 nmol MDA/mg protein). However, all necrotic cells in the centrilobular region stained positive for nitrotyrosine, an indicator of hepatocellular peroxynitrite formation. There was no significant difference in AAP-induced liver injury or hepatic nitrotyrosine staining between animals on the vitamin E diet compared to mice on the control diet. To verify a potential effect of the vitamin E-diet on drug-induced liver injury, animals were pretreated with 100 mg/kg phorone and 0.35 mmol/kg $FeSO_4$ before receiving 0.6 mmol/kg allyl alcohol. Massive LPO and liver injury was observed in livers of animals on the control diet as indicated by a 32-fold increase in MDA levels and plasma ALT activities of 2310 ± 340 University/L. Animals on the vitamin E-diet had 40% lower MDA levels and 84% lower ALT values. Conclusions: Despite ROS formation, AAP overdose did not cause LPO and consequently, enhancing hepatic vitamin E levels did not prevent liver injury. These results are consistent with our recent findings (Knight et al., J Pharmacol Exp Therap 303: in press, 2002) that peroxynitrite but not reactive oxygen formation per se is responsible for AAP-induced liver injury.

49 OXIDANT STRESS PRECEDES LIVER INJURY AFTER ACETAMINOPHEN IN CULTURED MOUSE HEPATOCYTES.

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The role of reactive oxygen species (ROS) in the pathophysiology of acetaminophen (AAP)-induced liver injury is controversial for many years. Superoxide can function as precursor for peroxynitrite formation or can dismutate to molecular oxygen and hydrogen peroxide, which can be reductively cleaved to the highly reactive hydroxyl radical. We identified mitochondria as the source of ROS formation after AAP treatment *in vivo* by monitoring the cellular and mitochondrial glutathione disulfide (GSSG) levels and the GSSG-to-GSH-ratio (Knight et al., Toxicol Sciences 62:212-220, 2001). A concern with this approach is the initial severe depletion of hepatocellular glutathione, which makes the quantitative assessment of the oxidant stress difficult. Therefore, we tested the hypothesis that mitochondrial ROS formation precedes the onset of cell injury in a cell culture model using 2, 7-dichlorodihydrofluorescein diacetate (DCFH) as marker for an intracellular oxidant stress. Hepatocytes were isolated from fasted C3Heb/FeJ mice. After adherence in collagen-coated wells, AAP was added (final conc.: 5 mM) and DCFH fluorescence, lactate dehydrogenase (LDH) release and trypan blue uptake were determined from 0 to 12 h. Fluorescence began to increase at 3 h (1, 640 ±390 % of baseline), reached a maximum at 6 h (2, 410 ± 490 % of baseline)and was still high at 9 h (1, 510 ±250 % of baseline). Cell injury, as indicated by increased cellular release of LDH and uptake of trypan blue, was not evident at 1.5 or 3 h. However, a significant release of LDH was observed at 6 h (19 ± 11 % release of total cellular LDH), at 9 h (39 ± 7 % release) and at 12 h (53 ± 11 % release). Co-treatment with 500 nM cyclosporin, an inhibitor of the mitochondrial membrane permeability transition, attenuated the fluorescence increase by 62 %. Conclusions. A substantial mitochondrial oxidant stress is an early event in AAP-induced cell damage that precedes the onset of injury by several hours.

50 DIVERGENT EFFECTS OF HYPEROXIA ON CC10 AND GLUTATHIONE REDUCTASE MESSENGER RNA LEVELS IN LUNGS OF MICE.

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Levels of Clara cell secreted protein (CC10) in trachael aspirate fluids (TAFs) increase in prematurely born human infants who survive and do not develop bronchopulmonary dysplasia (BPD), but not in infants who die or develop BPD

[Ramsay, AJRCCM 164:155, 2001]. Oxidative modifications of CC10, indicated by reactivities with 2, 4-dinitrophenylhydrazine (DNPH), appear to account for some of the decreases in TAF concentrations of CC10. The present studies were to test the hypothesis that hyperoxia also exerts pretranslational effects that may contribute to the diminished CC10 levels. Adult male ICR mice were placed in greater than 95 percent O2 for up to 96 h. Increases in lung weight-to body weight ratios were not observed through 72 h of hyperoxia, thus indicating minimal lung injury through this duration of exposure. Levels of CC10 mRNA were assessed by real time PCR, with 18S as reference. By 48 h of hyperoxia, lung levels of CC10 mRNA decreased to less than half of the levels observed in air-breathing control animals, and by 96 h, CC10 message levels were less than 5 percent of controls. In contrast, message levels of glutathione reductase in the lungs of animals in hyperoxia increased through 72 h to levels five times the levels in air-breathing control animals, then declined sharply between 72 and 96 h of hyperoxia. Lung GR activities were not increased in animals exposed to hyperoxia. The data indicate that hyperoxia can exert pretranslational effects that may contribute to the diminished levels of CC10 that we observed in premature human infants. The marked differences betwteen CC10 and GR mRNA responses indicate gene-specific effects and/or selective and early damage to the Clara cells in the lungs of animals and possibly in humans exposed to hyperoxia. Supported in part by GM44263.

51 HYPEROXIC LUNG INJURY IN MICE WITH GENETIC DEFICIENCIES IN GLUTATHIONE REDUCTASE ACTIVITIES.

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Glutathione-dependent mechanisms are vital to antioxidant defense mechanisms, and we have demonstrated previously that transgene-driven enhancement of glutathione reductase (GR) activities can increase dramatically cell resistance to oxidant toxicities *in vitro*. Conversely, enhanced sensitivities to oxidants are observed in cells with attenuated GR activities from antisense expression of the GR transgene. The toxicological relevance of GR *in vivo* is attributed to the effects on toxicities of BCNU, which inhibits GR *in vitro* and *in vivo*, but the specificity of BCNU is less than is often assumed. In the present studies, we investigated the susceptibilities to hyperoxic lung injury of a line of mice that exhibit GR activities in livers and lungs that are less than 10% of the respective activities observed in the parent strain (C3H) of mice. The founder mouse of this GR hypomorph strain was generated by administration of isopropyl methanesulfonate, and the trait bred to homogeneity (Pretsch, Genet Res Camb 73:1, 1999). The GR hypomorph animals do not exhibit any remarkable phenotype, and the trait has remained stable for over 20 generations. We obtained breeding pairs and re-derived progeny at our institution. Lung and liver GR activities in the GR hypomorph mice were 6 and 9% of control C3H mice, whereas respective glutathione peroxidase activities and GSH levels were not different. Adult male mice of the GR hypomorph strain (Gr1a1Neu) exposed to greater than 95 percent O2 exhibited increases in lung weight to body weight ratios by 72 h of exposure, whereas increased lung weights in the wild type C3H mice were observed only after 96 h of continuous exposure to hyperoxia. The greater susceptibility of the Neu than of the C3H mice to hyperoxia is consistent with significant contributions of GR to antioxidant functions, but the modest effects on injury of such marked differences in GR activities suggest compensatory responses. Supported in part by GM44263.

52 EXPRESSION AND LOCALIZATION OF P70 ALBUMIN PRECURSOR PROTEIN AND PHI AP3 IN OXIDATIVELY STRESSED VASCULAR SMOOTH MUSCLE CELLS.

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Redox signaling by benzo(a)pyrene (BaP) in vascular smooth muscle cells (vSMCs) involves activation of protein binding to a DNA regulatory sequence termed the antioxidant response element (ARE). ARE sequences (RTGAYNNNGCR) are similar to consensus TPA-response elements (RTGACTCA), cyclic AMP response elements (TGACGTCA) and Maf-response elements (TGCTGAGTCA), evidence of functional overlap in redox signaling among multiple cellular pathways. Recently, this laboratory identified p70 albumin precursor protein (APP) and Phi AP3 as novel components of the redox sensing machinery in vSMCs. The present studies were conducted to characterize the expression and cellular localization of these proteins in BaP-treated cells. Affinity-purified IgG was generated against APP and Phi AP3 peptide antigens. Two immune-reactive bands of Mr of 35- and 70-kDa were detected for APP in extracts of the cytosolic and nuclear compartments of vSMCs. Short-term oxidant treatment (0.5 hr) selectively increased p70 expression in the cytosolic compartment, while the p70 immune-reactive protein accumulated in the nucleus within 0.5 - 1 hr, along with a concomitant increase in p35 immunoreactivity. Anti-Phi AP3 immunoreactivity was detected at Mr 18-, 40 and 65-kDa in the nuclear fraction, while Mr 18- and 40- and 42-kDa proteins were predominant in the cytosolic fraction. Treatment of vSMCs with 3uM BaP abolished the nuclear p65 Phi AP3 signal and enhanced the appearance of p40 immunoreactivity. Fluorescence microscopy of BaP-treated cells showed diffuse Phi AP3 cytosolic staining, while APP immunofluorescence was confined to the nucleus. These results suggest that BaP alters the expression and localization of APP and Phi AP3, and implicate these proteins in redox signaling in vSMCs. (Supported by NIH Grants ES04849, ES04917, and ES09106).

53 NF-KB DYSREGULATION IN ATHEROSCLEROTIC VASCULAR SMOOTH MUSCLE CELLS: COMPLEX COMPOSITION AND REDOX SENSITIVITY.

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Repeated exposure to chemical oxidants leads to induction of atherogenic vascular smooth muscle cell (vSMC) phenotypes. These cells are characterized by heightened proliferative activity, altered integrin expression, and upregulated NF-κB activity. NF-κB, a dimer of Rel proteins, is a transcription factor typically located in the cytoplasm *via* association with an inhibitory protein IκB. After phosphorylation by the IKK complex, IκB is ubiquitinated and destroyed, allowing NF-κB dimers to translocate to the nucleus and effect transcriptional regulation of a variety of gene targets, including osteopontin, a cytokine implicated in the maintenance of atherogenic vSMC phenotypes. In the present studies, male Sprague-Dawley rats (175-200g) were gavaged once daily for 20 consecutive days with 70 mg/kg allylamine, a vascular specific pro-oxidant. Aortic smooth muscle cells were isolated by enzymatic digestion and maintained in serial culture. Cells were seeded at 100 cells/mm2, growth arrested for 72 hours, and then released into growth by addition of serum mitogens. Immunoprecipitated IKKa was phosphorylated to a greater extent in allylamine cultures than controls, indicating a higher level of NF-κB activation. Rel A/p65 proteins were phosphorylated to a lesser extent in allylamine cultures, suggesting changes in the ability of this protein to regulate gene induction. The levels of other NF-κB constituent proteins in nuclear extracts were altered, with p52 levels increased and RelB levels decreased, in the nuclei of allylamine cells relative to controls. Gel shift analysis using nuclear proteins from control and allylamine cultures treated with PDTC or N-acetyl cysteine showed that NF-κB activity in allylamine cells was more sensitive to antioxidant inhibition than respective controls. These results implicate altered redox homeostasis as a key regulator of NF-κB activation in chemical atherogenesis. (This work was supported in part by NIH grants HL62539 and ES09016).

54 ROLE OF BIP/GRP78 IN 11-DEOXY-16, 16-DIMETHYL PROSTAGLANDIN E2 MEDIATED CYTOPROTECTION IN RENAL EPITHELIAL CELLS.

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Treatment with 11-Deoxy-16, 16-dimethyl prostaglandin E_2 (DDM-PGE_2), a stable synthetic analog of PGE_2 protects renal proximal tubule epithelial cells (LLC-PK_1) against oncotic/necrotic cell death induced by hydrogen peroxide (H_2O_2), iodoacetamide (IDAM), and 2, 3, 5-tris(glutathion-S-yl)hydroquinone (TGHQ), but not against cisplatin or mercuric chloride induced apoptotic cell death. Utilizing mass spectral and western blot analyses we showed that cytoprotection was associated with the up-regulation of at least six proteins, including the major endoplasmic reticulum (ER) chaperone, glucose-regulated protein 78 (Bip/Grp78). To elucidate the role of Grp78 in cell injury, we investigated cytoprotection using LLC-PK_1 cells in which induction of *grp78* expression was disrupted by stable expression of an antisense *grp78* RNA (pkASgrp78). As anticipated, DDM-PGE_2 failed to induce Grp78 in pkASgrp78 cells, with a concomitant inability to provide cytoprotection against TGHQ, H_2O_2, or IDAM. In contrast, DDM-PGE_2 induced Grp78 and afforded cytoprotection against all three toxicants in cells transfected with empty vector (pKNEO). These data suggest Grp78 plays an important role in DDM-PGE_2 mediated cytoprotection. Furthermore, using 2D gel electrophoresis coupled with MALDI-TOF peptide mass mapping and post source decay, we compared the pattern of protein induction in pkASgrp78 and pKNEO cells following DDM-PGE_2 pretreatment. Our results revealed that DDM-PGE_2 induced several proteins in pKNEO cells, but not in pkASgrp78 cells, including S100 calcium binding protein A2, proteasome subunit C2, galectin-1, galectin-3, myosin light chain, and molecular chaperones such as heat shock protein 27 and chaperonin 10. The findings suggest that these proteins may act in concert with Grp78 during DDM-PGE_2 mediated cytoprotection against oncotic/necrotic cell death. (GM56321, ES07784).

55 TBHQ PROTECTS NEURONAL CELLS FROM OXIDATIVE INJURY THROUGH UPREGULATION OF THE ANTIOXIDANT RESPONSIVE ELEMENT.

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Activation of a cis-acting response element known as the antioxidant responsive element (ARE) has been shown to increase the expression of many phase II protective genes such as NAD(P)H:quinone oxidoreductase 1 (NQO1) and Glutamate-cysteine ligase (GCL). A transgenic reporter mouse line was previously derived by insertion of a 51 base pair segment of the rat NQO1 promoter containing the core ARE coupled to a heat-stable human placental alkaline phosphatase (hPAP) reporter gene construct. To determine if activation of the ARE confers protection against oxidative stress-induced cytotoxicity, primary mouse cortical neuronal cultures were pretreated with tert-butylhydroquinone (tBHQ). TBHQ is a potent activator of the ARE through its stimulation of NF-E2-related factor 2 (Nrf2, the major transcription factor required for ARE binding and activation) nuclear translocation. Pretreatment of cortical cultures for 48 hours with tBHQ attenuated oxidative stress-induced apoptosis resulting from both hydrogen peroxide and L-glutamic acid treatments in a dose-dependent manner. This protective effect directly correlated with the amount of ARE activation in the cultures as measured by an increase in hPAP reporter activity. TBHQ-pretreated cultures showed reduced numbers of TUNEL-positive cells and fewer nuclei displaying condensed or fragmented chromatin visualized by Hoescht 33258. The doses of H2O2 and L-glutamic acid used in this study selectively killed the neurons (β-tubulin III-positive) of this mixed culture system. TBHQ pretreatment caused a significant decrease in this neurotoxicity. Furthermore, preliminary studies suggest that treatment of immature cultures (3 DIV) with L-glutamic acid is attenuated by tBHQ and leads to NMDA receptor-independent toxicity. These results lend further support to the idea that activation of gene expression *via* the ARE increases the resiliency of cells against multiple insults known to cause programmed cell death.

56 DEVELOPMENT OF A MICROARRAY ELISA FOR CHARACTERIZING POTENTIAL MARKERS OF BREAST CANCER IN NIPPLE ASPIRATE FLUID.

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Nipple aspirate fluid (NAF) is obtained from nonlactating women and is likely to be a rich source of protein markers for breast cancer. Analysis of NAF is difficult due to small sample volumes (typically 10-50 μL) and a rudimentary knowledge of its protein composition. To address these problems, we have taken a dual approach of characterizing the NAF proteome and of developing a microarray ELISA. Using trypsin digestion and LCQ tandem mass spectrometry, 64 proteins were identified in NAF. At least 15 of these proteins have been reported to be altered in serum or tumor tissue from women with breast cancer. Using the microarray ELISA, hepatocyte growth factor (HGF) was quantitatively detectable to the sub-pg/mL level. Significant differences between HGF concentrations in sera of breast cancer patients was detected and the serum HGF values correlated (r2 = 0.90) with those obtained using a commercial 96-well ELISA assay. In multiplex studies, we simultaneously assayed 5 potential markers at biologically relevant levels. These studies indicated that excellent signal-to-background ratios could be obtained for each assay even though the concentrations of the different antigens varied 3000-fold. Overall, this research provides an initial characterization of the NAF proteome and develops a microarray ELISA that appears suitable for the high-throughput evaluation of potential cancer markers in NAF. This research was supported by the US Army's Breast Cancer Research Program, award #DAMD17-00-1-0132.

57 FINDING NON-INVASIVE BIOMARKERS USING MICROARRAY TECHNOLOGY.

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Finding non-invasive biomarkers of toxicity using microarray technology can be a challenge. Profiling of target organ toxicities often requires invasive procedures to obtain mRNA, gene expression changes are often transient, and differentially expressed transcripts often encode proteins that cannot be detected in accessible fluids, such as blood or urine. However, if expression data are mined with the intent of identifying biomarkers, these hurdles can be overcome. Here, we present a case study whereby microarray technology was utilized to identify a differentially expressed transcript that encodes a protein that is premonitory for a gastrointestinal toxicity observed in rat upon compound treatment. Analysis of microarray data identified the secreted serine protease adipsin as a transcript that was up-regulated early and whose expression levels were sustained throughout the compound treatment period. Expression changes in adipsin two hours post-treatment preceded gross pathological effects that required four days to manifest in the ileum of treated animals. Adipsin has previously been determined to encode an adipocyte-specific component of the alternative complement cascade. In investigating adipsin as a toxicity biomarker, however, we have identified a new regulatory mechanism for this gene that places it as a downstream target of the notch cell fate signaling pathway. Promoter analysis and protein expression using peptide antibodies confirm the array findings and mechanistically link this biomarker to off-target effects of adipsin. Furthermore, *in vitro* models used to confirm adipsin gene regulation and expression now allow screening for molecules for *in vivo* gastrointestinal effects using an *in vitro* transcription-based screen. In summary, we have employed a predetermined microaray data mining approach to identify a non-invasive toxicology biomarker using invasive microarray technology. Follow up studies have not only confirmed biologically what was observed transcriptionally, but identified a new regulatory control mechanism for the adipsin gene.

58 HIERARCHICAL CLUSTERING ANALYSIS OF 2-DIMENSIONAL PROTEIN GEL IMAGES: CORRELATION WITH TESTICULAR TOXICITY IN BEAGLE DOGS.

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Changes in protein expression that correlate with onset of histopathology are potentially useful as indicators, and possibly as early markers of target organ toxicity. The use of 2-dimensional (2D) gel electrophoresis for the examination of global changes in an organism's proteome has great potential to aid in the identification of novel markers of toxicity. However, this approach has proven to be difficult due to the immense amount of data obtained from individual gels. We investigated a statistical approach where hierarchical clustering methods were used to correlate the changes of individual protein levels across a number of gels. Male beagle dogs were dosed with ketoconazole, a potent anti-fungal with noted testicular toxicities, (40 mg/kg) for 1, 7 or 25 days. Testicular degeneration occurred as early as day 7 and continued through day 25. Cellular proteins were extracted from the testes and separated by 2-D protein gel electrophoresis. Proteins whose abundance was significantly changed as compared to control testes ($p<0.05$) were identified and subjected to hierarchical clustering analysis. Two different software packages clustered proteins according to the similarity of their expression profiles. Clusters of proteins whose expression pattern correlated with or preceded the onset of the lesion were identified. These protein spots will be selected for mass spectrometry identification and further analysis. By using hierarchical clustering, the number of protein spots analyzed from 2D gels was reduced from over 2000 to less than 150. This approach will allow us to focus on specific clusters of proteins based on their correlation with the development of drug-induced histopathological lesions.

59 USE OF GENE EXPRESSION PROFILING TO UNDERSTAND THE TRANSCRIPTIONAL PROGRAMME ASSOCIATED WITH ESTROGEN-INDUCED UTERINE GROWTH: IMPLICATIONS FOR THE USE OF SURROGATE MOLECULAR MARKERS IN TOXICOLOGY.

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Toxicogenomics, the measurement of gene expression changes in toxicant-exposed biological materials, is beginning to have a significant impact on toxicological research. The use of gene expression data in mechanistic and predictive toxicology is hindered by a lack of information on the relationships between transcriptional events and physiological and pathological changes. We have analysed this relationship using the rodent uterotrophic response as a model experimental system. Immature mice were administered with a single dose of estradiol (400 mg/kg) sufficient to induce a sustained increase in uterine weight, or with vehicle alone (corn oil), by sub-cutaneous injection. Uterine weight, histopathological parameters and gene expression levels were measured 1, 2, 4, 8, 24, 48, and 72hr after injection. Greater than 1, 000 of the 12, 500 genes analysed showed altered expression in response to estradiol treatment. Hierarchical clustering was used to group these genes into co-regulated gene clusters. By comparing the kinetics of gene regulation of each cluster with uterine weight and histopathological measurements, we were able to assess the contribution made by each cluster to the overall physiological response of the uterus. Using this approach, we have identified groups of co-regulated genes that may be involved in early (e.g. induction of growth-promoting transcription

factors, fluid imbibation), mid (e.g. increases in mRNA and protein metabolism) and late (e.g. induction of DNA replication and cell division) events. This study reveals novel insights into the molecular mechanisms that accompany estrogen-regulated uterine growth. Furthermore, by correlating gene expression and physiological changes we have identified alterations in gene regulation that may be used as molecular markers for early, mid and late uterine responses.

60 PROTEOMIC INVESTIGATION OF BODY FLUIDS.

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The use of surface-enhanced laser desorption ionization (SELDI) linked to time of flight (TOF) mass spectroscopy to determine protein expression profiles of body fluids is proving useful in informing diagnostic and therapeutic approaches to respiratory disease. Prior to initiation of such studies at the Lovelace Respiratory Research Institute, we developed standard protocols for the preparation of body fluid samples for analyses on the five major commercially available chips (anionic, cationic, hydrophobic, normal phase and metal affinity surfaces). As expected, there was considerable variation in the proteins bound by each chip type. Using these protocols we determined the reproducibility of the results between the eight surface areas on each chip, and between different chips with the same surface. Some variation was observed, suggesting that replicate analyses are advisable and internal standards are needed as reference values for quantitative analyses. We also determined the effect of large amounts of albumin (commonly present in body fluids) on the binding of other proteins to the surfaces. Albumin binding can prevent the binding of other proteins and mask their presence. Sample treatment including freeze/thawing and trichloracetic acid precipitation can produce apparent artifacts in the patterns of peaks seen in the TOF analyses. Preliminary analyses of human bronchoalveolar lavage fluid (BALF) suggested that a biomarker of developing chronic obstructive pulmonary disease (COPD) could be detected in smokers. Under conditions of the assay (SAX2 chip protein array, pH 7.0), the biomarker protein (Mw about 5 kDA) was not seen in BALF from non-smokers, variably seen from smokers without COPD, and present in large amounts from smokers with COPD. Additional samples are being analyzed to determine the consistency of this result. The results of these studies provide valuable information for the design of future investigations using proteomic analysis of body fluids. Research supported by the Lovelace Respiratory Research Institute.

61 USE OF PROTEOMIC TECHNOLOGIES FOR DISCOVERY OF NEW MARKERS OF SKIN IRRITATION *IN VITRO.*

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The development of predictive *in vitro* test systems for the identification of skin irritation hazards relies on a greater understanding of the mechanistic basis of the human skin irritation response. Proteomic technologies now allow investigation of important biochemical events in the processes of skin irritation and the discovery of potential new markers. This study was designed to profile and identify proteins involved in the skin irritation response, following exposure of a reconstructed human skin model (EpiDerm™ (MatTek)) to a range of skin irritants: sodium lauryl sulphate (SLS), benzalkonium chloride (BKC), nonanoic acid (NAA) and phenol. EpiDerm™ cultures were exposed (15min-24h) in triplicate to non-cytotoxic doses of the skin irritants (0.1mg/ml SLS, 0.01% (v/v) BKC, 0.025% (v/v) NAA and 0.25% (v/v) phenol) as determined by MTT assay and histological examination. Proteomics was performed using SELDI-TOF to investigate protein expression profiles following exposure to skin irritants. A number of proteins (MW 6-52kD) were found to be differentially expressed when profiled using a range of ProteinChip® arrays (Ciphergen). For example, proteins of MW 9.9 and 12.9kD exhibited upregulation following exposure to skin irritants at the majority of time points tested, while some protein changes were chemical specific (e.g. 13.7kD protein upregulated following 4h exposure to phenol). In addition, differential phosphorylation (80D mass shift) patterns correlating with exposure to skin irritants were also identified. 2D-gel electrophoresis was also used in combination with MALDI-TOF and a number of proteins including heat shock proteins, metallothioneins, calmodulin-like skin protein and involucrin demonstrated differential expression/post-translational modification following exposure to skin irritants. In conclusion, these results demonstrate the potential of proteomic technologies to investigate the differential regulation of proteins in response to skin irritants, some of which could represent potential new *in vitro* markers of skin irritation.

62 CHARACTERIZATION OF A BILIARY PROTEOMIC INJURY SIGNATURE IN RATS EXPOSED TO 1, 1-DICHLOROETHYLENE.

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Previous work has described the hepatotoxicity and metabolism of 1, 1-DCE, which causes a selective injury to the canalicular membrane. Bioactivation of 1, 1-DCE results in formation of reactive electrophilic intermediates that, in turn, are capable of protein alkylation. Protein alkylation following DCE treatment ultimately results in formation of S-carboxymethylated proteins. Proteins are excreted into the bile *via* the canalicular membrane. Analysis of biliary proteins provides insight into both intracellular and canalicular membrane changes. Bile was collected from rats both prior to and 3h after exposure to 50 mg/kg 1, 1-DCE. Proteins were separated by 1D-PAGE and subjected to in-gel digestion. Samples were analyzed by LC-MS-MS on a ThermoFinnigan LCQ-DECA mass spectrometer. Each sample was subjected to multiple analyses over 300 to 2000 amu, 300 to 700 amu, 700 to 1200 amu and 1200 to 1800 amu. Proteins were identified with the aid of SEQUEST and BLAST database searching, and verified by interpretation of the corresponding spectra. Moslen et al. observed a decrease in protein content after exposure to 1, 1-DCE; however, our data show a marked increase in the number of different proteins. Twenty-one proteins were identified in the control samples, whereas 40 were identified in the post-exposure samples. In addition, several S-carboxymethylated peptides were identified in samples collected after exposure. Since this modification does not occur endogenously, these modified peptides provide evidence for protein covalent modification by mechanisms previously proposed. This work describes the first comprehensive characterization of the rat biliary proteome both before and after exposure to a hepatotoxic chemical. (This work was supported by NIH Grants ES10056 and ES06694.)

63 A PROTEOMIC APPROACH TO IDENTIFY THE MOLECULAR TARGETS OF LEAD AND ACRYLAMIDE NEUROTOXICITY IN THE NEURONAL SNARE PROTEIN CIRCUITRY THAT UNDERLIES NEUROTRANSMITTER RELEASE.

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Our lab has developed a series of proteomic systems to identify protein:protein interactions that are affected by a toxin or drug. Here we present potential targets of the neurotoxins lead (Pb^{+2}) and acrylamide (ACR) identified from a library encoding millions of individual proteins. Our search was anchored by the neuronal SNARE protein complex, specifically SNAP-25. The system described is a proteomic approach to molecular toxicology based on the two-hybrid screen. The system is divided into three steps, the first screen identifying potential protein:protein complexes from the large human neuronal cDNA libraries. Secondary and tertiary screens are then used to limit artifacts and identify protein complexes that are disrupted by neurotoxin. The neuronal SNAREs are a special class of SNARE proteins, involved in the trafficking and calcium dependant fusion of neurotransmitter containing vesicles to the presynaptic neuronal membrane. Our approach, an intersection of proteomics, neuroscience, and molecular toxicology, is robust and inexpensive, providing a method to identify the molecular targets of a toxin or drug from complex libraries of proteins. For this study the neurotoxins Pb^{+2} and ACR provide excellent model systems, being associated with dysfunctions in neurotransmitter release, and membrane trafficking. We hypothesized that the neuronal SNARE protein circuitry (the neuronal SNARE complex and its associated proteins) was involved in this dysfunction. The experimental range of Pb^{+2} and ACR concentrations used had no toxic effects on the growth of control strains in the study. The primary and secondary screens of the cDNA library encoded proteins identified 43 potential protein:protein complexes. The tertiary neurotoxicity screen identified 12 candidate clones that were selected for sequencing and were independently tested for self-activation. Of the 12 candidate clones, 4 were previously identified as SNAP-25 partner proteins, 2 were SNARE proteins, 4 were known proteins, and 2 were unknown sequences.

64 BIOMARKERS OF HUMAN GLIOMA CELL EXPOSURE TO ELECTROMAGNETIC FIELDS.

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Several recent reviews on the potential health effects of electric and magnetic fields (EMFs) have concluded that power frequency EMF exposures are potentially carcinogenic to humans. Similarly, recent pooled analyses examining the relationship between magnetic fields (MF) exposure and childhood cancer indicate that exposure to residential MF exceeding 3 or 4 milligauss (mG) are associated with a 70-100% increased risk of acute lymphocytic leukemia, the most common form of

cancer in children. In light of these data, the National Institute for Occupational Safety and Health (NIOSH), together with Colorado State University (CSU) are investigating the effects of putative occupational carcinogens on cancer-linked molecular events in appropriate cellular systems. We are currently interested in the biological plausibility of EMFs as a putative carcinogen in SF767 human glioma cells. Here we study the effect of short-term environmental-level EMF exposure using cDNA microarrays. Following a 3 hr exposure we investigate changes in gene induction to determine appropriate genetic biomarkers of effect of exposure to EMF. At 70% confluence SF767 are exposed in the presence or absence of either epidermal growth factor (EGF) or melatonin, to a 12 mG, 60 Hz EMF. Ambient field strength in the cell culture incubator is maintained at 2 mG, 60 Hz. Total RNA was then isolated for use on the PerkinElmer oncogene array. Computer analysis and comparison of gene up and down regulation is performed across groups. These data suggest a limited yet complicated response of glioma cells to environmental field exposure. The number of oncogenes induced by EMF alone and in the presence or absence of melatonin and/or EGF range from 8 to over 40. The number of suppressed oncogenes ranges from 7 to more than 20. Current cluster analysis of this data may offer preliminary evidence of the possible role of EMF as a carcinogen in human glioma cells.

65 ORAL EXPOSURE TO INORGANIC MERCURY ALTERS T-LYMPHOCYTE PHENOTYPES AND CYTOKINE GENE EXPRESSION IN BALB/C MICE.

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Mercury is a well recognized health hazard and an environmental contaminant. Mercury is known to suppress immune responses, but the mechanisms responsible for this effect are still unclear. The aims of this study were to investigate the effect of mercury on immune parameters, such as hematology, lymphocyte phenotypes and cytokine gene expression. Male BALB/c mice were exposed continuously to 0, 0.3, 1.5, 7.5, or 37.5 ppm of mercury in drinking water for 14 days. Food and water consumption decreased in a dose-dependent manner in mice exposed to mercury. Body weight was reduced at the highest dose of mercury whereas the relative kidney and spleen weight were significantly increased. The dose-range of mercury used did not cause hepatotoxicity as indicated by circulating alanine aminotransferase and aspartate aminotransferase. Circulating blood leukocytes were elevated in mice treated with the highest dose of mercury. Single-cell splenocyte cultures were used to determine the effects of mercury treatment on mitogen-induced lymphocyte blastogenesis. Mercury at 1.5 ppm increased the PHA and LPS stimulation indices for T and B lymphocytes, respectively reflecting the observed decrease in basal splenocyte proliferation in mercury-treated mice. Exposure to 7.5 and 37.5 ppm of mercury decreased the CD8+ T lymphocyte population in thymus, whereas double positive CD4+/CD8+ and CD4+ thymocytes were not altered. Mercury ranging from 1.5 to 37.5 ppm dose-dependently decreased CD3+ T lymphocytes in spleen; both CD4+ and CD8+ single positive lymphocyte numbers were decreased. The population of CD45+ B lymphocytes was not changed. Mercury altered the expression of cytokines (tumor necrosis factor α, interferon γ, interleukin-12), c-myc, and major histocompatibility complex II in various organs. Results indicated that decreases in T lymphocyte populations in immune organs and altered cytokine gene expression may contribute to the immunosuppressive effects of inorganic mercury.

66 INDUCTION OF HEPATIC METALLOTHIONEIN BY VANADIUM.

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We have previously shown that the induction of hepatic metallothionein (MT) in mice by manganese (Mn) administration is entirely dependent on the production of inflammatory cytokine, interleukin-6 (IL-6). It is known that IL-6 is one of the mediators of MT induction. In the present study, we investigated the induction mechanism of MT synthesis by vanadium (V) in mice. Male ICR mice were injected subcutaneously with ammonium metavanadate (V[5]; 0.05-0.3 mmol/kg) and sacrificed 24 h after the injection. Although hepatic total vanadium concentration was lower than that in kidney, MT concentration was increased dose-dependently in the liver by V[5] administration, but not in the kidney. HPLC/ICP-MS analysis on the distribution of the metals in hepatic cytosol of V[5]-treated animals showed that the major metal bound to MT-I and MT-II was not vanadium, but zinc. A time-course study showed that hepatic total vanadium content, serum alanine aminotransferase (ALT) activity and serum IL-6 concentration reached the peak at 4-6 h after V[5] (0.3 mmol/kg) injection, and then declined quickly. Concentration of serum amyloid A (SAA), an acute-phase protein that is induced by IL-6, increased at 24 h after the injection. To confirm the involvement of IL-6 in MT induction by V[5], IL-6 null and control mice were administered with V[5]. In IL-6 null animals, no increase in SAA was observed, and MT induction by V[5] was significantly decreased to about 55 % of control animals. These data suggest that both IL-6-dependent and independent mechanisms are involved in MT induction by V[5] in mice.

67 ENHANCED GENOTOXICITY BY DIMETHYLARSINIC ACID IN METALLOTHIONEIN-I/II NULL MICE.

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To elucidate the protective role of metallothionein (MT) in the genotoxicity of dimethylarsinic acid (DMA), we examined the sensitivity of MT-I/II null mice to the genetic damage and the induction of apoptosis caused by DMA. Eight-week-old female MT-I/II null mice and wild-type mice were given p.o. injection of DMA (188, 375, 750 mg/kg). The blood, urine and liver were removed from each mouse under ether anesthesia at 24 hr after the injection. The DNA strand breaks in the peripheral blood cells and the 8-hydroxy-deoxyguanosine (8-OHdG) in the serum and urine were determined as indicators of genetic damage. The production of DNA strand breaks in both MT-I/II null mice and wild-type mice was elevated by DMA treatment in a dose-dependent manner. The increased the production of DNA strand breaks in MT-I/II null mice at 750 mg/kg DMA was significantly higher than that of wild-type mice. Moreover, 8-OHdG levels in the serum and urine of MT-I/II null mice were also increased by DMA treatment and they were significantly higher than those of wild-type mice. On the other hand, the DMA-induced apoptosis in the liver was not different between MT-I/II null mice and wild-type mice. MT concentrations in the liver of wild-type mice were increased by DMA treatment in a dose-dependent manner. There were no detectable amounts of hepatic MT in untreated MT-I/II null mice, and they could not be induced by DMA treatment. These results suggested that MT plays an important role in defense of DMA-caused genotoxicity.

68 METAL INDUCED ACTIVATION OF METALLOTHIONEIN GENE EXPRESSION.

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Metallothionein (MT) transcription is induced following exposure to elevated concentrations of a variety of transition metals. Metal-activated transcription is controlled *via* interactions between metal response elements (MREs) and the metal response element-binding transcription factor, MTF-1. It has been proposed that metals can initiate intracellular signaling cascades that result in altered states of MTF-1 phosphorylation, ultimately leading to the activation of MT transcription. COS-7 cells were transfected with one of three fusion chloramphenicol acetyltransferase (CAT) reporter genes: p-42-CAT (minimal mouse MT-1 promoter); pMREd5'-CAT (5 tandem copies of MREd' inserted upstream of the TATA box in p-42-CAT); or p-153-CAT (intact mouse MT-1 promoter). Cells were co-transfected with a β-galactosidase reporter plasmid, pSV-β-gal to control for transfection efficiency. Following a 4 hr metal exposure, the level of CAT protein was determined by a CAT-ELISA. Results were normalized with respect to the level of β-galactosidase activity. Of metals tested, only cadmium, copper, mercury, arsenic, antimony, silver, gold, and zinc were able to activate MT transcription *via* the MRE, while aluminum, indium, selenium, beryllium, tin, iron, cobalt, manganese, bismuth, molybdenum, lead, titanium, vanadium, chromium(III and IV), and nickel were not. CAT assay results were verified using reverse transcription PCR (RT-PCR) with MT primers and total RNA isolated from COS-7 cells exposed to the various metals for 4 hours. All real-time MT data was normalized to an actin standard. Of metals tested, cadmium, copper, zinc, mercury, silver, and arsenic exposures caused an increase in the steady state levels of MT mRNA. Inhibition studies were completed in order to test the hypothesis that phosphorylation of MTF-1 is involved in activation of MT gene transcription. These studies involved pre-treating cells for 30 minutes with 100μM of a broad range protein kinase C (PKC) inhibitor, H7. Inhibition results suggest that MT transcription induced by these metals occurs *via* a convergent signal transduction pathway involving PKC.

69 MT-3 OVEREXPRESSION INCREASES CHEMOTHERAPEUTIC RESISTANCE AND AFFECTS THE GROWTH OF BREAST CANCER CELL LINES.

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The third isoform of the human metallothionein (MT) gene family is unique compared to the 1 and 2 isoforms in that it has a more restricted pattern of tissue distribution. This laboratory has previously shown that overexpression of MT-3 in the

prostate cancer cell line PC-3 and in the breast cancer cell line MCF-7, inhibited the growth of these cells and increased their resistance to certain chemotherapeutic drugs. Since estrogen receptor (ER) status is a prognostic marker in the diagnosis of breast cancer, we were interested in determining the effect of overexpression of MT-3 in ER positive and ER negative cell lines. For this purpose, the MT-3 gene was stably transfected under the control of the CMV promoter in ER positive cell lines (MCF-7 and T-47D) and ER negative cell lines (MDA-MB-231 and Hs578T). Our previous work has shown that the MT-1E isoform is expressed in ER negative cells and not in ER positive cells. Therefore the MT-1E gene was transfected into the two ER positive cell lines. The expression of MT-3 and MT-1E gene and protein was confirmed in five independent clones using RT-PCR and immuno-blot analysis respectively. MT-3 overexpression decreased the growth rate of MCF-7 and Hs578T cells, whereas the growth rate of T-47D and MDA-MB-231cells was not affected. Overexpression of MT-3 also conferred resistance to the chemotherapeutic drugs cisplatin, doxorubicin, paclitaxel, 5-Fluorouracil and methotrexate. However, overexpression of MT-1E did not affect the growth rate of the cells but did confer drug resistance. This data suggests that the growth inhibitory activity is a function of MT-3 and is not dependant on the ER status of breast cancer cells but rather, it subdivides ER positive cells into subcategories based on the expression of MT-3 which may be important factor in the prognosis of the disease.

70 THE INABILITY TO PRODUCE THE MAJOR FORMS OF METALLOTHIONEIN RENDERS MICE HYPERSENSITIVE TO THE CHRONIC TOXIC EFFECTS OF LEAD, INCLUDING RENAL HYPERPLASIA, WHILE PREVENTING INCLUSION BODY FORMATION.

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Lead (Pb) is a high priority environmental hazard and defining factors that predispose populations to the chronic Pb toxicity is a key research issue. Recently, we found that mice unable to produce the major forms of the metal-binding protein metallothionein, specifically MT-I/II knockout (MT-null) mice, are hypersensitive to the subchronic toxic effects of oral Pb, like diminished renal function and nephromegaly, compared to wild type (WT) mice that express MT normally. The MT-null mice accumulated less tissue Pb and did not form Pb inclusion bodies. Since the MT-null phenotype reduces tissue Pb accumulation, it is unclear how this might impact chronic Pb toxicity. Thus, chronic Pb toxicity was compared in MT-null and WT mice. Male MT-null and WT mice (n = 25) received Pb (as Pb acetate) in the drinking water at 0, 1000, 2000, 4000 ppm for up to 104 weeks. Reduced survival occurred at 2000 and 4000 ppm in MT-null mice but only at 4000 ppm in WT mice. Renal preneoplasia (cystic hyperplasia) was much more common and severe in Pb-exposed MT-null mice (up to 60%) than WT mice (up to 21%). A metastatic renal carcinoma occurred in a Pb-treated MT-null mouse while no renal tumors occurred in WT mice. Chronic Pb-induced nephropathy, including tubular degeneration, necrosis and interstitial fibrosis, did not occur to a significant extent in WT mice, but was common (up to 72%) and dose-related in both incidence and severity in MT-null mice. MT-null mice did not form renal Pb-containing nuclear inclusion bodies (NIBs), which are thought to mitigate Pb toxicity, whereas NIBs were common in WT mice. Thus, the MT-null phenotype is unable to form NIBs even after protracted Pb exposure and this increases chronic Pb toxicity. Overall, MT appears to have an important role in chronic Pb toxicity and NIB formation, and the inability to produce MT may predispose populations to chronic Pb toxicity. (Funded in part by DHHS #NO1-CO-12400.)

71 EFFECT OF METALLOTHIONEIN-3 EXPRESSION ON CADMIUM TOXICITY IN THE HUMAN PROXIMAL TUBULE CELL LINE HK-2.

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The kidney and the proximal tubule in particular are the organ and cell type critically affected by chronic exposure to cadmium. Numerous studies have shown that metallothionein (MT) 1 and 2 family members play an important protective role against the chronic nephrotoxicity classically elicited by cadmium exposure. The third isoform of metallothionein, MT-3 can also bind and sequester cadmium similar to that of the MT-1 and 2 isoforms, and could have the potential to play a role in mediating toxicity that cadmium elicits on the renal proximal tubule cell. Previous work by this laboratory has shown that the immortalized human proximal tubule cell line HK-2, does not express the MT-3 gene. The goal of this study was to determine if over-expression of MT-3 in the HK-2 cell line would increase the resistance of these cells to the toxic effects of cadmium. The HK-2 cell lines and the MT-3 transfected cells were exposed to lethal and sub-lethal concentrations of cadmium chloride for various time periods. The effect of cadmium treatment on cell viability was determined by the automated counting of the cell nuclei stained with the nuclear binding dye, 4', 6-diamidino-2phenylindole (DAPI). Continuous exposure of HK-2 cells to various concentrations of cadmium for 8 to 48 hr resulted in cell death with extensive chromatin condensation and DNA fragmentation, features typical for cells undergoing apoptosis. This was further confirmed by agarose gel electrophoresis and caspase-3 assay. The MT-3 transfected cells did not show any increased resistance to cadmium compared to the HK-2 parental cells with very few cells showing morphological changes typical for apoptosis. Extended exposure of the parental and transfected cells for 16 days to cadmium had a similar effect. These results indicate that MT-3 expression may have a minimal role in providing protection against the toxic effects of cadmium.

72 EXPRESSION OF THE MT-3 PROTEIN IN THE NORMAL BREAST EPITHELIAL CELL LINE MCF-10 IS DEPENDANT UPON THE PRESENCE OF THE HEAVY METAL CADMIUM.

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Metallothioneins (MT) are a family of low molecular weight (6Kb) cysteine rich proteins that participate in a variety of functions such as detoxification of heavy metals and homeostasis of essential metals. They can also act as scavengers of free radicals. MT-1 and 2 isoforms are ubiquitously expressed, whereas the expression of the third isoform is limited to the neural tissue. Our laboratory has recently shown that MT-3 is not expressed in normal breast tissue, but is overexpressed in certain breast cancers and that the overexpression is associated with tumors having a poor prognosis. The goal of this study was to determine the effect of MT-3 overexpression in breast epithelial cells using a cell culture model. For this purpose, the normal breast epithelial cell line MCF-10 was stably transfected with the MT-3 gene under the control of the CMV promoter. Expression of MT genes and protein were determined by RT-PCR and immuno-blot analysis respectively. The MT-3 transfected cells expressed the MT-3 mRNA but surprisingly did not express any MT-3 protein. In order to explain this finding, the MT-3 transfected cells, vector only transfected cells and the parental MCF-10 cells were exposed to various concentrations of cadmium chloride from 4 to 48 hours. Exposure to cadmium resulted in a significant increase in the gene expression of MT-1E, 1X and 2A isoforms in all the cell types. As expected, there was no expression of MT-3 in the parental or the vector only transfected cells. However, in the MT-3 transfected cells exposure to cadmium caused a 10 fold induction in MT-3 protein levels. This data suggests that the MT-3 protein is highly unstable in the absence of heavy metals and is rapidly degraded. However, heavy metals such as cadmium bind to the protein, stabilize it and prevent their degradation.

73 CADMIUM OR ZINC IS REQUIRED FOR THE EXPRESSION OF MT-3 PROTEIN IN THE MT-3 GENE TRANSFECTED UROTSA CELL LINE.

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Metallothioneins (MT) are cysteine rich intracellular proteins that bind transition metals with high affinity and have a wide pattern of tissue distribution. However the third isoform of metallothionein, MT-3 is shown to have a restricted pattern of tissue distribution with expression confined to the neural tissue. Previous work done by this laboratory has shown that MT-3 is not expressed in the normal bladder but is over-expressed in bladder cancer with levels correlating to the type and grade of tumor. Recently, a new bladder epithelial cell line UROtsa has been characterized that does not express the MT-3 gene and may serve as a useful invitro model system of the normal human urothelium. The goal of this study was to see the effect of MT-3 over expression in the normal human bladder epithelial cell line. For this purpose, the UROtsa cell line was stably transfected with the MT-3 gene under the control of the CMV promoter, or the blank vector without the MT-3 gene. The MT-3 gene expression was determined by RT-PCR and the protein levels were detected by immuno-dot blot. The MT-3 transfected cells expressed the mRNA for the MT-3 gene but did not express any protein. One hypothesis to explain why the over expressed RNA does not result in increased protein level is that the protein is rapidly degraded if it is not saturated with heavy metal. To test this hypothesis, the parental cell line UROtsa, the vector blank and the MT-3 transfected cells were exposed to 1, 5 and 9mM cadmium chloride for 4 to 48 hr and MT-3 levels were determined. Treatment with cadmium did not increase the level of MT-3 mRNA or protein in the parental cell as well as the blank vector control. However, in the MT-3 transfected cells, the protein levels increased from 1.5 ng to 6ng/mg of the total protein. Exposure to zinc sulfate had a similar effect on these cells. This data suggests that the MT-3 protein is highly unstable and heavy metals such as cadmium and zinc could stabilize the protein and prevent it from undergoing rapid degradation.

74 EXPRESSION OF METALLOTHIONEIN ISOFORMS IN THE NORMAL HUMAN PROSTATE EPITHELIAL CELL LINE, 267-B1, EXPOSED TO CADMIUM.

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Cadmium is a known prostate carcinogen. The assessment of this metal's ability to transform epithelial cells of the prostate is complicated by the presence of metallothionein (MT). This protein can protect against the toxic effects of cadmium by metal sequestration, and exposure to this metal can drastically increase the cellular levels of MT. To study the involvement of MT in the genesis of prostate cancer, the expression profile of the MT isoforms was characterized in the normal human prostate cell line, 267-B1, in response to cadmium exposure. 267-B1 cells were exposed to 18 μM cadmium for up to 4 hr and recovered in normal growth medium for 24 hr, or cells were exposed to 8 μM cadmium continually up to 16 days. Cultures were harvested at various time points and the levels of MT isoform mRNA were assessed by reverse-transcription polymerase chain reaction (RT-PCR), and the levels of MT protein were measured by immuno-dot blot. Basal expression of MT-2A, -1E, and -1X mRNA was detected at levels near that of the house-keeping gene glyceraldehyde phosphate dehydrogenase whereas the levels of MT-3 mRNA were considerably lower. This was similar to the expression profile found previously in normal human prostate tissue. Cadmium increased the levels of MT-1E, 3 fold, and 1X slightly, but there was no induction of MT-2A. Cadmium elicited the expression of -1F in both exposure protocols, whereas the expression of MT-1A and -1G appeared only in the exposure and recovery protocol. Cadmium was unable to induce the levels of MT-3 mRNA or protein in either cadmium exposure protocol. The total MT-1 and -2 protein level was increased from 2.59 +/- 0.45 ng/ μg protein to a transient level of 16.11 +/- 2.85 ng/ μg protein during the exposure and recovery protocol, and to a level of 25.46 +/- 0.38 ng/ μg protein after 16 days of continuous exposure to cadmium.

75 LEAD MODULATES BOTH OSTEOBLAST AND OSTEOCLAST ACTIVITY: A PARADIGM FOR ENHANCED BONE LOSS.

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Many adults over the age of fifty have a substantial bone lead burden. This is due to the long half life of lead in the skeleton and to the sequestration of lead in the mineral compartment of bone. While the concern over lead toxicity in soft tissues may be decreasing, the cells in the bone compartment remain exposed to high levels. Our findings document that both osteoblast and osteoclast activity is depressed under the influence of lead. Primary cultures of osteoblasts and osteoclasts isolated from neo-natal rat calvaria and long bone marrow aspirates (respectively) were used for these experiments. Osteoblasts cultured in the presence of lead demonstrated a dose and time dependent decrease in alkaline phosphatase activity and collagen synthesis, both hallmarks of bone formation. The effect was statistically significant at 0.1uM and higher. This effect is mediated by a decrease in the mRNA levels for the requisite osteoblast transcription factor Runx2. Also, we have shown that lead rapidly and potently decreases TRIP-1 mRNA levels. TRIP-1 is a key effector in the TGF beta pathway which controls osteoblast differentiation. The effect of lead on osteoclast function is also inhibitory. This was demonstrated by a decrease in total surface area resorbed by isolated osteoclasts. The effect is mediated by a decrease in RANK ligand (the final paracrine factor involved in osteoclast activation) production and an increase in OPG (osteoprotegerin, a decoy receptor for RANK ligand) levels. A meta analysis of all of the osteoblast and osteoclast data indicate that the effect on osteoblasts occurs at a lower lead level than on osteoclasts. This implies that bone formation is more adversely affected than bone resorption. Lead exposure to bone cells in culture depresses both bone formation and resorption. However, bone formation is more profoundly affected than resorption. If this qualitative difference exists *in vivo*, then it might explain the decrease in skeletal density seen in animals and humans exposed to lead.

76 URANIUM AND CELL DEATH IN THE RAT KIDNEY.

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We investigated the histopathological course of uranium-induced acute tubular necrosis in adult male Sprague-Dawley rats, focusing on pathogenic events. Rats were sacrificed 8 hours, 24 hours, and 168 hours following intraperitoneal administration of 0, 1 or 10 mg/kg of uranium given as uranyl acetate in saline. Mean total uranium concentration (in ng/ml, n = 3-5) in serum was as follows: 1 mg/kg dose- 20.9 at 8 hours, 4.5 at 24 hours; 10 mg/kg dose- 200 at 8 hours, 30.5 at 24 hours. Light microscopic studies revealed diffuse brush border loss and necrosis of renal proximal tubular epithelium, most marked in rats given 10 mg/kg, confirming earlier work documenting the toxic effect of uranium (Sanchez et al., Biol Trace Elem Res 84:139; Lim et al., Yonsei Med. J 28:38). This lesion was notable at 8 hours post-dosing in the outer stripe of the medulla, and extended through the cortex by 24 hours, best seen in rats given 10 mg/kg. We have demonstrated that apoptosis is a feature of this event, most prominent at 24 hours post-dosing, using the TUNEL assay (1:25) (TUNEL Label, Roche Diagnostics). Glomeruli appeared intact. By 168 hours, proximal tubular epithelial regeneration was evident in the cortex and outer medullary stripe, more prominent in the 1 mg/kg dosage group. This was characterized by cells having basophilic cytoplasm and not infrequently, mitotic figures. We demonstrate that apoptotic events occur during uranium-induced renal tubular injury and that regeneration is a prominent sequel to such a heavy metal effect. (Supported by: US Army Medical Research and Materiel Command DAMD17-01-1-0775. This abstract does not necessarily reflect the position or policy of the US Government.)

77 ARSENIC ALTERS HORMONE-MEDIATED POSITIVE, BUT NOT NEGATIVE, REGULATORY EFFECTS OF STEROID RECEPTORS.

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Arsenic (As) is an agent of considerable human health concern in the US and worldwide. We previously reported that As+3 (arsenite) can act as a potent endocrine disruptor, inhibiting hormone-mediated gene activation by glucocorticoid receptor (GR) and estrogen receptor in cultured cells and *in vivo*. We have investigated these effects further in the current work. Expression constructs for GR, mineralocorticoid receptor or androgen receptor were co-transfected into EDR3 hepatoma cells along with luciferase expression constructs containing appropriate hormone receptor DNA binding sites. As altered the ability of each of these steroid receptors to activate expression of receptor-regulated gene constructs following treatment with dexamethasone, aldosterone or testosterone, respectively. As had little or no effect on basal expression of these constructs in the absence of hormone. The lowest doses of As used (0.1 - 0.5 μM) potentiated the hormone-induced gene expression by 2- to 3-fold over that with hormone alone. In contrast, slightly higher doses of As (1-3 μM) caused a dose-dependent suppression of induction. These results in combination with previous studies indicate that As has quantitatively and qualitatively similar effects on the entire family of steroid hormone receptors. GR can also act as a negative regulator of gene expression through both DNA-dependent and -independent mechanisms. In the latter case, hormone-activated GR can inhibit AP-1 or NF-κB gene activation in a dominant negative manner. Using several different cell culture systems we observed that activated GR suppressed AP-1 and NF-κB-mediated gene induction by phorbol ester (TPA) or TNFα, respectively. However, As treatment of cells had no effect on GR negative regulation in these systems. These results indicate that As affects DNA-dependent gene activation by GR but not its DNA-independent negative regulatory effects, suggesting that the endocrine disrupting effects of As occur primarily at the level of hormone receptor-mediated gene transcription (NIEHS ES07373).

78 ROLE OF THE CALPAIN PATHWAY IN ARSENITE-MEDIATED DECREASES IN CYP3A IN CULTURED RAT HEPATOCYTES.

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We have previously shown that arsenite decreases CYP3A inductions by both Dexamethasone (DEX) and phenobarbital post-transcriptionally in cultured rat hepatocytes, by a mechanism not involving decreased availability of heme. Here we investigated, in primary cultures of rat hepatocytes, whether arsenite decreased CYP3A by increasing degradation of the protein. Three possible routes of protein degradation are the lysosomal, proteosomal and calpain pathways. CYP3A does not contain the pentapeptide consensus sequence recognized by the lysosomal pathway. The ubiquitin-dependent proteosomal pathway has been shown to play a role in the degradation of inactivated CYP3A in rat hepatocytes However, arsenite has been shown to inhibit the ubiquination pathway. Therefore, we first investigated the role of the calpain pathway in arsenite-mediated decreases in CYP3A protein. We established conditions for monitoring calpain activity in intact cells in serum-free medium, using t-butoxycarbonyl-Leu-Met-7-amino-4-chlorimethylcoumarin as a substrate. CYP3A protein induced by DEX was increased further in the pres-

ence of calpeptin, an inhibitor of the calpain pathway. These results suggest that CYP3A is partially degraded *via* the calpain pathway. Combined treatment with DEX, arsenite and calpeptin resulted in greater decreases in CYP3A protein compared to treatment without calpeptin. None of the treatments altered protein synthesis or reduction of MTT, indicating no toxicity. The results indicate that arsenite is not increasing CYP3A protein degradation *via* the calpain pathway. The results also suggest that a labile protein potentiates the effect of arsenite to decrease CYP3A. This work was supported by NIH-ES10426(JS) and the Department of Veterans Affairs.

79 INORGANIC ARSENIC INCREASES VASOCONSTRICTION THROUGH CALCIUM-SENSITIZATION IN VASCULAR SMOOTH MUSCLES.

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Chronic exposure of arsenic is well known to be the cause of cardiovascular disease such as hypertension. In order to investigate the effect of arsenic on blood vessels, we examined whether arsenic affected agonist-induced contraction of aortic rings in organ bath system. Treatment with arsenite increased vasoconstriction induced by phenylephrine or serotonin in a concentration-dependent manner. Similar effects were also shown in the aortic rings without endothelium, suggesting that vascular smooth muscle played a key role in enhanced vasoconstriction induced by arsenite. Arsenite is the most potent form among arsenic species tested. These alterations were well correlated with myosin light chain (MLC) phosphorylation induced by arsenite in smooth muscles. Direct calcium measurement using fura-2 dye in aortic rings revealed that arsenite enhanced contraction by high K+ without further increase in intracellular calcium levels. Calcium-sensitization of contractile machinery, therefore, may contribute to the enhanced vasoconstriction by arsenite. Consistent with these *in vitro* results, intravenous administration of 1.0 mg/kg arsenite augmented blood pressure increase induced by phenylephrine in conscious rats. These results suggest that arsenite increases agonist-induced vasoconstriction and calcium-sensitization in smooth muscles was one of the key mechanisms for the arsenite-induced hypercontraction in blood vessels.

80 ARSENIC-INDUCED DYSFUNCTION IN RELAXATION OF BLOOD VESSELS.

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Several epidemiological studies have suggested that exposure to arsenic is strongly correlated with the development of cardiovascular diseases such as hypertension. To determine whether arsenic affects vasomotor tone in blood vessels, we investigated the effect of arsenic on vasorelaxation using isolated rat aortic rings in an organ bath system. Treatment with arsenite inhibited acetylcholine-induced relaxation of the aortic rings in a concentration-dependent manner, while several other arsenic species did not have any effect. Consistent with these findings, the levels of cGMP in the aortic rings were significantly reduced by arsenite treatment. In cultured human aortic endothelial cells, treatment with arsenite resulted in a concentration-dependent inhibition of endothelial nitric oxide synthase (eNOS). In addition, higher concentrations of arsenite decreased the relaxation induced by sodium nitroprusside (a NO donor) and 8-Br-cGMP (a cGMP analog) in aortic rings without endothelium. These *in vitro* results indicate that arsenite is capable of suppressing in blood vessels by inhibiting eNOS activity in endothelial cells and by impairing the relaxation machinery in smooth muscle cells. *In vivo* studies revealed that the reduction of blood pressure by acetylcholine infusion was significantly suppressed after arsenite was administered intravenously to rats. These data suggest that an impairment of vasomotor tone due to arsenite exposure may be a contributing factor in the development of cardiovascular disease.

81 CLONING, EXPRESSION, AND CHARACTERIZATION OF RAT S-ADENOSYL-L-METHIONINE: ARSENIC(III) METHYLTRANSFERASE (CYT19).

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S-adenosyl-L-methionine: arsenic(III) methyltransferase, an enzyme purified from rat liver, catalyzes the formation of methyl and dimethyl arsenic from trivalent inorganic arsenic. Because the predicted amino acid sequence of this enzyme resembles those of predicted products of cyt19 genes in the human and mouse genomes, the rat protein is designated cyt19. Rat, mouse, and human cyt19 contain common amino acid motifs of non-nucleic acid methyltransferases. Expression of cloned rat cyt19 yields a protein that methylates trivalent inorganic arsenic, producing methyl and dimethyl arsenic as minor and major metabolites, respectively. Omission of S-adenosyl-L-methionine or of a reductant abolishes its catalytic activity. Dithiothreitol and tris-(2-carboxyethyl)phosphine are approximately equipotent as reductants. A thioredoxin-thioredoxin reductase generation system can also function as a reductant. The capacities of rat cyt19 to catalyze both the oxidative methylation of arsenicals and the putative reduction of arsenicals to the trivalent oxidation state suggest that this protein has both methyltransferase and reductive functions. Selenite and methylselenol are potent inhibitors of cyt19-catalyzed formation of methylated arsenicals from inorganic arsenic. Selenite (Ki = 1.4 μM) is a more potent inhibitor than is methylselenol (Ki = 19 μM) and neither dimethylated or trimethylated selenium compounds inhibit cyt19. Alteration of cyt19-catalyzed production of methylated arsenicals by selenium-containing compounds could be a locus of interaction between these metalloids. (This abstract does not necessarily reflect EPA policy.)

82 INORGANIC AND METHYLATED TRIVALENT ARSENICALS INHIBIT GLUCOSE UPTAKE BY MURINE ADIPOCYTES.

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Chronic exposures to inorganic arsenic (iAs) are associated with increased incidence of various forms of cancer and noncancerous diseases, including non-insulin dependent (Type 2) diabetes mellitus. Although mechanisms by which iAs induces diabetes have not been identified, the nature of the disease indicates that iAs or its metabolites may interfere with insulin signaling or with critical steps in glucose metabolism. We have examined effects of iAs and methylated arsenicals on basal and insulin-stimulated glucose uptake by cultured 3T3-L1 adipocytes. Inorganic and methylated pentavalent arsenicals did not affect either basal or insulin-stimulated glucose uptake. Trivalent arsenicals, arsenite (iAsIII), methylarsine oxide (MAsIII), and iododimethylarsine (DMAsIII), inhibited insulin-stimulated glucose uptake in a concentration-dependent manner. Exposures to 20 μM iAsIII, 1 μM MAsIII or 2 μM DMAsIII decreased insulin-stimulated glucose uptake by 35 to 45% but did not affect cell viability. Basal glucose uptake was significantly inhibited only in cells exposed to MAsIII. All three trivalent arsenicals suppressed expression of protein kinase B (PKB/Akt), a component of insulin-activated signal transduction pathway. In addition, iAsIII and MAsIII, but not DMAsIII, inhibited phosphorylation of PKB/Akt. Notably, exposures to methylated trivalent arsenicals, but not to iAsIII, decreased the intracellular concentration of glutathione (GSH). Treatment of adipocytes with GSH-ethylester increased cellular GSH levels and prevented MAsIII- and DMAsIII-induced inhibition of glucose uptake. In contrast, treatment with GSH-ethylester did not prevent the inhibition of glucose uptake by iAsIII. These results suggest that trivalent arsenicals inhibit the PKB/Akt-dependent activation of glucose uptake by insulin. This mechanism may be, in part, responsible for the development of Type-2 diabetes in individuals chronically exposed to iAs.

83 COMPENSATORY HEME PATHWAY RESPONSES IN INTERACTION STUDIES OF LEAD, CADMIUM AND ARSENIC TOXICITY.

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In addition to dose, duration of exposure is a critical determinant in assessing the toxicity of chemicals. This factor is particularly important for low-dose, chemical mixture situations such as those found near Superfund sites. The present studies were undertaken to evaluate changes in a number of parameters in rats exposed to lead, cadmium and arsenic combinations in deionized drinking water at empirically determined LOEL dose levels (Pb, 25ppm, Cd, 10ppm, As(As^{3+}), 5ppm). These studies employed a factorial statistical design to evaluate interactive effects at 30, 90 or 180 day (in progress) sacrifice time points. Histopathological evaluation of kidneys from animals in all treatment groups showed an absence of marked changes relative to controls at the 30 and 90 day time points. However, marked, statistically significant, inhibition of the heme pathway enzyme, delta-aminolevulinic acid dehydratase (ALAD) activity was observed in blood and kidney. Erythrocyte zinc-protoporphyrin (ZPP) concentrations were also observed to be statistically increased at 30 days for all treatment groups relative to controls. In contrast, at 90 days, blood ZPP were not statistically different from controls. In general, ALAD activities in the mixture treatment groups were more markedly different from controls at 30 days than at 90 days although all treatment groups were statistically different from controls at both time points. Interaction treatment group-specific differences

and kidney versus blood differences were also observed for this parameter. These data suggest differential *in vivo* compensation in response to these treatments for several heme pathway parameters by the hematopoietic system and kidneys at the 90 day intermediate time point and underscore the need for a detailed consideration of exposure duration in chronic low-dose mixture studies. It will be of interest to evaluate whether rats in the ongoing 180 day study are able to maintain this compensatory response or crossover into more overt toxic responses. (Supported in part by EPA STAR grant # 827161).

84 SUBLETHAL ARSENIC EXPOSURE ALTERS FOCAL ADHESION AND CYTOSKELETAL ORGANIZATION IN CULTURED H9C2 CELLS.

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Focal adhesions are sites where the extracellular matrix is connected to the actin cytoskeleton *via* integrins. They play an important role in cell morphology and migration. Alterations in the balance of cytoskeletal and focal adhesion protein expression and phosphorylation have been shown to affect many cellular processes including migration, proliferation and death. Changes in cytoskeletal and focal adhesion organization of cultured H9C2 cells that were exposed to a sublethal dose of sodium arsenite for 48 hours were observed. Arsenic reduced the amount of total F-actin content present in the cells. Immunolocalization of actin stress fibers and focal adhesions showed fewer focal adhesions present and a disorganization of the actin cyotskeleton in arsenic treated cells. When arsenic exposed cells were incubated with magnetic beads coated with a RGD peptide to promote integrin binding and the subsequent formation of an artificial focal adhesion, there was a delay in the recruitment of the focal adhesion proteins FAK, vinculin and paxillin. Immunoprecipitation using a phospho-tyrosine antibody showed that arsenic was able to dephosphorylate several focal adhesion proteins. Time-lapse video microscopy showed a reduction in the rate of cell motility and cell spreading in response to arsenic treatment. These data imply that arsenic disrupts focal adhesion and cytoskeleton organization and subsequently interferes with the cellular processes that rely on the proper organization of these structures.

85 ARSENITE INHIBITION OF THE HOMOLOGS α-KETOGLUTARATE DEHYDROGENASE AND PYRUVATE DEHYDROGENASE.

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The toxicity and carcinogenicity of arsenite have been ascribed to its ability to bind protein sulfhydryls, notably the pyruvate dehydrogenase (PDH) enzyme complex. One of the subunits of this complex is post-translationally modified by the addition of lipoic acid cofactors. The adjacent 1, 3-sulfhydryls of this cofactor are believed to provide an arsenite binding moiety. Against this background of literature, we have studied the ability of arsenite to inhibit the highly homologous α-ketoglurate dehydrogenase (KGDH) enzyme complex. This enzyme complex contains fewer subunits that have been modified by the addition of lipoic acid and fewer lipoic acid cofactors added to each subunit that is modified. This enzyme is, however, a control point for the citric acid cycle. Classic inhibition kinetic studies were performed on the purified enzymes. Both enzymes were inhibited in the low micromolar range. Mechanistically, arsenite inhibited PDH in an uncompetitive manner but KGDH exhibited mixed inhibition. Loss of dithiobisbenzoic acid reactivity indicated that inhibition was due to a modification of sulfhydryl groups, however probing the enzyme with a lipoic acid polyclonal antibody did not indicate sufficient modification at this site to be detected by this assay. The lipolylated peptide fragment could be detected by high resolution capilliary mass spectrometry. This fragment was diminished upon arsenite treatment. Western blot analysis indicated a change in mobility of the arsenite-treated protein subunit, indicating a significant change in charge of the protein. It is anticipated that the characterization of these modifications will provide a potential biomarker of arsenic toxicity.

86 CADMIUM PERTURBATION OF MICROTUBULES THROUGH ITS INTERACTION WITH PROTEIN SULFHYDRYLS.

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Previous studies demonstrated cadmium (Cd) depolymerization of microtubules (MT), a key element of the cytoskeleton. Certain cysteine residues of tubulin, the principal protein of MT, are critical for MT assembly. To further explore mechanisms of Cd damage to MT, we have examined effects of this metal ion on the sulfhydryl status of tubulins in MT polymerization *in vitro* and in cultured cells. Preincubation of "pure" tubulins free of MT associated proteins (MAPs) with $CdCl_2$ inhibited the binding of [^{3}H]-N-ethylmaleimide (NEM), a potent sulfhydryl(—SH) group reagent, to tubulins in a dose-dependent manner indicating Cd^{2+} binding to tubulins through—SH groups. Turbidity assays of MT assembly *in vitro* at 27°C in a reaction mixture containing 3.5 mg/ml tubulin, 1 mM GTP, 0.175 mM Mg^{2+}, 0.175 mM EGTA, and 0.1 M Pipes, pH, 6.6, showed that the initial rates of MT assembly were decreased to 44%, 29% and 12%, respectively, of the control in the presence of 15, 45 and 75 mM $CdCl_2$. Importantly, adding 1 mM dithiothreitol, a potent thiol reducing agent, to the Cd-pretreated tubulin mixture markedly blocked the Cd inhibitory effects on MT assembly *in vitro*. These results were further confirmed by EM examination of the polymerization products taken at the reaction plateau. Moreover, we also demonstrated using ^{109}Cd and [^{3}H]-NEM as probes, that taxol, an agent promoting MT assembly and stability, protected tubulin—SH groups from Cd and NEM binding thus antagonizing the Cd or NEM-induced MT disassembly *in vitro* and in cells. (Supported in part by an NIH grant, R01-ES 11164.)

87 DOWNREGULATION OF TUBULIN AND MICROTUBULE-ASSOCIATED PROTEINS (MAPS) IN CULTURED LUNG FIBROBLASTS EXPOSED TO CADMIUM.

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Cadmium (Cd) is an established human lung carcinogen and induces severe injury to pulmonary cells. Our previous studies demonstrated that Cd targeted the microtubules (MT) system causing their depolymerization in exposed cells. To further understand the mechanisms of Cd damage to MT, we have examined Cd binding to tubulin proteins, tubulin synthesis and its mRNA expression, and the calcium (Ca)-released MAPs in Cd treated lung fibroblasts (RFL6 cells). ^{109}Cd labeling assays with isotope-pulsed cells showed Cd binding to tubulin proteins isolated by the Vallee's taxol assembly method such that 70-80% of total tubulin-bound radioactivities were associated with the free tubulin fraction while 20-30% remained in the MT polymers fraction. High concentrations of Cd also induced decreases in [^{109}Cd]-labeled tubulin polymers reflecting strong disassembly of MT in these cells. Immunoprecipitation of [^{35}S]-methionine-labeled proteins using an anti-βtubulin antibody revealed a dose dependent inhibition by Cd of newβtubulin synthesis in RFL6 cells. Further, northern blots indicated the inhibition of newβtubulin synthesis was concomitant with downregulation ofβtubulin RNAs in Cd exposed RFL6 cells. In addition, 2-D gel analysis of detergent-extracted and Ca-released cytoskeletal proteins in [^{35}S]-methionine labeled RFL6 cells illustrated significant reductions in MAPs of 53/5.9, 53/6.5, 55/6.3, 56/6.6 and 86/8.5 (apparent MW in kDa/isoelectric point) by 51%, 56%, 61%, 64% and 88%, respectively, in addition to markedly deminished levels ofαandβtubulins in Cd-treated RFL6 cells. In view of the feedback inhibition exerted by free tubulin pools in tubulin synthesis and MT stabilization by MAPs, these results suggest that downregulation of tubulin (at both protein and mRNA levels) as well as MAP proteins may be an important mechanism for further injury to MT following the initial Cd binding to and disassembly of this key cytoskeletal element. (Supported in part by an NIH grant, R01-ES 11164.)

87a CYTOTOXIC POTENTIALS OF NICKEL COMPOUNDS FOLLOWING| *BITAL*|IN VITRO *EXPOSURE OF HUMAN BLOOD LYMPHOCYTES: ROLE OF SPECIATION.*

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Although nickel and its compounds are known to be toxic and carcinogenic, the information regarding the effects of nickel speciation on such toxicities is very limited at present. Therefore, the present investigation was carried out to evaluate the cytotoxic potentials of different nickel compounds using human blood lymphocytes from normal healthy volunteers. Isolated human lymphocytes in RPMI-1640 medium were exposed *in vitro* for 0-4h at 37° C to either nickel carbonate hydroxide (0-1 mM), or nickel subsulfide (0-2 mM), or nickel sulfate (0-4 mM), or nickel oxide (0-6 mM) in an atmosphere of 95% oxygen and 5% CO2. The cytotoxicity (cell death) was evaluated by trypan blue exclusion test. All these nickel compounds showed both concentration- and time-dependent cytotoxic potentials. Furthermore, the extent of such cytotoxicity was found to depend on the chemical form (speciation) of nickel. Thus, after a 4-hr treatment of human lymphocytes the percent of cell death (or, cytotoxicity) were 100, 67.66, 61.33 and 68.36% with 1 mM nickel carbonate hydroxide (2NiCO3.3Ni(OH)2.4H2O), 1 mM nickel subsulfide (Ni3S2), 2 mM nickel sulfate (NiSO4.6H2O), and 6 mM nickel oxide (NiO), respectively. The preliminary results suggest that the cytotoxic potency of

nickel decreases in the following order: nickel carbonate hydroxide > nickel subsulfide > nickel sulfate > nickel oxide. Such cytotoxic potency follows the same order as the nephrotoxic potency of these nickel compounds previously observed in our laboratory (Toxicol. Lett. 122:235-244, 2001).

88 REGIONAL DISTRIBUTION OF URANIUM IN RAT BRAIN.

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It has been suggested that depleted uranium exposure contributed to symptoms of Gulf War Illness. Previous studies have demonstrated that depleted uranium alters hippocampal neuron function and indicate that uranium causes neurotoxicity. However, it is not clear to what extent uranium accumulates in various brain regions, which is likely to determine the pattern of neurotoxicity. The distribution of brain uranium was quantified in male Sprague-Dawley rats that were treated with a single injection of 1 or 10 mg uranium/ kg as uranyl acetate by intraperitoneal injection. To determine if physiological stress has an impact on uranium distribution or accumulation, a subset of animals underwent periods of forced swimming for 5 days prior to uranium injection. The concentration of uranium in serum, hippocampus, striatum, cerebellum, and frontal cortex was determined by ICP-MS at 8 hours, 24 hours, 7 days and 30 days after exposure. Both doses of uranium increased uranium content of all brain regions tested. In vehicle controls, uranium concentrations in cerebellum, cortex, hippocampus, and striatum were 0.18, 0.66, 2.0, and 1.4 ng/g respectively. Twenty-four hours after administration of 1 mg uranium/kg, values for these tissues were 17.8, 2.8, 8.9, and 3.7 ng/g, respectively, while in animals given 10 mg uranium/kg, tissue concentrations were 12.7, 13.3, 19.7, 9.9 ng/g respectively. In both dose groups, stress tended to reduce brain concentrations without markedly affecting serum values. These studies demonstrate that soluble uranium rapidly enters the brain and exhibits regional distribution. (Supported by: US Army Medical Research and Materiel Command DAMD17-01-1-0775. This abstract does not necessarily reflect the position or policy of the US Government.)

89 URANIUM UPTAKE IN RAT BRAIN ENDOTHELIAL CELLS AND THE POSSIBLE LINK TO DIVALENT METAL TRANSPORTER 1.

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Many causes of "Gulf War Syndrome" have been proposed including depleted uranium (DU). Given recent evidence suggesting uranium accumulation in the rat brain, as well as neurobehavioral changes in US veterans retaining DU shrapnel as a result of friendly fire injury, we embarked on a series of studies to identify a putative uranium transporter on blood-brain barrier (BBB) endothelial cells. We tested the hypothesis that transendothelial uranium transport is mediated through divalent metal transporter 1 (DMT1; also known as Nramp or DCT1). We conducted western blot analysis of DMT-1 in an immortalized cell line of the BBB referred to as rat endothelial cells 4 (RBE4). Addition of desferrioxamine (DFO), an iron chelator, to the medium for 24 hours lead to a significant increase in DMT1 expression (264% of control; $p<0.004$). The 15 and 30 minute uptake of uranium (8 mM) in DFO treated RBE4 cells was analyzed with neutron activation analysis (NAA). The studies confirm that RBE4 cells readily take up uranium (0.3 ug University/mg protein). There was no statistically significant difference in the uptake of uranium between the two time periods (15 and 30 min), and an 18.26% decrease in uptake for cells treated with DFO compared to RBE4 cells treated with regular media. The studies corroborate the expression of DMT-1 in RBE4 cells and that its level of expression is modulated by iron levels in the medium. Treatment with DFO upregulates the protein expression of DMT-1 in RBE4 cells, but it is yet to be determined whether this increase is associate with increased uranium uptake. Finally, DMT-1 transfected Chinese Hamster Ovary (CHO) CHO cells have been selected with G4-18 antibiotic and the forward clones express greater DMT-1 protein than reverse or control cells, allowing for future studies on the role of DMT-1 in uranium transport. (Supported by DOD Award 3 DAMD17-01-1-0685).

90 BRAIN REGIONAL UPTAKE OF MANGANESE (Mn) AS AFFECTED BY SUBCHRONIC *IN VIVO* MN EXPOSURE IN SPRAGUE-DAWLEY RATS.

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Mechanism whereby Mn enters the brain remains uncertain. It has been suggested that Mn and/or its binding moiety with transferrin (Tf) may be transported into the brain, presumably by a saturable process. However, little is known about brain regional differences in uptake of Mn. This study aimed to compare properties of Mn transport by various brain regions. Brains of controls or rats received ip.-injections of 6 mg Mn/kg daily for 14 days, were dual-perfused with a Ringer solution containing trace of ^{54}Mn *via* common carotid arteries. Brain areas, e.g., cerebellum (CB), brain stem (BS), choroid plexus (CP), frontal cortex (FC), striatum (ST), and hippocampus (HP), were removed and cerebral capillaries obtained by a capillary depletion method. AAS of tissue Mn showed that Mn levels were significantly increased in all brain regions after Mn exposure with 2.1 fold increase in CP, 3.6 in CB, 4.5 in BS, 4.8 in FC, and 4.9 in ST as compared to controls ($n=8$-12, $p<0.05$). Mn exposure did not alter serum ferritin level, but increased serum transferrin by 16% ($p<0.001$). The temporal pattern of ^{54}Mn uptake in brain capillaries was linear up to 10 min. The uptake volume (Vi) following a 10-min perfusion was 5.01+/-0.09 ml/100g (mean+/-SEM) and 3.26+/-0.55 ml/100g in control and Mn-exposed rats respectively, a reduction of 34%. Accordingly, the uptake by parenchyma of the whole brain at 10 min was reduced from 0.06+/-0.02 ml/100g in controls to 0.02+/-0.001 ml/100g in Mn-dosed animals. Among brain areas studied, the uptake of ^{54}Mn by striatum was significantly diminished by 73% in Mn-treated rats. However, Mn exposure did not significantly alter the influx of Mn to other brain regions including CSF. These results indicate that there appeared to exist a region-specific down regulation of brain influx of Mn as a result of Mn exposure. (Supported in part by ES-08146 and Burrows-Wellcome Foundation)

91 HETEROGENEOUS MANGANESE ACCUMULATION IN THE IRON DEFICIENT DEVELOPING RAT BRAIN IS LINKED TO DIVALENT METAL TRANSPORTER LEVELS.

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Iron deficiency (ID) is a prevalent nutritional disorder affecting nearly 2 billion people worldwide. Recently, our laboratory showed that ID causes increased manganese (Mn) concentrations in several brain regions, with regional accumulation in the hippocampus, caudate putamen, globus pallidus and substantia nigra. We hypothesized that this ID-associated Mn accumulation corresponds with changes in iron associated proteins [transferrin receptor (TfR) and divalent metal transporter (DMT-1)]. Prior studies showed that the brain regions most susceptible to Mn accumulation are those that display the greatest response to ID (i.e., increased transferrin and TfR). Thus, we sought to test our hypothesis by examining brain regional levels of TfR and DMT-1 in rats exposed to dietary ID and varying Mn levels and correlating these changes with regional Mn concentration. Twenty-one day old male Sprague-Dawley rats were fed for 6 weeks one of four semi-purified diets (AIN-93-G), (CN n=7), (ID n=7), (IDMn+ n=7), and (CNMn+ n=7). After 6 weeks of dietary treatment the rats were euthanized, and the brains dissected into seven brain regions. Brain regions were analyzed for [Mn] with neutron activation analysis (NAA), and for DMT-1 and TfR levels *via* immunoblot analysis. While TfR levels are detectable in all the regions tested, DMT-1 is specifically detected in the caudate putamen and globus pallidus and to a lesser extent in the hippocampus and substantia nigra with a distinct band at approximately 66 kDa. ID increases both TfR and DMT-1 in most brain regions, however increases in DMT-1 correlate with ID-associated Mn accumulation in the caudate putamen and hippocampus, but this is not the case for TfR. These data suggest that increases in DMT-1 due to ID may be the likely facilitator in the region-specific Mn accumulation associated with ID. (Supported by NIEHS 10563).

92 INCREASED MANGANESE UPTAKE IN IRON DEPRIVED AND IRON OVERLOADED PRIMARY ASTROCYTE CULTURES IS DUE TO INCREASED DIVALENT METAL TRANSPORTER.

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Neurotoxicity due to excessive brain manganese (Mn) can occur due to environmental (air pollution, soil, water) and/or metabolic aberrations (decreased biliary excretion). The role of astrocytes in managing the levels of extracellular constituents in the brain is critical for normal functioning. Given the putative role of astrocytes in regulating the movement of metals across the blood brain barrier, we sought to examine the relationship between iron status and manganese transport in astrocytes. Furthermore, our study examined the effect of iron status on astrocytic transferrin receptor (TfR) and divalent metal transporter (DMT-1) levels and their relationship to Mn uptake. All experiments were carried out in primary astrocyte cultures after they reached full confluency (approx. three weeks). Astrocytes were incubated for 24 hours in astrocyte growth media (AGM) containing 200 uM desferroxamine (ID), 400 uM iron dextran (Fe3+), 300 uM MnCl2 (Mn2+) or AGM (CN). The next day, five minute 54Mn uptake was measured and protein was harvested from parallel culture plates for DMT-1 and TfR immunoblot analysis. Both

iron deprivation (ID) and iron overload (Fe3+) caused significant increases in 54Mn uptake in astrocytes. As expected, MnCl2 treatment caused a significant decrease in astrocytic 54Mn uptake. TfR levels were significantly increased due to ID and decreased in astrocytes exposed to Fe3+ and Mn2+ treatments. As expected, DMT-1 was increased due to ID and decreased due to Mn2+. Interestingly, DMT-1 levels were also increased due to Fe3+ treatment, but not to the extent of ID. The decreased TfR associated with Fe3+ treatment and the increased DMT-1 levels, suggest that DMT-1 is a likely transporter of Mn in astrocytes. (Supported by NIEHS 10563)

93 MOLECULAR MECHANISM OF MANGANESE-INDUCED DISRUPTION OF IRON TRANSPORT AT THE BLOOD-CSF BARRIER.

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Previous studies indicate that Mn exposure appears to induce a compartmental shift of iron (Fe) from the systemic circulation to the CSF, possibly by acting on Fe transport machinery localized at the blood-brain barrier and/or blood-CSF barrier. Since Mn exposure increases the amount of mRNAs encoding transferrin receptor (TfR), which possesses 3'-UTR stem sloops, we hypothesize that Mn may alter the binding affinity of iron regulatory protein-1 (IRP1) to TfR mRNAs thereby influencing Fe transport at the blood-CSF barrier. A primary culture of choroidal epithelial cells was adapted to grow on a freely permeable membrane sandwiched between two culture chambers to mimic blood-CSF barrier. Trace ^{59}Fe was added to the outer chamber and radioactivity in both inner and outer chambers monitored to determine ^{59}Fe transepithelial transport. Following Mn treatment (100 uM for 3 days), the initial influx rate constant (K_i) was increased from the controls (135 dpm/ml/min) to Mn-treated cells (156 dpm/ml/min), a rise of 16%. Accordingly, the Css of ^{59}Fe was increased by 10%. Mn exposure, however, reduced ^{59}Fe retained in cells by 58%. Gel shift assay using S100 cytosolic extracts indicated that Mn exposure (100 uM) caused a time-related alteration of binding IRPs to stem loop-containing mRNAs; the IRP1-mRNA binding was initially reduced following an 8-hr Mn exposure. It was subsequently increased at 24- and 72- hr exposure interval. The protein-mRNA interaction was reduced after 6-day exposure. Binding of IRP2 to mRNAs was increased following 6 day Mn treatment. Western blot showed a significant increase of cellular TfR in Mn treated group (100 uM) starting at 8 hr treatment. The increase of TfR continued even at 6-day treatment. The data suggest that Mn upon entering the cells appears capable of altering IRPs binding ability to mRNAs with 3' or 5' UTR-stem loops. The disrupted protein-mRNA interaction in choroidal epithelial cells may underlie the increased Fe transport by the blood-CSF barrier. (Supported by NIEHS RO1 ES08146)

94 CHARACTERISTICS OF CARRIER-MEDIATED BRAIN MANGANESE UPTAKE IN ENDOTHELIAL CELL CULTURES AND INTO PERFUSED RAT BRAIN.

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Manganese (Mn) is essential to healthy brain development and function. High brain Mn concentrations are neurotoxic and can lead to manganism, a neuropathy with parkinson-like symptoms. Previous studies indicated that brain Mn^{2+} influx across the rat blood-brain barrier (BBB) is carrier-mediated. This study determined characteristics of $^{54}Mn^{2+}$ uptake to identify the possible transporter(s) at the BBB. It was hypothesized that Mn^{2+} brain influx occurs *via* one or more divalent metal transporters or channels. $^{54}Mn^{2+}$ uptake into bovine brain microvascular endothelial cells (bBMECs) was found to be concentrative after 30 minutes and increased with decreasing proton concentration. Ferrous (Fe^{2+}) iron (100 µM) did not inhibit Mn^{2+} (0.375 µM) influx into rat brain in experiments using the *in situ* brain perfusion method. This suggests that Mn^{2+} brain entry is not solely mediated by the divalent metal transporter 1 (DMT-1), which co-transports protons and for which Fe^{2+} is a competing substrate. Ouabain, a Na^+/K^+-ATPase inhibitor, did not affect Mn^{2+} uptake into bBMECs. Pretreatment with 1 mM vanadate, an inhibitor of P-type ATPases, decreased $^{54}Mn^{2+}$ uptake to 40% of control uptake into bBMECs in a 30-minute incubation. However, vanadate (1 or 10 mM) did not inhibit influx into brain tissue or the lateral ventricular choroid plexus within the short duration (90 or 180 seconds) of *in situ* brain perfusion. These results suggest that vanadate does not directly block the uptake of Mn^{2+} at the BBB. In conclusion, DMT-1 does not appear to be solely responsible for brain entry of Mn^{2+} in the absence of transferrin. A vanadate-sensitive divalent ATPase may play a role in Mn^{2+} brain influx, but does not exclusively mediate Mn^{2+} influx. Supported by Health Effects Institute Agreement #99-10.

95 THE DIVALENT METAL TRANSPORTER-1 IS NOT ESSENTIAL FOR BRAIN MANGANESE UPTAKE.

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Manganese (Mn) is an essential mineral in the brain, however excessive brain Mn is neurotoxic. Maintenance of brain Mn entry might depend on a process that can be regulated. Brain uptake of the Mn^{2+} ion, Mn transferrin and Mn citrate is carrier-mediated. It has been hypothesized that the divalent metal transporter (DMT-1) plays a role in brain Mn uptake, as it has been shown to mediate Mn uptake into DMT-1 transfected oocytes and to be expressed at the blood-brain barrier. To test this hypothesis, brain uptake of ^{54}Mn from an arterial perfusion containing the Mn^{2+} ion or Mn transferrin in ~ 60 day old Belgrade rats was compared to their sex-matched littermates and Wistar rats. Belgrade rats were derived from Wistar rats and are -/- for DMT-1. Their littermates are +/-. Mn in 9 brain regions and the choroid plexus was determined using the *in situ* brain perfusion method. One side of the brain was perfused for 90 seconds with a solution containing 0.375 µM Mn and ~ 2 µCi/ml ^{54}Mn (as the ion or as Mn transferrin) and 1 µCi/ml ^{14}C-sucrose, an indicator of vascular volume and brain entry by diffusion. All rats were maintained on a diet containing 210 mg Fe/kg for 2 weeks prior to study. Brain ^{54}Mn following perfusion with ^{54}Mn ion was not consistently lower in the Belgrade rats than their +/- littermates or the Wistar controls. ^{54}Mn was lower in some brain regions in Belgrade rats after ^{54}Mn transferrin perfusion than their +/- littermates but ^{54}Mn was seen in all brain regions. In summary, the absence of DMT-1 decreased Mn uptake from brain perfusate containing Mn transferrin. Mn was seen in the brain following perfusion of the Mn ion and Mn transferrin in Belgrade rats. These results suggest one or more carriers mediate Mn uptake into the brain that are not DMT-1 or DMT-1 linked. Partially supported by Health Effects Institute Agreement #99-10.

96 MANGANESE CELL CULTURE EXPOSURE PARAMETERS AND THEIR IMPLICATIONS FOR TOXICITY.

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Manganese (Mn) is an essential element that has been shown to be a neurotoxin at elevated exposures. Since Mn is an essential nutrient, studying fundamental mechanisms of toxicity can be difficult. In cell culture models of toxicity these difficulties have been confounded by a range of cell culture conditions (e.g. serum conc.) and manganese concentrations. These differences make inter-study comparisons difficult. To address these difficulties and to make dose-response relationships more relevant, total cellular accumulation should be assessed when doing cell culture. To demonstrate this fundamental consideration, we explored the hypothesis that lower cell culture medium protein levels and increased Mn exposure duration, combined with increases in Mn exposure magnitude would result in greater cellular Mn uptake. PC-12 cells were exposed to $MnCl_2$ (0 to 500µM), for 24 and 48-hours in RPMI 1640 growth medium supplemented with either 5% or 15% fetal bovine serum (FBS). Levels of lactate dehydrogenase (LDH) were measured as an indicator of overt cytotoxicity. Cellular Mn uptake was measured using GFAAS. As predicted, total cellular Mn uptake increased with exposure duration (2-fold at 10µM, and 6-fold at 200µM). The high Mn accumulation following 200µM exposure (e.g., 24hrs, 5% FBS) is attributed to overt cellular dysfunction and cytotoxicity, based on significant increases in medium LDH concentrations at this Mn dose. Cellular Mn levels also increased significantly (4-fold) for 200µM exposure for 24-hours in the 5% FBS supplemented medium compared to the 15% FBS medium. This is consistent with a greater bioavailability of Mn in the exposure medium supplemented with 5% FBS compared to 15% FBS, and is substantiated by a higher relative partitioning of Mn in the <3000mw fraction of exposure medium supplemented with 5% FBS. These data demonstrate the importance of cell culture conditions on the cellular accumulation of toxic metals, and they provide a fundamental framework to facilitate comparisons between studies that used different culture conditions.

97 2-D DIGE PROTEOMIC ANALSYES OF Mn EXPOSURE IN DOPAMINE AND GABA PRODUCING CELL LINES: IMPLICATIONS FOR M*N* NEUROTOXICITY.

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There is substantial evidence demonstrating neurotoxicity of moderate to elevated manganese (Mn) exposures. While several underlying mechanisms of toxicity have been purported (e.g., mitochondrial effects, oxidative stress, dysregulation of cellu-

lar iron), the unequivocal identification of specific mechanisms operating *in vivo* remains unclear. This fact is due, in part, to the complex multi-factoral nature of Mn regulation, functionality, and toxicity within organisms and specific cell types. To better understand the complexity of cellular responses to elevated Mn exposures, we are utilizing a 2-dimensional (2-D) differential in-gel electrophoresis (DIGE) method to evaluate changes in proteome expression patterns in dopamine producing (PC12) and GABA producing (M213) cell types. These cell types were selected, since studies from our laboratory and others have suggested differential susceptibility of dopaminergic versus GABAergic systems to Mn toxicity. The 2-D DIGE approach relies on minimal labeling of cellular proteins from control and treated cells with distinct fluoroprobes (Cydyes, Amersham Biosciences), followed by 2-D electrophoresis on a common gel, and MALDI-TOF analyses. Multiplexing of 2-D gel experiments and the use of in-gel internal standards allows for statistical analyses of replicated treatments. Our preliminary studies (pH 3 to 10 IPG, 12% acrylamide gel) have indicated that Mn exposures (200uM) produce significant changes in protein expression patterns in PC-12 cells, as expected. However, experimental replication and statistical analyses markedly reduced the number of measurably affected proteins. Further analyses across various Mn doses in both PC-12 and M213 cells, and the MALDI-TOF identification of selected proteins are underway. These data will facilitate identification both of specific cellular targets, and changes in protein expression patterns due to elevated Mn exposure, thereby increasing our understanding of Mn neurotoxicity across target cells.

98 CHARACTERIZATION OF NIGRO-STRIATAL DEFICITS IN A MOUSE MODEL OF MANGANESE-INDUCED PARKINSONISM.

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Chronic exposure to manganese (Mn) produces neurological deficits resembling those in idiopathic Parkinson's disease (PD). A mechanistic basis for Mn-induced neurodegeneration is not well established but involves excitoxicity and disruption of mitochondrial function within basal ganglia. The rationale of the present study was to develop a mouse model of Mn-induced parkinsonism to elucidate cellular and neurochemical targets of Mn in the nigro-striatal system. Female C57Bl/6J mice were exposed to saline or MnCl2 (50 or 100 mg/kg/day) by oral gavage once daily for 8 weeks. At the cessation of treatment animals were evaluated for locomotor activity, catacholamine levels, and histopathological changes in the striatum and substantia nigra. Locomotor activity was assessed by open field activity. DA and DOPAC were quantified by HPLC. Serial sections from the striatum and substantia nigra were analyzed for neuronal viability, expression of tyrosine hydroxylase (TH) and glial acidic fibrillary protein (GFAP), and for nitro-tyrosine protein adducts. Striatal DA content was decreased by 26% and 55% in animals exposed to 50 and 100 mg/kg/day, respectively. The ratio of DOPAC:DA increased by 43% in the 100mg/kg/day group. Animals treated with 100 mg/kg/day MnCl2 displayed a 40% decrease in locomotor activity and a 76% increase in margin time. 100 mg/kg/day MnCl2 caused a 55% decrease in nissl-positive neurons in the striatumand a concomitant increase in dying, fluorojade-positive neurons. The expression of GFAP was increased in both the striatum and substantia nigra in animals exposed to 100 mg/kg/day MnCl2 and was paralleled by increased nitro-tyrosine protein adducts. Decreased TH staining was only observed in the striatum of animals exposed to 100 mg/kg/day MnCl2. It is concluded from these studies that Mn-induced depletion of striatal DA levels occurs both from an increase in DA turnover as well as from degeneration of neurons and nerve terminals within the striatum subsequent to glial activation and overproduction of NO.

99 MANGANESE EXPOSURE INDUCES AN INCREASE IN INTRACELLULAR GABA IN IMMORTALIZED RODENT STRIATAL CELLS.

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Chronic elevated manganese (Mn) exposure has been associated with neurological disorders. The introduction of the anti-knock additive MMT to gasoline has been approved in the US, leading to concern about the potential for Mn neurotoxicity in sensitive populations. Published whole animal rat studies from our laboratory have suggested that moderate Mn exposure causes a significant increase in striatal GABA levels, which may represent an important cell/tissue based outcome underlying Mn toxicity. To further explore this hypothesis, we investigated the toxic effects of Mn exposures on two immortalized rodent GABAergic striatal cell lines (M213-2O and M213-2O/clone 2, generously provided by W. Freed, NIH). These cells are similar except that M213-2O/clone 2 additionally contains the human glutamate decarboxylase (GAD)-67 gene. Cells were exposed to $MnCl_2$ (0, 10, 50, and 200 μM) in DMEM:F12 culture medium containing 5% FBS for 24 hours. Lactate dehydrogenase (LDH) activity was measured in the exposure medium, followed by measurement of intracellular Mn concentration, and HPLC analysis of intracellular amino acids γ-aminobutyric acid (GABA), glutamate, glutamine, and aspartate. Results show that only the 200μM Mn dose was cytotoxic, with LDH levels 200-300% of control ($p<0.001$). Intracellular Mn concentrations in M213-2O cells increased from a control level of 0.07±0.01 nmol Mn/mg protein (mean ± SE, n=3) to 0.18±0.01, 0.34±0.01, and 0.66±0.04 nmol Mn/mg protein in 10, 50, and 200 μM Mn exposed cultures, respectively. Consistent with *in vivo* observations, Mn exposure (10 - 50μM) significantly increased GABA levels by 200%±20 and 250%±26 of control in M213-2O and M213-2O/clone 2 cells, respectively. Intracellular Glu, and Asp levels in M213-2O cells increased a significant 170%±8, and 140%±18 of control respectively (10μM Mn dose). These results indicate significant modulation of amino acid metabolism in both cell lines in the presence of Mn concentrations well below levels that induce overt cellular toxicity.

100 THE RELATIVE EFFECTS OF Mn(II) AND Mn(III) ON CELL FUNCTION.

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Chronic elevated manganese (Mn) exposure has been associated with neurological disorders. The introduction of the anti-knock agent MMT to gasoline has lead to concern about the potential for increased Mn exposures and neurotoxicity. Since the combustion products of MMT include Mn-oxides and phosphates of various Mn oxidation states (Mn(II), Mn(III) and Mn(IV)), there is justifiable need to investigate how the oxidation state of Mn exposure mediates toxicity. Therefore, the objective of this study was to evaluate the relative metabolic and toxic effects of Mn(II) and Mn(III) on an established cell line (PC12s). Cells were exposed to Mn(II)-chloride or Mn(III)-pyrophosphate at doses of 0 to 200uM *via* culture medium for 24 hrs. Total cellular Mn concentrations(concs.) were measured *via* GFAAS to quantify the intracellular Mn dose. Cellular function following exposure was evaluated by measuring dopamine (DA) and its metabolites, serotonin (5-HT), and ATP cellular concs. Cell stress was evaluated by measuring lactate dehydrogenase (LDH), total 8-isoprostanes, and cellular levels of ROS enzymes. Results indicate that total cellular Mn increased in a dose-dependent fashion, as expected, although Mn(III) exposed cells accumulated significantly ($p<0.001$) more Mn compared to cells exposed to Mn(II) for a given Mn exposure dose. When compared based on total intracellular Mn concs., Mn(III) led to a significantly greater decrease (<20% of control, $p<0.001$) in 5-HT concs. compared to effects of Mn(II) (~70% of control) at the higher range of exposures. Cellular levels of DA were also reduced by Mn, though the effects of Mn(II) were slightly greater (~50% of control) than the effects of Mn(III) (~70% of control). Other cellular outcomes measured had less dramatic responses. We can surmise from this data that Mn(III) may have a greater effect on cellular function than Mn(II). Consistent with previous studies (Reaney et. al. 2002, Chem. Res. Toxicology.). Furthermore, the effects seen on DA and 5-HT are independent of more broad measures of toxicity and may be specific to Mn in PC12s.

101 MANGANESE AUGMENTS CYTOKINE- AND LPS-INDUCED NO PRODUCTION IN GLIAL CELLS THROUGH ACTIVATION OF MAP KINASE AND NF-κB.

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Activation of glial cells and subsequent production of inflammatory mediators contributes to the degeneration of dopaminergic neurons in idiopathic Parkinson's disease (PD). Injury to the nigro-striatal system in humans chronically exposed to manganese (Mn) involves mitochondrial dysfunction and increased production of nitric oxide (NO), though the mechanisms underlying these phenomena are unclear. We therefore postulated that Mn potentiates lipopolysaccharide- (LPS) and cytokine-induced expression of inducible nitric oxide synthase (iNOS) in glial cells through stimulation of protein tyrosine kinase signaling cascades and activation of the transcription factor NF-κB. Wide field fluorescence microscopy was used to assess NO production and mitochondrial function in live C6 glioma cells. Activation of NF-κB was determined by electrophoretic mobility shift assay using a consensus κB sequence. Expression of iNOS and phosphorylation of p44/p42 mitogen-activated protein kinase (MAPK) was determined by western immunoblotting. Exposure to 100 μM MnCl2 potentiated LPS-induced NO production in C6 cells 1.7-fold compared to LPS alone. The effect of Mn on mitochondrial membrane potential ($\Delta\Psi$mt) and mitochondrial calcium ([Ca2+]mt) levels was not altered by LPS. LPS-induced expression of iNOS was dramatically increased by Mn and required NF-κB (p65/p50) activation. Overexpression of a mutant IκB, the inhibitory subunit of NF-κB, blocked p65/p50 DNA binding, iNOS expression, and production of NO. Exposure to concentrations of Mn as low as 1 μM increased phosphorylation of p44/p42 MAPK and p65. Exposure to 1-10 μM MnCl2 synergistically increased TNFα- and IFNγ-induced MAPK and p65 phosphorylation. It is concluded from these studies that Mn-induced NO production in glial cells involves both direct effects on mitochondrial calcium and activation of MAPK signaling pathways that increases inducible expression of the iNOS gene through an NF-κB-dependent mechanism.

102 TEMPORAL RESPONSES IN THE DISRUPTION OF Fe REGULATION BY Mn.

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Previous work in our lab has shown that acute low level manganese (Mn) exposure interferes with cellular iron (Fe) regulation (Kwik-Uribe et al. 2002, Toxicology. Sciences. 66:204). To expand our understanding of these events, the effect of both Mn dose (1, 10, 50, and 200 uM Mn) and duration of exposure (12, 24, 36, and 48 hrs) on (i) total intracellular and labile iron concentrations, (ii) the cellular abundance of transferrin receptor (TfR), H- and L-ferritin, and mitochondrial aconitase proteins, and (iii) IRP binding activity were systematically evaluated in undifferentiated PC12 cells. While total intracellular Fe concentrations were largely unaffected, the Phen Green SK (Molecular Probes) sensitive labile Fe pool was dramatically increased by exposure to Mn. This increase occurred in concert with changes in the cellular abundance of TfR, ferritin, and mitochondrial aconitase, protein changes consistent with an Fe deficiency response which favored Fe uptake and increased intracellular availability. In addition, an analysis of IRP binding activity and abundance demonstrated that Mn could alter the dynamics of IRP-1 binding and the intracellular abundance of IRP-2. Notably, significant effects were observed as early as 12 hrs exposure and at Mn doses as low as 1uM. The overall pattern of effect suggests that Mn produced an inappropriate cellular response akin to Fe deficiency that the cells were able to mount a compensatory response, while the highest Mn dose (200uM) produced an Fe deficiency response that the cells were unable to compensate for. Together, these data show that dose and exposure duration significantly impact cellular Fe regulation, supporting that this dysregulation may be one contributory mechanism underlying Mn neurotoxicity.

103 INFLUENCE OF SUBACUTE AND SUBCHRONIC MANGANESE SULFATE ON GLIAL FIBRILLARY ACIDIC PROTEIN.

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The potential of manganese sulfate (MnSO4), given daily by gavage for either 14 or 90 days, to alter the glial fibrillary acidic protein (GFAP), was studied in rats as an early sign of neurotoxicity. Male and female rats were randomly given suspensions containing 0.1, 1.0, 10, 30, or 100 milligrams of manganese/kg or the vehicle control. GFAP were performed with ELISA on the hypothalamus and corpus striatum. The results show that 10, 30, and 100mg/kg significantly elevated the GFAP levels in the hypothalamus of male rats after two weeks of daily gavage. Furthermore, the GFAP levels in the hypothalamus of male rats after 90 days of daily gavage with 30, and 100mg/kg were still significantly elevated relative to rats injected with 6mg/kg midazolam or 1mg/kg manganese. In female rats, 30 and 100mg/kg of manganese significantly elevated the GFAP levels in the hypothalamus after two weeks of daily gavage relative to rats injected with 6mg/kg midazolam. In contrast, the GFAP levels in the hypothalamus of female rats were not altered by 90 days of daily gavage with MnSO4. In addition, neither 14 nor 90 days of daily gavage with MnSO4 altered the GFAP levels in the corpus striatum of male and female rats. The above results indicate that an early sign associated with neurotoxicity, activation of glial cells in the hypothalamus, could be produced by just two weeks of oral exposure of 10 to 30 mg/kg manganese (from MnSO4), but not in the corpus striatum of rats. (Support by ATSDR grant U50/ATU398948).

104 DIFFERENTIAL INDUCTION OF DNA REPAIR CAPACITY AFTER ARSENIC EXPOSURE ALLOWS FOR REGIONAL SPECIFIC ACCUMULATION OF DNA DAMAGE.

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Although exposure to arsenic has been primarily associated with the incidence of skin, lung, and bladder cancers, recent reports indicate that exposure to arsenic may have detrimental effects on CNS function. Chronic consumption of arsenic causes defects in operant learning in adult and young rats, alters locomotor activity, and changes in brain neurochemistry. Arsenic, by virtue of its reactivity with sulfhydryl groups of enzymes involved in cellular respiration, can alter the redox status in the cell causing free radical accumulation with the subsequent damage to cell macromolecules. Cells have in place a set of defense mechanisms to ameliorate the nocive effects associated with elevated free radicals. Amongst these, DNA repair mechanisms have been shown to up-regulate in specific brain regions after induction of oxidative stress. The objective of this study was to evaluate the status of oxidative damage to DNA, assessed by determining the levels of 8-hydroxy-2-deoxyguanosine (8-oxodGuo), and the activity of the DNA repair enzyme 8-oxoguanine DNA glycosylase 1 (Ogg1), responsible for removing the 8-oxoGuo DNA lesion, in specific brain regions after chronic exposure to arsenic. Mice were given sodium arsenite in the drinking water for a period of 20 weeks. The levels of 8-oxodGuo were found to be elevated in hippocampus and cortex but not in cerebellum of animals exposed to arsenic. Arsenic exposure also led to a region-specific induction in the activity of Ogg1. Region specific elevation of Ogg1 activity was in inverse relationship to the regional specific elevation of 8-oxoGuo. These results could help explain the intrinsic factors that would enhance the vulnerability of specific neuronal populations, and shed light in the molecular events associated with toxic effects of arsenic to the CNS. Supported by: NSF-EPSCoR (EPS-0091995)

105 DIMERCAPTOSUCCINIC ACID IS INEFFECTIVE IN THE TREATMENT OF ACUTE THALLIUM POISONING.

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Introduction: Despite being banned as a pesticide, thallium still results in human and animal poisonings. Current recommended treatments include the use of the chemical Prussian Blue. Limitations in its availability may result in Prussian Blue not being obtainable in the thallium poisoned patient. The chelator 2, 3-Dimercaptosuccinic acid (DMSA) is currently FDA approved for use in childhood lead poisoning and has been reported to be beneficial in treating other heavy metal poisonings. The objective of this study was to determine the efficacy of DMSA as a treatment for thallium poisoning by studying reduction in mortality and whole brain concentrations in thallium poisoned rats. Material and Methods: Rats were gavaged with 30 mg/kg of thallium. After 24 hours they were randomized to DMSA (n=20) 50 mg/kg twice daily for 5 days, Prussian Blue (n=20) 50 mg/kg twice daily for 5 days or control (n=30). Animals were monitored twice daily for weight loss and mortality. Animals losing greater than 20% of their starting weight were euthanized and counted as a mortality. All surviving rats at 120 hours had their brains harvested and digested and underwent subsequent thallium analysis *via* atomic absorption spectrophotometry. Results: There was no difference in the rate of survival (45% vs 21%, p=0.07) or mean whole brain thallium concentrations (3.4 vs 3.0 μg/g, p=0.06) between DMSA and control rats Prussian Blue treated rats had significantly improved survival (70% vs 21%, p <0.01) and lower whole brain thallium concentrations (1.6 vs 3.0 μg/g, p<0.01 tissue) compared to controls. Conclusion: DMSA is ineffective in the treatment of acute thallium poisoning in rats.

106 DIFFERENT RESPONSES OF NEURONS AND GLIA TO Cu ELEVATION IN ROS GENERATION.

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Copper (Cu) is an essential trace element in the development and function of the central nervous system (CNS). Its high redox potential is a potential cause of cytotoxicity in various neurodegenerative diseases in which Cu homeostasis is disrupted, including Alzheimer's disease. Disturbances of reactive oxygen species (ROS) homeostasis are also associated with these diseases. Neurons and glia are two main types of cells in the CNS and may have different responses to Cu elevation due to their different structural and functional properties. In this report, we used human SY5Y neuroblastoma cells as a neuronal model and human CCF-STTG1 astrocytoma cells as a glial model. Both cell lines were exposed to CuSO4 (0-10 μM) in serum-free medium and ROS was measured with pro-fluorescent molecules. Data showed that glial ROS significantly increased by 50% when cells were exposed to 10 μM Cu for 1 hr and neuronal ROS showed no change at any Cu concentration tested. Similar results were observed in rat C6 glioma and PC12 cells. To address this difference, expression of genes that contribute to Cu buffering capacity (ATP7A and hCtr1) and superoxide dismutation (Mn-SOD) was profiled by quantitative PCR. Both SY5Y and CCF-STTG1 cells had the same level of hCtr1 expression. However, SY5Y had much higher ATP7A expression than did CCF-STTG1. In contrast, CCF-STTG1 had higher Mn-SOD expression than SY5Y cells. These data suggest that glia are more sensitive to Cu elevation than are neurons in ROS enhancement. Furthermore, ATP7A and Mn-SOD probably play roles in Cu-induced ROS generation.

107 AGE-RELATED VASCULAR CHANGES IN THE CEREBRAL WHITE MATTER OF DOGS.

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Dogs are known to share a variety of morphological age-related brain changes with humans: the changes include neuronal loss and amyloidosis in the cerebral cortex, dystrophic axonal changes and ubiquitin deposition in the cerebral white matter.

Degenerative changes in the white matter appear to be more prominent than those in the cortex. Based on our recent observation of iron accumulation and ferritin immunoreactivity in the aged dog brain, we suspected that iron-induced oxidative injury may be related to pathogenesis of the severe age-related morphological changes in the cerebral white matter. In this study, we performed immunohistochemical analysis using antibodies against laminin and fibrinogen to determine whether age-related morphological changes of blood-brain-barrier (BBB) occur in the white matter, resulting in an exudation of serum materials (*ie.* iron) which may cause oxidative injury. In addition, we examined iron deposition and astrocytic reaction for oxidative stress with the use of Prussian blue DAB and post DAB enhancement (PBD-PDE) method and superoxide dismutase (SOD) immunohistochemistry (IHC). Brains from 9 young dogs, ranging in age from 6 months to 5 years, and 22 old dogs from 10 to 18 years, were used. In young dogs, there were no significant findings in histological and immunohistochemical examinations. In contrast, a decrease of laminin immunostains was shown in the basement membranes of some capillaries and venules in the white matter of aged dogs. Fibrinogen IHC showed positive immunoreactions in some macrophages accumulating in perivascular spaces and some astrocytes. PBD-PDE method technique for iron demonstrated positive stains in some macrophages and astrocytes. There were some astrocytic processes positive for SOD in the white matter. These results suggest a possibility that a collapse of vascular basement membranes may occur during aging, leading to a disturbance of BBB function and thereby to perivascular exudation of serum materials including an iron. The vascular changes may be related to severe degenerative changes in the white matter.

108 HEAVY METALS AND PCBS PROMOTE BETA-AMYLOID AGGREGATION AND ITS CYTOTOXCITY IN PC12 CELLS.

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Alzheimer disease (AD) is a gradual and irreversible, progressive neurodegenerative disorder which is characterized with the accumulation of amyloid fibrils. The major constituents of amyloid fibrils in the AD brain are 39-43 amino acid amyloid β (Aβ) peptides, which are protein fragments snipped from a larger protein called amyloid precursor protein (APP). The amyloid hypothesis states that Aβ deposition is a major causative factor for the onset of AD. This is further supported by other studies, which showed that Aβ is toxic only when it is aggregated. The factors that cause the aggregation of Aβ are not clearly known, however it has been proposed that exposure to environmental toxic agents such as heavy metals and persistent organic pollutants may exaggerate the aggregation of this peptide. The present study is aimed to screen the role of various environmental agents, which could influence Aβ aggregation. Synthetic Ab peptide (1-40) was dissolved in urea, diluted with PBS (pH 7.4) and incubated in the presence of 0, 0.1 μM, 1 μM and 10 μM of Ba/Hg/Pb/Zn/PCBs. Peptide aggregation was studied by fluorometric determination with bis-ANS as well as measuring the sedimentation of the aggregated peptide by a protein assay. The protein assay showed that Pb, PCBs and Zn significantly promoted the aggregation of Aβ, whereas Ba and Hg had minimal effects. Similar results were obtained with the bis-ANS studies. Control studies conducted using both techniques and another protein, bovine serum albumen (BSA), showed non-significant effects on aggregation. Furthermore, the cytotoxicity of the Abeta peptide in the presence of these toxicants was investigated in PC12 cells. It was observed that toxicants that promoted peptide aggregation also enhanced the cytotoxicity of Aβ. These findings suggests that environmental agents are potential risk factors for the development of AD by promoting the aggregation of Abeta and/or enhancing its cytotoxicity.

109 ALUMINUM MALTOLATE-INDUCED CYTOTOXICITY IN NEURO-2a CELLS INVOLVES APOPTOSIS AND NECROSIS.

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Aluminum maltolate (Al-malt) has been shown to cause neurodegeneration following *in vivo* exposure and apoptosis plays a prominent role. The objective of this study was to define the mode of cell death induced by Al-malt. Neuro-2a cells were treated with Al-malt for 24 h in the presence or absence of pretreatment with a variety of pharmacological agents. Al-malt concentration-dependently increased cell death as indicated by lactate dehydrogenase (LDH) release and the appearance of cells with hypodiploid DNA content. Flow cytometry using acridine orange (high-live, low-apoptotic) and ethidium bromide (necrotic) dual staining showed that the mode of cell death was a combination of apoptosis and necrosis. Treatment with Al-malt resulted in caspase 3 activation and the externalization of phosphatidyl serine, both indicative of apoptosis. In addition, nuclear condensation and fragmentation were evident in Hoechst 33258 stained nuclei from Al-malt-treated cultures. Interestingly, pretreatment with cyclohexamide (CHX) markedly reduced Al-malt-induced apoptosis indicating that this mode of death is dependent on *de novo* protein synthesis in the current culture model. Necrotic cell death was not prevented by CHX suggesting that only apoptosis was dependent on gene expression. Pretreatment with antioxidants and kinase inhibitors (mitogen activated protein kinases and PKC) did not reduce Al-malt toxicity suggesting independence from oxidative stress and major kinase signaling pathways. The results provide insight into the mechanisms of Al-malt neurotoxicity and support the involvement of this metal in neurodegeneration.

110 ALUMINUM-INDUCED NEURODEGENERATION INVOLVES DIFFERENTIAL REGULATION OF PROINFLAMMATORY CYTOKINE AND NEUROTROPHIN GENE EXPRESSION.

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The etiology of human neurodegenerative diseases including Alzheimer's disease (AD) is exceedingly complex and our understanding of the mechanisms involved is far from complete. The experimental neurotoxicology of aluminum has been shown to recapitulate virtually every pathophysiological feature of AD and therefore represents a useful model to study the mechanisms involved in neurodegeneration. The present study investigated the effects of aluminum maltolate (Al-malt) on the delicate balance that exists between pro-inflammatory cytokines and neurotrophins using primary brain rotation-mediated aggregate cultures. Aggregates were treated with Al-malt (5-150 μM) on day 15 *in vitro* for 72 h. Cell death increased in a time- and concentration-dependent manner reaching significance in aggregates treated with 150 μM Al-malt in 48 h and 50 μM by 72 h. Analysis of gene expression at 72 h revealed a concentration-dependent increase in tumor necrosis factor α and macrophage inflammatory protein-1α suggestive of a state of inflammation. In contrast, a dramatic concentration-dependent decrease in the expression of nerve growth factor (NGF) and brain derived neurotrophic factor was observed. In fact, NGF expression could not be detected in aggregates treated with 50 and 150 μM Al-malt. These changes in gene expression correlated with a decrease in aggregate size and an increase in neurodegeneration as indicated by Fluoro-Jade B staining. The results indicated a differential regulation of pro-inflammatory cytokines and neurotrophins in brain tissue following treatment with Al-malt. Such findings provide insight into the possible involvement of deregulation of the cytokine/neurotrophin balance in the etiology of neurodegeneration.

111 ALUMINUM IN DRINKING WATER PROMOTES NEURO-INFLAMMATORY INDICES *IN VIVO*.

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Several epidemiological studies indicate a positive relationship between the incidence of Alzheimer's Disease (AD) and aluminum (Al) concentration in drinking water. However, others have not found such an association and the controversy regarding the role of aluminum in AD is still ongoing and unresolved. Existing evidence suggests that Al is capable of causing inflammation systemically and in the central nervous system. In order to determine if a biologically relevant amount of Al is capable of producing neuroinflammation *in vivo*, we exposed mice for 10 weeks to Al lactate (0.01, 0.1, 1 mM) in drinking water. The pro-inflammatory cytokine TNF-α was increased in the brain of mice treated with the highest dose of Al lactate. The inflammation related transcription factor NF-κB was increased in the mice brains dosed with the lowest concentration of Al. NF-κB activation was not observed at the highest Al dose. This may be due to homeostatic processes which down regulate gene expression of pro-inflammatory elements by negative feedback. No effect on these inflammatory markers was seen in the liver or serum. Therefore, the effects of Al observed were not due to a systemic response, but rather, suggest a selective neurological reaction.

112 EFFECTS OF ALUMINUM ON MEMBRANE PROPERTIES AND BIOGENIC AMINE METABOLISM IN RESTING PC-12 CELLS.

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Effects of aluminum (Al) on membrane properties of excitable cells are not fully understood. Evidence suggests that oxidative stress and membrane alterations play important roles in Al neurotoxicity. The present study was conducted to investigate

the relationship between membrane effects of Al and altered neuronal function, namely biogenic amine metabolism. Resting rat pheochromocytoma (PC-12) cells, a model that exhibits neuron-like properties, were used. Treatment of PC-12 cells with Al (>0.01 mM) resulted in a concentration-dependent decrease in membrane fluidity. Similar concentrations of Al increased the rate of extracellular acidification, measured by a cytosensor microphysiometer, indicating stimulation of proton extrusion from cells. Al caused a rapid and concentration-dependent hyperpolarization of the cell membrane as determined by decreased fluorescence of a potential-sensitive dye, *bis*-[12]trimethine oxonol [$Dibac_4$(3)]. These membrane alterations corresponded with an increased production of reactive oxygen species, indicated by increasing dihydrorhodamine 123 oxidation. We previously demonstrated a selective decrease in hypothalamic DA and metabolites in mice treated with different concentrations of Al in drinking water for 4 weeks. Therefore, it was desirable to relate the observed alterations in membrane properties caused by Al to metabolic function of PC-12 cells, a model previously employed for neurotransmitter synthesis and release. Incubation of PC-12 cells with up to 3 mM Al for 6 h caused no alterations in the production or release of neurotransmitters or their metabolites. Results indicated that acute exposure to Al modifies membrane properties of neuron-like cells but had no effect on neurotransmitter biogenic amine pathways under the current treatment conditions.

113 VANADIUM INHALATION INDUCES NEURONAL ALTERATIONS IN *CORPUS STRIATUM*. AN EXPERIMENTAL MODEL IN MICE.

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Metals air pollution is a persistent problem world wide. In Mexico City, and maybe in other areas with high gasoline consumption, Vanadium (V) may be increased in air suspended particles. This element is more toxic when inhaled. Very little information is accessible about V and Nervous system modifications. Only relation of V exposure with a decrement in the catecholamines and with depression has been suggested. Here we report neuronal morphological changes found in an inhalation model in mice, looking for changes in those areas in the nervous system with high concentrations of catecholamines, such as the corpus striatum. CD-1 male mice 35g were exposed by inhalation to 0.02 micrometers V2O5, for 4 weeks, two hours twice a week. Mice were sacrificed after the 2nd, 4th, 6th, and 8th inhalation. The striatum was placed in Golgi stain for cytological analysis which consisted in counting the dendritic spines in 10 dendrites of 20 medium spiny neurons. Our results showed that there was a significant reduction of dendritic spines after the exposition during the different times; we found that the exposed groups had almost the half of dendritic spines than the control group. Our data reveal that the inhalation of this element causes severe neuronal damage in the corpus striatum.

114 DIBROMOACETIC ACID-INDUCED ELEVATIONS OF ESTRADIOL IN BOTH CYCLING AND OVARIECTOMIZED/ESTRADIOL-IMPLANTED FEMALE RATS.

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Haloacetic acids are one of the principal classes of disinfection by-products generated by the chlorination of municipal drinking water. Two members of this class, dibromoacetic acid (DBA) and dichloroacetic acids, have been reported to affect sperm production and gonadal hormonal activity in the male rat. In females, 2 wk oral exposures have been found to disrupt estrous cyclicity, and an alteration in estradiol (E2) concentrations may underlie the effect. Using ovariectomized (OVX) Sprague-Dawley rats implanted with E2 capsules over the final 3 days of a 2 wk oral exposure to DBA (0-270 mg/kg), dose-related elevations in serum E2 were present that accompanied a reduction in the daily induced surge of luteinizing hormone seen in this type of protocol. For an evaluation of intact, regular 4-day cycles, females were again gavaged for 2 wks, this time at doses (0, 30, 60 or 120 mg/kg, in water, pH adjusted to 6.8) unlikely to impair estrous cyclicity. Over the last 4 days of dosing, daily tail nick samples of blood from each rat (1400-1500h) showed that while serum E2 on diestrus and proestrus were comparable to controls, rats in the 60 and 120 mg/kg groups had E2 levels that remained elevated on vaginal estrus instead of exhibiting the marked fall typical of 4-day cycles. Results from both intact and OVX rats also suggest that over time the extended elevations of E2 could have contributed to the disruptions in cyclicity previously noted. To explore further whether the elevation in E2 seen in the OVX/E2-implanted rats was due to an impairment in E2 metabolism, females were gavaged with DBA (0 or 270 mg/kg) for 2 wks. Over the final 3 days, they were given phenobarbital (PhB) either in the drinking water (0.1%) or by IP injection (20 mg/kg) to increase hepatic E2 metabolism. Neither PhB route caused a lowering of the DBA-induced E2 elevations, suggesting that the effects on E2 were linked to an alteration in clearance of the hormone or a suicide inhibition in hepatic P450 activity. (This abstract does not necessarily reflect EPA policy.)

115 THE EFFECTS OF CYCLOPHOSPHAMIDE (CPA) ON THE SYNTHESIS OF GLUTATHIONE (GSH) IN RAT OVARIES.

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GSH, the most abundant non-protein thiol in mammalian cells, plays many important roles, including cytoprotection against exogenous and endogenous toxicants. CPA is a widely used chemotherapeutic drug that causes premature ovarian failure in many women who are treated with it. Although the precise mechanism by which CPA causes ovarian failure is not known, CPA's active metabolites are conjugated by GSH. Therefore, this study examined the effects of CPA on GSH synthesis in rat ovaries. It was hypothesized CPA would deplete ovarian GSH and cause a compensatory upregulation of protein and mRNA expression of the rate limiting enzyme in GSH synthesis, glutamate cysteine ligase (GCL). Adult female rats were injected intraperitoneally with 300 mg/kg or 50 mg/kg of CPA in 0.9% saline with 10% DMSO or saline/DMSO alone on the proestrous stage of the estrous cycle, as determined by vaginal cytology. The animals were sacrificed 24 hours later and ovaries were extracted. One ovary from each animal was used for measurement of total ovarian GSH by a modified Tietze method. The other ovary was used for analysis of either protein or mRNA levels of the catalytic or modulatory subunits of GCL (GCLc and GCLm), by Western or Northern blot, respectively. Total ovarian GSH levels decreased significantly with increasing CPA dose (p=0.009). GCLc mRNA increased non-significantly with increasing dose. GCLc and GCLm protein levels were not affected by CPA administration. These results suggest that the ovary is not able to up-regulate GSH synthesis in the face of CPA depletion of ovarian GSH. GSH depletion may play a role in the ovarian toxicity of CPA. Supported by NIEHS K08-ES10963-01 and the Center for Occupational and Environmental Health, UC Irvine.

116 CIGARETTE SMOKE EFFECTS HAMSTER OOCYTE CUMULUS COMPLEX PICKUP RATE, ADHESION, AND CILIARY BEAT FREQUENCY.

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Maternal smoking is associated with adverse effects on reproduction. Women who smoke are at increased risk for decreased fertility and ectopic pregnancy. Our lab has shown that the hamster oviduct is a target of cigarette smoke and that oocyte pick-up rate (OPR) is decreased after acute *in vitro* exposure to mainstream or sidestream smoke. Oocyte pick-up requires both ciliary beating and adhesion between the oocyte cumulus complex (OCC) and oviductal cilia. Since inhibition of OPR cannot be explained by changes in ciliary beat frequency (CBF) alone, the purpose of this study was to test the hypothesis that cigarette smoke decreases OPR by altering adhesion between the OCC and oviductal cilia. An assay, developed to measure adhesion between the OCC and oviduct, was used in conjunction with *in vitro* assays to measure OPR and CBF. Experiments were done to determine the effect of mainstream and sidestream whole, gas, and particulate smoke solutions on OPR and adhesion before and after exposure of OCC or oviducts. CBF was measured before, during, and after oviducts were exposed. Results showed that all smoke solutions except sidestream particulate decreased OPR and increased adhesion when OCC were treated. All six types of smoke solutions decreased OPR and increased adhesion after oviducts were exposed. Mainstream whole had no effect on CBF, however, both mainstream gas (MSG) and particulate (MSP) decreased CBF during exposure. MSG, but not MSP, recovered to control levels after exposure. All sidestream smoke solutions increased CBF during exposure, and sidestream whole and gas recovered to control levels after exposure. The results show that pretreatment of either the OCC or the oviduct with mainstream or sidestream smoke solutions increases adhesion and that decreases in OPR correlate more closely with increases in adhesion than with changes in CBF.

117 LOCALIZATION OF GLUTAMATE CYSTEINE LIGASE CATALYTIC SUBUNIT (GCLC) PROTEIN IN IMMATURE RAT OVARY AFTER TREATMENT WITH PMSG.

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Glutathione (GSH) is an antioxidant tripeptide that may play a protective role in ovarian follicles. We have previously observed that GSH and protein levels of the catalytic and modulatory subunits of the rate-limiting enzyme in GSH synthesis,

GCLc and GCLm, increase in immature rat ovaries after treatment with pregnant mare's serum gonadotropin (PMSG) in saline. The objective of this study was to localize GCLc protein in the rat ovary using immunohistochemistry. We hypothesized that gonadotropin treatment increases ovarian GSH synthesis by stimulating expression of GCLc protein in growing follicles. Six 25-day-old female Spraque Dawley rats were treated with 0.9% saline (n=3) or 10 IU PMSG in saline (n=3) subcutaneously. Animals were euthanized and both ovaries were dissected out at 24 hours after treatment. Immunohistochemistry with a polyclonal rabbit anti-GCLc antibody was performed on every 4th 8μm section of one ovary, and total GSH concentrations were measured in the other ovary using a modified Tietze assay. As has been previously reported, there were significant increases in ovary weight and GSH levels in PMSG-treated ovaries compared to saline-treated ones. Also, as expected, there were more large antral follicles in PMSG-treated ovaries. The GCLc staining was strongest in granulosa and theca cells of follicles in both saline and PMSG-treated groups. Moreover, there was a trend towards positive GCLc staining in a higher proportion of follicles in PMSG-treated ovaries than saline-treated. The trend was similar when follicles were categorized by type (primary, secondary, and antral). These results support the hypothesis that gonadotropin increases ovarian GSH synthesis by increasing GCLc subunit protein levels in granulosa cells of growing ovarian follicles. (Supported by NIEHS K08-ES10963-01 to UL).

118 BENZYL ISOTHIOCYANATE INDUCED FUNCTIONAL ABERRATION OF ISOLATED UTERINE STRIPS.

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Benzyl isothiocyanate (a chemopreventive phytochemical found in cruciferous vegetables) has also been used as an antibiotic for the treatment of urinary tract infections (UTI). In order to evaluate the potential hazard of benzyl isothiocyanate (BITC) on parturition, we studied its effect on uterine activity *in vitro*. BITC (5 mM) caused time dependent modulation of spontaneous contractions of myometrial biopsy specimens obtained from women undergoing lower segment caesarian sections (with approval). The modulating effect of BITC on human myometrial contractions was characterized by progressive reduction in the amplitude and at the same time, increase in the frequency and tone of contractions leading to irreversible uterine quiescence within 2 hours. Furthermore, on isolated whole uterus of non-gravid rats, BITC (10-320 $_{\mu}$M) caused irreversible, concentration-dependent inhibition of spontaneous, prostaglandin $F_{2\alpha}$ (5 $_{\mu}$g/ml) and oxytocin (10 mU/ml) induced contractions of the uterus. At 20 $_{\mu}$M of BITC, slight increase in the contractile tones was observed. Pre-incubation of the rat uterus in Ringer Locke solution containing 100 $_{\mu}$M of BITC (at 37^0C; gassed with carbogen for 1 hour) before suspension in tissue baths also caused significant depression of the maximum force of spontaneous and KCl (60mM) induced uterine contractions relative to the control (P<0.01). Cryosections of BITC-treated rat uterus (hematoxyline and eosin stained) examined under light microscope revealed structural disintegrity with marked cytoplasmic vacuolation. BITC- induced degenerative changes in the uterus could hence be responsible for the altered uterine functions. The present findings suggest that BITC could modify spontaneous and drug-induced uterine contractions. Should BITC alter uterine functions *in vivo*, normal/drug-induced labor will be impaired. Though the normal level of BITC in vegetables is believed to have no ill effect, concentrated BITC in dietary supplements or as a remedy for UTI during pregnancy could have deleterious effect on parturition.

119 NEONATAL ESTROGENIZATION INDUCES HYPOSPADIA AND INFERTILITY IN FEMALE RATS.

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Emerging data from studies in both humans and experimental animals suggest that *in utero* or neonatal exposure to estrogens may induce adverse effects on later reproductive function in the mature female. The present study was designed to determine the effects of neonatal exposure to a model estrogen on fertility in female rats. Female Sprague-Dawley rats received subcutaneous injections of 25 ug estradiol benzoate (EB) at 1, 3 and 5 days of age. Rats were observed throughout juvenile development and into adulthood to identify gross alterations in reproductive anatomy. Fertility in adult female rats receiving neonatal EB was then compared to fertility in age-matched controls. Control and neonatally estrogenized females were individually co-habitated with untreated males for up to 8 weeks. After mating, all females were allowed to undergo natural parturition; dams that failed to deliver pups by gestation day 25 were evaluated for the presence of implantation sites. 29/29 female rats receiving neonatal EB exhibited hypospadia or malpositioning of the urethral opening. In comparison to age-matched controls, female rats receiving neonatal EB demonstrated significant decreases in the number of successful matings (defined as the number of pregnancies), and decreased fertility index. 28/30 mated female controls demonstrated live implants and successfully delivered pups. By contrast, 0/29 female rats that received neonatal EB delivered live pups. No implantation sites were present in any neonatally estrogenized female rat, suggesting a failure in the process of conception. Infertility in neonatally estrogenized females could result from hypospadia or other anatomic alterations in the genitalia; these alterations could interfere directly with mating and/or fertilization. Alternatively, the effects of neonatal estrogenization on fertility in adult animals could be secondary to long-term alterations in estrus cyclicity or other hormonal mechanism. [Supported by N01-CN-15110 from the National Cancer Institute.]

120 IDENTIFICATION OF COMPOUNDS IN CIGARETTE SMOKE THAT INHIBIT HAMSTER OVIDUCTAL FUNCTIONING.

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Our past studies have shown that chemicals in cigarette smoke inhibit oviductal functioning *in vivo* and *in vitro*. The purposes of this study were to identify the individual toxicants in cigarette smoke solutions that inhibit oocyte pickup rate, ciliary beat frequency, and infundibular smooth muscle contraction and to determine their effective doses using *in vitro* bioassays. Solid phase extraction and gas chromatography-mass spectrometry were used to identify individual chemicals in the mainstream and sidestream cigarette smoke solutions that were active in the above assays. Pyridines, pyrazines, indoles, quinolines, and phenols were identified in the solutions of mainstream and sidestream cigarette smoke. Commercially available standards of the identified compounds were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The lowest observable adverse effect level and efficacy were determined for each compound using the oocyte pickup rate, ciliary beat frequency, and infundibular muscle contraction assays. Previously, we have shown that several pyridine compounds including 2-methylpyridine, 4-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, and 4-vinylpyridine were inhibitory at picomolar concentrations in all three bioassays. Further studies have shown that compounds in the pyrazine group: 2-methylpyrazine, ethylpyrazine, 2-methoxy-3-methylpyrazine, 2, 5-dimethylpyrazine, and 2, 3, 5-trimethylpyrazine, were inhibitory in pico or nanomolar doses. Both quinoline and isoquinoline were inhibitory in picomolar doses. 5-Methylindole showed inhibition in the nanomolar range. Indole, which is found in large quantities relative to other compounds in the smoke, showed inhibition at 10^{-15}M. The phenolic compounds were not as inhibitory as the other classes of compounds in the bioassays, although hydroquinone and 4-ethylphenol were inhibitory at nanomolar doses. This work is important because it shows that very low doses of cigarette smoke components significantly inhibit proper oviductal functioning raising questions regarding the safety of these compounds.

121 FOLLICLE GROWTH IN THE MOUSE OVARY IS MEDIATED BY THE ARYL HYDROCARBON RECEPTOR.

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The ligand-activated aryl hydrocarbon receptor (AhR) binds various environmental contaminants, mediating toxic outcomes. A physiological role for the AhR has remained elusive, though AhR deficient mice (AhRKO) have helped to better understand its mechanisms. In the reproductive system, a lack of AhR results in mice with reduced litter sizes, and difficulties rearing pups to weaning and maintaining conceptuses. Previous laboratory studies have shown that the AhR regulates folliculogenesis, with AhR deficiency causing fewer preantral and antral follicles, without changes in primordial and primary follicle numbers in adult life. Our hypothesis is that AhR deficiency may cause fewer preantral and antral follicles by slowing follicular growth and/or inducing atresia, contributing to reduced ovulatory capacity from follicular growth changes. Follicular growth was compared in wild type (WT) and AhRKO ovaries using morphometric techniques and by measuring the ability of the ovaries and follicles to respond to pregnant mare's serum gonadotropin (PMSG). Atresia was compared using morphometric techniques, TUNEL assays, and 3' end-labeling of fragmented DNA. In addition, ovulation in WT and AhRKO mice was compared by assessing the number of corpora lutea per ovary. Our results indicate that follicle growth and ovulation were reduced in AhRKO ovaries compared to WT ovaries. AhRKO ovaries exhibited a 1.5-fold decrease in number of preantral and antral follicles between postnatal day (PND) 32 and PND 45, were less responsive to PMSG, and contained fewer corpora lutea than WT ovaries. No significant differences in follicular atresia or apoptosis were found in WT and AhRKO ovaries. These data suggest that the AhR regulates preantral and antral follicle growth and ovulation, but not atresia of preantral and antral follicles in the mouse ovary. (Supported by NIH grants HD38955, T32 ES07263-13, and ES01332).

122 GENE EXPRESSION PROFILE INDUCED BY 17-α-ETHYNYL ESTRADIOL IN THE PRE-PUBERTAL FEMALE REPRODUCTIVE SYSTEM OF THE RAT.

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The various effects of 17 β-estradiol in the reproductive and other systems, are mediated mostly by the regulation of temporal and cell-type specific expression of different genes. To better understand the molecular events associated with the activation of the estrogen receptor (ER), here we have used microarray technology to determine the transcriptional program and dose-response characteristics of exposure to 17 α-ethynyl estradiol (EE), in the reproductive system of pre-pubertal rats. Changes in patterns of gene expression were determined in the uterus and ovaries of Sprague-Dawley rats on postnatal day 24, 24 h after exposure to EE, from 0.001 to 10 μg EE/kg/day (s.c.), for four days. Transcript profiles were compared between treatment groups and controls using oligonucleotide arrays to determine the expression level of approximately 7000 annotated rat genes and over 1740 expressed sequence tags. Quantification of the number of genes whose expression was modified by the treatment, for each dose of EE tested, showed clear evidence of a dose-dependent treatment effect that follows a monotonic response, concordant with the dose-response pattern of uterine wet weight gain and luminal epithelial cell height. At the highest dose tested of EE, we determined that the expression level of over 300 genes was modified significantly ($p \leq 0.0001$). A dose-dependent analysis of the transcript profile revealed a set of 88 genes whose expression is significantly and reproducibly modified (increased or decreased) by EE exposure ($p \leq 0.0001$). Our results demonstrate that, exposure to an ER agonist during pre-pubertal maturation changes the gene expression profile of estrogen-sensitive tissues. The product of the EE-regulated genes identified in these tissues, have a physiological role in different intracellular pathways, information that will be valuable to determine the mechanism of action of estrogens. Those genes could be used as biomarkers to identify chemicals with estrogenic activity.

123 ALTERED GENE EXPRESSION IN THE MURINE UTERUS FOLLOWING DEVELOPMENTAL TREATMENT WITH GENISTEIN, A SOY PHYTOESTROGEN.

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Permanent alterations follow developmental exposure to synthetic and naturally occurring estrogens. Long-term consequences of diethylstilbestrol (DES) or genistein on days 1-5 include uterine carcinoma. Thus, the developing reproductive tract appears to be uniquely sensitive to environmental estrogens. To study mechanisms involved in genisteins toxic effects, estrogen-signaling pathways were examined in outbred CD-1 mice following treatment on days 1-5 with genistein (0.5-50 mg/kg). These dose levels span the range for infants consuming soy based infant formula. Expression of estrogen receptor (ER)α, ERβ, and estrogen responsive genes in the uterus including lactoferrin (LF) and c-fos were compared to untreated controls at 5, 12, and 19 days. Ribonuclease protection assays (RPA) and immunohistochemistry showed no expression of ERβin the uterus of control or genistein-treated mice; however, ERα was increased following the lowest genistein dose. LF was increased on day 5 following neonatal genistein in a dose dependent manner. LF induction was blocked by the anti-estrogen, ICI 182 780 suggesting LF expression was mediated through ERα since ERβ was not present; c-fos followed similar patterns as LF. Another group of mice was treated with genistein on days 1-5 and subsequently stimulated at 17 days with 3 daily injections of estrogen (DES; 10μg/kg/day). On the 4th day, uterine wt./body wt. ratios were determined. Mice exposed to the lowest dose of genistein showed an enhanced response while higher neonatal doses dampened the effects of estrogen at puberty; altered gene expression paralleled uterine wet weight response. Reproductive assessment at 2, 4, and 6 months showed alterations at all genistein doses. These findings suggest exposure to genistein during development alters estrogen responsive genes that may play a role in subfertility and long-term toxicity.

124 EXPOSURE TO 1-BROMOPROPANE DISRUPTS DEVELOPMENT OF OVARIAN FOLLICLES IN ADULT RATS.

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Although 1-bromopropane has been used in chemical and electronic industries as an alternative to ozone layer-depleting solvents, its toxicity on female reproductive organs has not been fully elucidated. The aim of this experimental was to determine the effect of 1-bromopropane on female reproductive function in rats. Forty female Wistar rats were divided into four equal groups. Each group was exposed daily to 0, 200, 400, or 800-ppm 1-bromopropane for eight hours a day. After exposure for 7 weeks, all rats in the 800-ppm group became seriously ill and were sacrificed during the 8th week. The other dose groups were exposed for 12 weeks. In the 800-ppm group, but not in the other two exposed groups, body weight was significantly less than the control at each time point from 2 to7 weeks after the beginning of exposure. Tests of vaginal smears showed a significant increase in the number of irregular estrous cycles with extended diestrus in the 400 and 800-ppm groups. Histopathological examination of the ovary showed a significant dose-dependent reduction of the number of normal antral follicles and a decrease in the number of normal growing follicles in the 400-ppm group. No significant change was found in plasma concentrations of LH or FSH in any group compared with the control. Our results indicate that 1-bromopropane can induce a dose-dependent ovarian dysfunction in non-pregnant female rats associated with disruption in follicular growth process.

125 METHOXYCHLOR MAY REGULATE ESTROGEN RECEPTOR LEVELS IN THE MOUSE OVARY.

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The effects of estrogenic pesticides on the female reproductive system are not well understood. We previously reported that the estrogenic pesticide, methoxychlor (MXC), increases the number of atretic ovarian follicles, alters ovarian estrogen receptor α (ERα) and bcl-2 levels, increases the thickness of the ovarian surface epithelium, and disrupts estrous cyclicity in mice. The purpose of the current study was to determine whether MXC alters the levels of both ERα and estrogen receptor β (ERβ) in the mouse ovary. To test this hypothesis, we dosed adult CD-1 mice (39 days) for 10 and 20 days with sesame oil (control) or MXC (8, 16, 32mg/kg/d). All animals were terminated during estrus because ER levels in the ovary differ throughout the estrous cycle and we wanted to minimize this variability. The ovaries then were removed and snap frozen for western blot analysis of ERα and ERβ levels. At the 10-day time-point, MXC (8-32 mg/kg/d) increased ovarian ERα levels compared to controls, but it did not appear to change ovarian ERβ levels. At the 20-day time-point, 8 mg/kg/d MXC increased ovarian levels of ERα, while 32mg/kg/d MXC decreased ovarian levels of ERα compared to controls. At this same time-point, MXC appeared to increase ovarian ERβ levels in a dose-response fashion. These studies suggest that MXC may differently affect the levels of ERα and ERβ in the mouse ovary. (Supported by NIH HD 38955 and the Women's Health Research Group at the University of Maryland).

126 EFFECTS OF BROMODICHLOROMETHANE (BDCM) ON *EX VIVO* LUTEAL FUNCTION IN THE PREGNANT F344 RAT.

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We have reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption, in F344 rats when treated on gestation day (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period. BDCM-induced pregnancy loss was associated with reductions in serum progesterone (P) and corresponding decreases in LH on GD 10, suggesting BDCM disrupts the maternal hypothalamic-pituitary-gonadal axis. These and other data indicate that BDCM affects the hypothalamus or pituitary gland; however, an effect on luteal responsiveness to LH had not been definitively excluded. To address this data gap, we used an *ex vivo* approach to assess luteal function. Dams were dosed by gavage on GD 6-9 (plug day = GD 0) at 0 or 100 mg/kg/d (n=11, 12). One hour after the GD-9 dose, rats were sacrificed, blood was collected and corpora lutea (CL) were incubated with or without hCG, an LH agonist, to stimulate P secretion. During the 24 h incubation, media were periodically sampled for hormone analysis by dissociation enhanced lanthanide flouroimmmunoassay (DELFIA®). Luteal responsiveness was unaffected; both groups displayed a 2.4-fold increase in P secretion in response to hCG challenge. Paradoxically, the BDCM-exposed CL showed >2-fold increases in P secretion *ex vivo* regardless of the presence of hCG; whereas the same animals, i.e., the CL donors, had decreased serum P and LH levels *in vivo*. It is unclear if this 'rebound' effect reflects the removal of the CL from a possible direct inhibitory influence of BDCM, or a response to the diminished LH stimulation *in vivo*. Regardless, the lack of effect on luteal responsiveness is further evidence that BDCM-induced pregnancy loss in the rat is due to reduced pituitary LH

secretion. *Funded by the EPA/UNC Toxicology Research Program, Training Agreement CT827206 Curriculum in Toxicology, UNC-Chapel Hill. This abstract does not necessarily reflect EPA policy.*

127 FTIR PATTERN SHIFT IN TOXICANTS INDUCED CYTOSKELETON REARRANGEMENT CAN IDENTIFY *IN SITU* AND INVASIVE CARCINOMA OF CERVICAL CELLS.

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Cervical cells were swabbed and scanned on Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectrum shifts were compared between normal cells against in situ- and invasive-carcinoma cells. It was found that phosphodiester group in nucleic acid band significantly shifted from 1080 cm-1 in normal cells to 1085 cm-1 in invasive carcinoma cells. Glycogen band also found significantly shifted from 1025 cm-1 in normal cells to 1029 cm-1 in in situ carcinoma cells. Similar phosphodiester bond changes detected by FTIR were found in spleen cells of rats exposed to methomyl, a carbamate insecticide which could induce apoptosis and cell cycle arrest at G0/G1 phase in leukocytic cell lines *via* IL-6 regulation. Colchicine, an antitubulin, can also cause shifts in FTIR pattern of spleen cells. Cytoskeletal-dynamic polymerization provides cell shape control through cellular mechanotransduction, which involves the interconnection of mechanical load network among adhesion molecules and actin, microtubules, and intermediate filaments. It was proposed that mechanisms of signal transduction by local stress from epidermal growth factor to membrane were through adhesive molecules and various integrins, and/or compartmentalization of cAMP-signaling by cAMP phosphodiesterase isoenzymes. Regulation of capillary blood flow, uptake by phagocytosis and synthesis of extracellular matrix may also participate. We proposed that FTIR pattern shifts may provide additional tool for hazard identification of compounds having effects on cytoskeletal dynamic polymerization, as well as an innovative tool for separating in situ and invasive carcinoma of cervical cells.

128 *IN VITRO* BIOACTIVATION OF 4-VINLYCYCLOHEXENE MONOEPOXIDE IN CULTURTED OVARIES FROM B6C3F1 MICE.

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The occupational chemical, 4-vinylcyclohexene (VCH) causes loss of small preantral ovarian follicles in B6C3F1 mice. Structure activity studies have shown that VCH requires hepatic metabolism to the diepoxide (VCD) to cause ovotoxicity. Whether the ovary is also capable of this bioactivation is unknown. VCD is known to be metabolized to an inactive tetrol metabolite (4-[1, 2-dihydroxy]ethyl-1, 2-dihydroxycyclohexane) by microsomal epoxide hydrolase (mEH), and the mouse ovary has been shown to express high levels of mEH activity. Because VCH must be bioactivated by Cyp 450 enzymes to form the mono-epoxide (VCM) and subsequently the bioactive diepoxide form (VCD), the following study was designed to test whether VCM can cause ovotoxicity *in vitro* in cultured ovaries from neonatal mice. Whole ovaries from female post natal day 4 B6C3F1 mice were incubated (15d) in control medium or medium containing VCM (125, 250, 500, 750, and 1000mM) +/- the mEH inhibitor, cyclohexene oxide (CHE; 2mM), or VCD (15 and 30mM). Following incubation, ovaries were prepared for histological evaluation, and follicles were counted. VCD depleted ($p<0.05$) primordial (control, 554 ± 58; 15mM, 0; 30mM, 0.7 ± 0.7) and small primary (control, 84 ± 7; 15mM, 2 ± 2; 30mM, 0) follicles. VCM at higher concentrations reduced ($p<0.05$) the number of primordial (control, 554 ± 58; 750mM, 230 ± 84; 1000mM, 149 ± 81) and small primary (control, 84 ± 7; 1000mM, 22 ± 18) follicles, compared to control. Furthermore, inhibition of mEH by adding CHE along with VCM at 500mM caused loss of ($p<0.05$) primordial follicles (500mM VCM, 512 ± 77; 500mM VCM ± 2mM CHE, 258 ± 93). These results demonstrate that the mouse ovary can participate in the conversion of VCM to the ovotoxic form VCD. Future studies will utilize the ovarian culture system to demonstrate direct metabolism of VCM to VCD. (ES08979; Center Grant ES06694)

129 APOPTOSIS AS A MECHANISM OF 8-MOP-INDUCED OVARIAN TOXICITY.

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Apoptosis, or programmed cell death, regulates the number of follicles ovulated through a distinct framework of gene regulation and signaling pathways. Previous experiments have demonstrated that foreign agents may induce apoptosis in a distinct follicular stage, depending on the inducing agent. Xanthotoxin, a naturally occurring photosensitizer and drug used to treat various skin disorders, has been implicated in a number of adverse reproductive effects in Wistar rats, including decreased birth rate as a direct result of decreased ovulations. Histological examination of ovaries in treated females determined that xanthotoxin targets the follicle and its granulosa cells at the antral stage and reduces 17-beta estradiol production. The present study investigates the potential for programmed cell death as the mechanism of xanthotoxin-mediated ovarian toxicity. Female Wistar rats were dosed (180 mg/kg, p.o.) for twelve weeks. Biomarkers of apoptotic activity were compared in control and treated subjects through Western Blotting. Levels of aromatase protein, (cytochrome P450 19), were measured as both a marker of follicular apoptosis and possible origin of decreased 17-beta estradiol. Levels of caspase 3 protein, the most common executioner of ovarian atresia, were also examined. A significant decrease in protein levels of both aromatase and caspase-3 was observed in xanthotoxin-dosed female rats compared with the control. Histological analysis also confirmed follicular damage typical of apoptotic-stimulating agents in antral follicles of xanthotoxin-dosed rats. These findings are consistent with increased levels of oxidative DNA damage and moreover, disruption of the hypothalamic-pituitary-gonadal axis. The significance of these results is far-reaching and may explain some adverse reproductive effects reported in humans undergoing psoralen photochemotherapy (PUVA).

130 HUMAN HEALTH RISK ASSESSMENT FOR PHENYL GLYCIDYL ETHER TO DETERMINE DRINKING WATER ACTION LEVELS.

C. D. Gillilland, G. L. Ball, C. J. McLellan and M. C. Sanders. *Toxicology Services, NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A risk assessment to determine acceptable levels of phenyl glycidyl ether in drinking water was conducted according to Annex A of NSF/ANSI Standard 60/61. As the only repeated-dose studies suitable for risk assessment were conducted by the inhalation route, several long-term inhalation studies were reviewed. The critical study chosen was a two-year rat inhalation study, in which groups of 100 Chr-CD rats per sex per exposure level were subjected to whole-body exposure of phenyl glycidyl ether at human equivalent doses of 0, 0.08, or 1.0 mg/kg-day for females or 0, 0.13, or 1.5 mg/kg-day for males. At the high dose, rhinitis, squamous metaplasia of the nasal epithelium, and nasal tumors were observed, all of which likely developed due to the irritant and cytotoxic properties of phenyl glycidyl ether and its reactivity with biological macromolecules. These effects were not seen at the low dose. Results were positive in several mutagenicity assays but negative in *in vivo* mouse micronucleus assays. In deriving drinking water action levels, a margin-of-exposure approach was used because the data suggested that there was a threshold for the observed nasal tumors. Benchmark dose modeling, based on the initial tumor precursor effect of rhinitis, was used to determine a BMDL of 0.09 mg/kg-day (95% confidence level), using the gamma, logistic, quantal linear, and Weibull models. The margin-of-exposure calculation took into account uncertainties of 3x for interspecies extrapolation and 10x each for intraspecies extrapolation and database deficiencies, resulting in a total margin of 300x. Based on this, a maximum exposure level of 0.0003 mg/kg-day was calculated. The Short-Term Exposure Level was calculated to be 0.03 mg/L, and the chronic exposure levels of the Total Allowable Concentration and the Single Product Allowable Concentration were calculated to be 0.002 mg/L and 0.0002 mg/L, respectively.

131 RISK ASSESSMENT OF IODINE FOR THE DEVELOPMENT OF DRINKING WATER ACTION LEVELS.

A. D. Phelka, L. L. Bestervelt, C. J. McLellan and M. C. Sanders. *Toxicology Services, NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A human health risk assessment was conducted by NSF International to determine safe short-term and long-term exposure limits for iodine in drinking water in accordance with NSF/ANSI Standard 60/61, Annex A. Very few applications have been authorized for the use of iodine outside of emergency drinking water and swimming pool applications even though the element is a proven effective water disinfectant. The use of iodine-containing products by international travelers, military personnel and the US Space Shuttle Program, has initiated a great deal of research on the ability of iodine to serve as a multi-purpose drinking water disinfectant. As an essential element, iodine intakes ranging from 40 to 200 μg/day are estimated to be necessary for maintenance of normal thyroid function. Longer-term exposure to iodine levels in excess of those necessary for normal thyroid function shows little visible effect on the thyroid in most individuals due to highly effective regulatory mechanisms. While individuals with preexisting thyroid conditions, as well as children and aged individuals, can be significantly more susceptible to elevated iodine levels, the presence of underlying thyroid disease and/or the age of an individual does not guarantee an adverse reaction to elevated iodine

concentrations. NSF has calculated an oral RfD of 0.01 mg/kg-day from the NOAEL of 1 mg/L (0.03 mg/kg-day) from an ongoing prison disinfection study initiated by Freund et al. (1966) and a total uncertainty factor of 3X. Because the response to excess iodine is highly individual, it is suggested that the risk values calculated by NSF be used with caution and only after consideration of the environment into which iodine supplementation is being introduced.

132 RISK ASSESSMENT OF ARSENIC IN DRINKING WATER FOR THE CALIFORNIA PUBLIC HEALTH GOAL.

J. P. Brown, R. A. Howd, A. M. Fan, A. Smith and P. Lopipero. *University of California, Berkeley, CA.*

Arsenic is known to be carcinogenic, atherogenic, genotoxic, and teratogenic, and may cause other adverse developmental effects in children. OEHHA has conducted a risk assessment of arsenic to support a public health goal for drinking water. Both cancer and noncancer endpoints were evaluated. Primary reliance was placed on human data in this assessment. The cancer risk estimate for arsenic in drinking water was based on mortality of arsenic-induced lung and urinary bladder cancers in studies of populations in Taiwan, Chile, and Argentina. Risks were determined from the equation Rx = R0(xB) where Rx represents the predicted risk to persons exposed to level x in drinking water, R0 represents the background lifetime risk of dying from lung or bladder cancer without arsenic exposure, and B is the slope or unit risk of the linear relative risk model. For lung and bladder tumors, the proposed unit risk estimate is 2.7x10-4 (micrograms/L)-1 based on a linear extrapolation of relative risks. The mode of carcinogenic action is not fully understood, but actual risks of low-level exposure are unlikely to exceed this value but could be lower or even zero. For noncancer effects, several studies were evaluated that indicated dose-response relationships between arsenic intake *via* drinking water and a variety of vascular and related diseases. A health protective value of 0.9 micrograms/L was developed based on one of these studies and endpoints, namely human cerebrovascular disease (Chiou et al., 1997). A benchmark dose approach was used with the quantal linear regression model and an overall uncertainty factor of 30 to account for interindividual variation and extrapolation from the lower bound on effect levels to negligible effect levels for the non-cancer effect.

133 HUMAN HEALTH RISK ASSESSMENT FOR t-BUTANOL TO DETERMINE DRINKING WATER ACTION LEVELS.

J. M. Russell, G. L. Ball, L. L. Bestervelt, C. J. McLellan, M. C. Sanders and A. P. Phelka. *Toxicology Services, NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A risk assessment to determine acceptable levels of t-butanol in drinking water was conducted according to Annex A of NSF/ANSI Standard 60/61. A chronic oral study in rats and mice was used as the critical study, in which the key effects were absolute and relative kidney weight increases in female rats. t-Butanol was administered in drinking water to male and female F344/N rats (ten rats per sex per dose) at a dose range of 90-650 mg/kg-day, and to male and female B6C3F1 mice (60 mice per sex per dose) at a dose range of 510-2110 mg/kg-day. In female and male rats, kidney weights were affected, and in male rats, significant nonneoplastic and neoplastic lesions of the kidney were also observed. Male rat kidney effects were discounted due to the presence of α-2 microglobulin accumulation, an effect that is not relevant to human health. Male and female mice showed thyroid follicular cell hyperplasia, adenoma, and carcinoma in the chronic study, and also showed mild kidney effects in a separate subchronic study. Evidence suggested that t-butanol is neither genotoxic nor a developmental toxicant. A two-generation reproductive study of methyl t-butyl ether, which is metabolized to t-butanol and formaldehyde, showed no effects that could be attributed to t-butanol. The oral RfD based on kidney effects in female rats was used for the risk assessment calculations, because it was lower than the oral RfD based on thyroid effects in mice. Benchmark dose modeling resulted in a BMDL of 133 mg/kg-day based on the power model (95% confidence level). Uncertainty factors of 10x each for intraspecies and interspecies variability, resulting in a total UF of 100x, were used to calculate an oral RfD of 1 mg/kg-day (rounded). The calculated Short-Term Exposure Level was 40 mg/L. The calculated chronic exposure levels were the Total Allowable Concentration of 9 mg/L and the Single Product Allowable Concentration of 0.9 mg/L.

134 HEALTH RISK TO FETUSES, INFANTS AND CHILDREN FROM STAGE 1 DISINFECTANTS AND DISINFECTANT BY-PRODUCTS (D/DBPS).

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The Safe Drinking Water Act (SDWA) of 1996, mandates EPA to consider the effects of contaminants in drinking water on the general population and on sensitive sub-groups such as infants, children, pregnant women and the elderly. Under the Executive Order 13045 of April 21, 1997, the Federal Health and Safety Standards must include an evaluation of potential risks to children from microbes and D/DBPs. Before the D/DBP rule was finalized in December 1998, a report was developed on The Health Risk to Fetuses, Infants and Children. This report considered the following issues: whether the D/DBPs caused developmental or reproductive effects; for carcinogenic D/DBPs, are children more likely to be affected than adults; and for noncarcinogenic effects, are children more sensitive than the adults.To answer these questions we evaluated toxicology studies, developmental and reproductive studies, and carcinogenicity and other systemic toxicity studies for the D/DBPs. We also evaluated epidemiology studies for carcinogenicity and developmental and reproductive effects. For Chloroform, Bromodichloromethane, Bromoform, Dichloroacetic Acid and Bromate the MCLG of zero for carcinogenic effects is protective of children's health. The Chloramine NOAEL/LOAEL is lower than NOAEL/LOAEL for developmental effects. Chlorine Dioxide and Chlorite MCLGs are based on developmental toxicity. The MCLGs for Chlorine and Dibromochlorometane are based on other non-carcinogenic effects. Based on our evaluations, it was concluded that for the Stage 1 D/DBPs the MCLGs are "protective" for developmental and reproductive effects. (The opinions expressed in this abstract are those of the authors and not necessarily those of EPA).

135 THE CUMULATIVE RISK OF DRINKING WATER DISINFECTION BY-PRODUCTS: ADEQUACY OF DATA AND APPROACH.

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Human exposure to chemicals is complex, involving mixtures of chemicals and multiple exposure routes. The cumulative relative potency factor (CRPF) approach is applied to mixtures of chemicals, when toxicity of at least one member is well-characterized. The toxic potency of other chemicals is scaled to that of the index chemical, taking into account the dose-response relationships evident. The disinfection of drinking water (DW) produces a myriad of chemicals (disinfection byproducts - DBP). Humans are exposed to DBP through the oral, dermal and inhalation routes, making the health risk issue one of cumulative risk. We have combined data on their concentration in DW, information on their physicochemical properties, human water use and activity patterns, indoor air flow modeling, and physiologically based pharmacokinetic (PBPK) modeling to derive internal doses for adults and children for trihalomethane, haloacetic acid and haloacetonitrile DBP. Fiftieth-percentiles for the 24-hr absorbed doses of dichloroacetic acid and bromodichloromethane in adult females, adult males and 6-year old children were 2.73, 3.14 and 1.12 E-2 mg; and 8.00, 8.43, 4.38 E-2 mg, respectively. This approach allows internal doses, rather than exposure concentrations, to be employed in the cumulative relative potency factor (CRPF) approach.

136 HEALTH RISK ASSESSMENT FOR MONOCHLOROACETIC ACID (MCA).

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MCA is one of the five haloacetic acids considered for regulation under EPA's proposed Stage 2 Disinfectants and Disinfection Byproducts Rule. A risk assessment of MCA showed that MCA exposure induces cardiovascular, kidney, and liver damage in fatal cases of MCA poisoning in humans. Subchronic and chronic oral-dosing studies suggest that the primary targets for MCA-induced toxicity include the heart and nasal epithelium in rats. In a 104 weeks drinking water study in rats, DeAngelo et al. (1997) noted increased spleen weight at 3.5 mg/kg/day, while decreased body weight, decreased liver and kidney weights, and increased testes weight were observed beginning at 26.1 mg/kg/day. Based on a Lowest-Observed-Adverse-Effects-Level (LOAEL) of 3.5 mg/kg/day for increased spleen-weight changes (DeAngelo et al., 1997), and applying an uncertainty factor (UF) of 1000, the RfD is 0.004 mg/kg/day. The UF accounts for the following: a factor of 10 for inter-individual variability in humans; 10 for extrapolation from an animal study; as well as two factors of 3 for extrapolation from a minimal LOAEL; and inadequacy of the data base (including the lack of adequate developmental toxicity studies in two species, and the lack of multi-generation reproduction study). MCA has produced mixed results in genotoxicity assays, but has not produced a carcinogenic response in a chronic gavage study in rodents (NTP, 1992), and a more recent chronic study in rats (DeAngelo et al., (1997). EPA has classified MCA as Group D: not classifiable as to human carcinogenicity. Under EPA's 1999 Draft Guidelines for Carcinogen Risk Assessment, MCA can be best described as having "inadequate data for an assessment of human carcinogenic potential". (The opinions expressed in this abstract are those of the author and not necessarily those of EPA.)

137 REGULATORY DETERMINATION FOR MANGANESE IN DRINKING WATER.

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As required by the 1996 Safe Drinking Water Act amendment, USEPA published a contaminant candidate list (CCL) in 1998 for the purpose of making regulatory determinations. Regulations are recommended only when all the following three

criteria are satisfied: 1) have adverse health effects, 2) occur or are likely to occur in drinking water at concentrations of health concern, and 3) regulation provides a meaningful opportunity to reduce human risk. Mn is ubiquitous in the environment, comprising about 0.1% of the earth crust. It is an essential trace element, necessary for normal growth and function of animals as well as human beings. Although high exposure *via* inhalation has been shown to cause CNS effect in miners, by the oral route, the available data do not show Mn in water presents a public health concern in the US Under normal circumstances, Mn intake is mainly from dietary sources, very little from water or air. Taking the median concentration of detections from the National Inorganic and Radionucleotide Survey data in drinking water (0.01 mg/L), and assuming a daily intake of 2 L of drinking water by an adult, the average daily dose would be 0.02 mg/person-day. The corresponding dose for a child consuming 1 L/day of drinking water would be 0.01 mg/child-day. These values are far below those expected from a normal diet. Further, Mn has a unique low staining threshold; it stains laundry at or below 0.05 mg/L (lower than the health reference level or HRL of 0.3 mg/L). Because of a lack of adequate epidemiological study, the HRL is based on dietary surveys showing no adverse effect at a daily dose of 10 mg/day or greater. Vegetarian diets can provide as high as 15 or 18 mg Mn per day for a long time with no adverse effect. Thus, regulation of drinking water for Mn does not provide a meaningful opportunity to reduce the risk of adverse health effects. The opinions expressed are that of the author and do not necessarily reflect USEPA policy.

138 EVALUATION OF THE ORAL REFERENCE DOSE FOR VANADIUM.

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USEPA has recently reevaluated human and animal health effects data for vanadium. The current database for vanadium compounds has sufficient chronic and subchronic data for development of an oral reference dose (RfD). A provisional RfD of 8E-4 mg V/kg/day has been calculated based on a drinking water study in rats. This value was derived from a marginal NOAEL of 0.25 mg V/kg/d in rats exposed to sodium metavanadate for 3 months.The NOAEL is considered marginal because mild histological changes evident in the spleen, kidneys and lungs of all exposed animals in the study. These changes were mild at the low dose, but were more evident in animals receiving a higher dose of the compound. Selection of 0.25 mg V/kg-day for derivation of the RfD is supported by results of a human study which identified a LOAEL value of 0.32 mg V/kg-day based on gastrointestinal effects. A composite uncertainty factor of 300 was used in the calculation of the RfD for protection of potentially sensitive subpopulations, interspecies extrapolation, study duration, and use of a marginal NOAEL. Based on this provisional RfD value, the Lifetime Health Advisory for vanadium is 6 ug/L. This value can be compared to concentrations of vanadium in US drinking water. Nationally representative data from the National Inorganic and Radionuclide Survey indicate that vanadium was detected in approximately 15% of public water systems served by ground water (median concentration 0.01 mg/L), affecting 7 million people nationally. Average concentrations of vanadium in smaller studies ranged from 0.0043 to 0.0461 mg/L. These data indicate that the occurrence levels of vanadium in drinking water are comparable to the Lifetime Health Advisory level. Disclaimer: The opinions and conclusions expressed in this abstract are those of the authors and do not necessarily reflect those of the affiliated institutions

139 ATSDR'S ACUTE MINIMAL RISK LEVEL FOR COPPER.

L. Ingerman[1] and A. S. Dorsey[2]. [1]*Environmental Science Center, Syracuse Research Corp, Syracuse, NY and* [2]*ATSDR, Atlanta, GA.* Sponsor: P. McGinnis.

ATSDR is currently re-evaluating the noncancer toxicity of copper and has prepared a draft for public comment version of the toxicological profile for copper. One of the most commonly reported adverse health effects of copper is gastrointestinal distress. Most of the available data on gastrointestinal effects comes from case reports of individuals ingesting beverages stored in copper or untinned brass containers or first draw water. Vomiting, nausea, and/or abdominal pain are usually reported shortly after ingestion. These symptoms are not usually persistent or associated with other effects. The case reports provide limited information on exposure levels, however, recently conducted studies identify a threshold for gastrointestinal effects. These studies, as well as the case reports, provide a strong basis for an acute-duration oral minimal risk level (MRL) for copper. An acute MRL is an estimate of daily exposure to a substance that is likely to be without an appreciable risk of adverse effects over a period of 14 days or less. In studies involving a single exposure to copper, adverse gastrointestinal effects (nausea, vomiting, abdominal pain, and/or diarrhea) have been observed at copper concentrations of 4 mg/L and higher (=0.01 mg Cu/kg). In repeated exposure studies, vomiting, nausea, and/or abdominal pain have been observed at 5 mg Cu/L (0.073 mg Cu/kg/day); no adverse effects were observed at concentrations of 1 or 3 mg/L. Animal studies support the identification of the gastrointestinal tract as a sensitive target of toxicity. A 2-week human exposure study (Pizarro et al. 1999) was selected as the basis for the acute MRL. No adverse effects were observed in women ingesting 3 mg Cu/L (0.027 mg Cu/kg/day). To estimate total copper exposure, the copper in the drinking water was added to the reported average dietary copper intake of 0.0266 mg Cu/kg/day. The total copper dose of 0.0538 mg/kg/day was considered a NOAEL for gastrointestinal effects. The NOAEL was divided by an uncertainty factor of 3 (to account for human variability) to yield an acute-duration oral MRL of 0.02 mg Cu/kg/day.

140 SELECTION OF PRE-CONTAMINANT CANDIDATE LIST CHEMICALS FROM UNIVERSE OF DRINKING WATER CONTAMINANTS: A STRUCTURE-ACTIVITY APPROACH.

S. S. Kueberuwa. *Office of Water, USEPA, Washington, DC.* Sponsor: F. Adeshina.

The 1996 Safe Drinking Water Act (SDWA) established that regulations are recommended only for those drinking water contaminants that have adverse effects, occur or are likely to occur in drinking water at concentrations of concern, and for which regulation provides meaningful opportunity to reduce risk. To meet this need, the United States Environmental Protection Agency (USEPA) is directed to publish a list of contaminants (referred to as the Contaminant Candidate List, or CCL). The National Research Council (NRC) has advised that the EPA consider using a "prototype" computerized classification tool in conjunction with expert judgment to produce the CCL from a selected pre-CCL. The pre-CCL must be selected from the "universe" of all known contaminants based on the likelihood to occur or have the potential to occur in drinking water and cause or have the potential to cause adverse effects, using a priority screening criteria and expert judgment. Because this "universe" of potential hazardous drinking water contaminants may number in the tens of thousands, it is proposed that this priority screening criteria and expert judgment should be achieved with an objective and systematic tool for reproducibility and transparency. Structure-Activity Relationships (SAR) can be used for producing the pre-CCL from the "universe" of drinking water contaminants. SAR, as a tool to predict toxicity and exposure, is based on molecular structure and physico-chemical properties that provides a rapid and inexpensive method for screening data-poor contaminants for rule-making prioritization. The SAR method is an EPA approved practice and is the basis of the data found in the EPA's Assessment Tools for The Evaluation of Risk (ASTER) and toxicity equivalency factors (TEF) for chlorinated dibenzo-p-dioxins and -dibenzofurans (CDDs and CDFs). [The opinions herein are those of the author and do not necessarily reflect the opinions of the US Environmental Protection Agency (USEPA)].

141 REGULATORY DETERMINATION FOR NAPHTHALENE IN DRINKING WATER.

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The 1996 Safe Drinking Water Act established a new paradigm for determining the need for drinking water regulations. Under this new paradigm, regulations are recommended only for those drinking water contaminants that have adverse health effects, occur or are likely to occur in drinking water at concentrations of concern, and for which regulation provides a meaningful opportunity to reduce total risk. On June 3, 2002, the USEPA published a preliminary determination that naphthalene did not satisfy the criteria for regulation. This determination was based primarily on a low occurrence of naphthalene at the Health Reference Level (HRL) in representative cross-sections of monitoring data collected under the Unregulated Contaminant Monitoring Program. The HRL (140 mg/L) was derived from the Reference Dose (RfD) for naphthalene after determination that the data are inadequate for an assessment of human carcinogenic potential for the oral route of exposure. The national cross-sections were developed using data collected from public water systems in up to 24 states during the time periods from 1988 to 1992 and 1993 to 1998. During the time periods covered by the monitoring data, there were only two instances where naphthalene was present above the HRL or half the HRL. In fact, only 0.43 and 0.24 % of the drinking water samples collected for Round 1 and Round 2, respectively, exceeded the detection limit for the analytical method used. Additional consideration of environmental fate and potential impacts on sensitive populations, including infants and children with an inherited defect in glucose-6-phosphate dehydrogenase, supported the conclusion that regulation of drinking water for naphthalene did not provide a meaningful opportunity to reduce the risk of adverse health effects. [The opinions expressed represent those of the authors and do not necessarily reflect the opinions of the US Environmental Protection Agency.]

142 EXACERBATION OF CARDIOVASCULAR PATHOLGIES IN FEMALE SPRAGUE- DAWLEY RATS FOLLOWING CHRONIC TREATMENT WITH 3, 3, 4, 4, 5-PENTACHLOROBIPHENYL (PCB126) OR 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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Epidemiologic studies have linked human dioxin exposure to increased severity and mortality of ischemic heart disease. The effect of chronic exposure to dioxin-like compounds on the cardiovascular system was investigated in female Harlan Sprague- Dawley rats as part of an ongoing NTP initiative to evaluate the relative potency of chronic toxicity and carcinogenicity of polychlorinated dioxins, furans, and biphenyls. Animals were treated by daily gavage with up to 1000 ng PCB126/kg/d or up to 100 ng TCDD/kg/d for up to 2 yr. Control animals received corn oil: acetone (99:1) vehicle alone. A stop-study group that received the highest dose of each chemical for 30 wk and then vehicle for the remainder of study was also included. A complete necropsy was performed on all animals, and a full list of tissues was collected and examined microscopically. Administration of each compound was associated with treatment-related increases in the incidence of degenerative cardiovascular lesions. Microscopically, incidences of cardiomyopathy, a degenerative myocardial change seen commonly in rats, and chronic active arteritis in a number of tissues were increased in a dose-related manner in all treated animals in both the PCB126 and TCDD studies. A trend for recovery was noted in the stop studies. Average severity of cardiomyopathy was minimal in all dose groups, including controls, in both studies. Chronic active arteritis occurred primarily in the mesentery and pancreas, although other tissues, including rectum, liver, heart, ovary, uterus, and glandular stomach in the PCB126 study and liver and ovary in the TCDD study were affected in a few dosed animals. Our investigations indicate that the rat cardiovascular system is a target for dioxin exposure, which increases the incidence and severity of spontaneous cardiomyopathy and arteriopathy.

143 BONE INJURY IN A CHEMICALLY INDUCED RAT MODEL OF HUMAN HEMOLYTIC DISORDERS ASSOCIATED WITH THROMBOSIS - COMPARATIVE PATHOLOGICAL AND MRI INVESTIGATION.

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Bone injury occurs in human hemolytic disorders associated with thrombosis, such as beta-thalassemia and sickle cell disease. Exposure of rats to 2-butoxyethanol (BE) has been associated with hemolytic anemia, disseminated thrombosis, and infarction in multiple organs including bones. This model apparently mimics acute thrombophilia in humans. To elucidate the extent of bone injury, male and female Fischer F344 rats were exposed to 4 daily doses of 250 mg BE/5ml water/kg BW and studied the tail vertebrae by histopathology and magnetic resonance imaging (MRI). Thrombosis and infarction were seen in both sexes with females more severely affected. Acute necrosis with little cellular reaction occurred at the epiphyseal, physeal, metaphyseal, and diaphyseal regions of the distal tail. Animals held untreated for an additional 24 days after the 4 daily treatments showed extensive areas of medullary fat necrosis, granulomatous inflammation with fibroplasia, and areas of new woven bone formation adjacent to necrotic bone trabeculae. Growth plate alterations were characterized by focally extensive degeneration of chondrocytes within the central region of the growth plate. MRI mean and standard deviation tissue-density data for both sexes indicated significant ($p \leq 0.05$) decrease following 4-days treatment and a significant increase ($p \leq 0.05$) following an additional 24 days without treatment. Thus, MRI was useful in predicting the BE-induced bone injury, being predominantly necrotic at the early sacrifice and regenerative with proliferation of connective tissue and bone following recovery.

144 EFFECT OF FAMOTIDINE, H2 HISTAMINE BLOCKER, ON HERG CURRENT AND ACTION POTENTIAL OF CARDIAC VENTRICULAR MYOCYTES.

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(Background) Numerous noncardiac drugs prolong QT interval in ECG, resulting in a distinctive polymorphic ventricular tachycardia. Furthermore, it is known that drug-induced QT prolongation and polymorphic ventricular tachycardia is more frequent in women than in men. Recently, it has been reported that a H2 histamine blocker, famotidine, causes QT prolongation leading to torsades de pointes in women. The present study was undertaken to determine whether famotidine has a cardiac proarrhythmic effect on HERG current in HERG (human ether a go-go related gene)-transfected cells and action potential duration (APD) in isolated guinea pig cardiac ventricular myocytes using the patch-clamp technique. (Methods and Results) Measurement of ion current: In HERG-transfected cells, ion currents were measured by the patch-clamp technique. Action potential: The hearts of male guinea pigs (4-12 weeks) were harvested and perfused with Ca2+ free Tyrode solution containing collagenase through the coronary artery using Langendorff apparatus. The left ventricle was cut out and dispersed to isolate ventricular myocytes. The AP of the ventricular myocytes (VM) was measured by the patch-clamp technique. Famotidine (10-100μM) prolonged APD90 by 15-46%, but had little effect on HERG current. Similarly, E-4031 (3 μM) prolonged APD90 by 40%, and inhibited on HERG current by 100%. 17β-estradiol (10 μM) did not prolong APD90. (Conclusion) These data indicate that prolongation of APD by famotidine might be not attributed to inhibition of HERG current, IKr. In conclusion, these results demonstrate that both evaluations in HERG current and APD of cardiac myocytes are very useful for assessment of cardiac proarrhythmic effect of new drugs.

145 THE ROLE OF GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE INDUCED BY 3H-1, 2-DITHIOLE-3-THIONE IN PROTECTING RAT AORTIC SMOOTH MUSCLE CELLS AGAINST 4-HYDROXYNONENAL-MEDIATED CYTOTOXICITY.

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4-Hydroxynonenal (4HNE) is a major aldehydic product of lipid peroxidation and is believed to contribute significantly to the pathogenesis of cardiovascular diseases, particularly atherosclerosis. 4HNE adducts have been detected in atherosclerotic lesions. One of the major metabolic transformations of 4HNE involves conjugation with glutathione (GSH) catalyzed by GSH S-transferase (GST). In this study we have characterized the induction of GSH and GST by 3H-1, 2-dithiole-3-thione (D3T) and their protective effects against 4HNE-mediated toxicity in rat aortic smooth muscle A10 cells. Incubation of A10 cells with micromolar concentrations of D3T resulted in a marked concentration- and time-dependent induction of both GSH and GST. Consistent with the increased GSH level, enhanced mRNA expression of ~{&C-}-glutamylcysteine synthetase occurred prior to the significant increase of GSH. To examine the protective effects of D3T-induced GSH and GST against 4HNE-mediated toxicity, A10 cells were pretreated with D3T and then exposed to 4HNE. Pretreatment of A10 cells with D3T led to a marked decrease of 4HNE-induced toxicity as determined by MTT reduction assay as well as light and electron microscopy. To further demonstrate the involvement of GSH and GST in protecting against 4HNE-induced toxicity, buthionine sulfoximine (BSO) and sulfasalazine were used to inhibit cellular GSH biosynthesis and GST activity, respectively. Either depletion of GSH by BSO or inhibition of GST by sulfasalazine caused great potentiation of 4HNE-mediated toxicity. Moreover, D3T failed to protect A10 cells against the toxicity induced by 4HNE in the presence of BSO. Taken together, this study demonstrated that D3T can induce both GSH and GST in aortic smooth muscle cells, and that this augmented cellular defense affords protection against 4HNE-induced cardiovascular cell injury.

146 CHEMICAL INDUCTION OF ENDOGENOUS ANTIOXIDANTS IN RAT CARDIOMYOCYTES: PROTECTION AGAINST OXIDATIVE CELL INJURY.

X. Peng, S. Cheng, L. D. Trombetta and Y. Li. *Pharmaceutical Sciences., St. John, Jamaica, NY.*

It is well known that reactive oxygen species (ROS) are involved in the pathogenesis of many human diseases, including cardiovascular diseases. Accumulating evidence shows that exogenous antioxidative compounds provide protection against oxidative cardiac injury. However, whether induction of endogenous cellular antioxidants by chemicals (drugs) also offers protection against oxidative cardiac injury has not been carefully studied. In this study, we have investigated the induction of cellular antioxidants by the unique chemoprotective agent, 3H -1, 2-dithiole-3-thione (D3T) and the protective effects of the D3T-induced cellular antioxidants against oxidative cell injury in rat cardiomyocyte H9C2 cells. Incubation of H9C2 cells with 25-100uM of D3T for 24 h resulted in a significant induction of a series of cellular antioxidants, including reduced glutathione (GSH), GSH peroxidase, GSSG reductase, catalase and GSH S-transferase. To determine whether the induced endogenous antioxidants can protect against oxidative cell injury, H9C2 cells were pre-treated with D3T and then incubated with xanthine oxidase (XO) plus xanthine, a system that generates ROS, or 4-hydroxy-2-nonenal (HNE), a major lipid peroxidation product formed during oxidative stress. We observed that D3T pre-treatment of H9C2 cells led to significant protection against XO/xanthine- and HNE-induced cytotoxicity as determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) reduction and morphological changes.

Moreover, immunoblot analysis showed that D3T-pretreatment of H9C2 cells resulted in a marked reduction of HNE-mediated protein adduct formation. Taken together, this study demonstrates that endogenous antioxidants in H9C2 cells can be induced by D3T, and that the induced antioxidant defenses afford significant protection against oxidative cardiac cell injury.

147 INHALATION TOXICITY OF BREVETOXIN 2 IN SPONTANEOUSLY HYPERTENSIVE AND F344 RATS.

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Brevetoxins (PbTxs) are neurotoxins produced by the marine dinoflagellate, *Karenia brevis*. Acute exposure to PbTxs in sea spray is known to cause respiratory tract irritation and may induce broncho-constriction in asthmatic individuals. Histopathologic examination of manatees dying from repeated exposure to brevetoxins during red tide events suggests that the respiratory, nervous, hematopoietic, and immune systems are potential targets for toxicity. The purpose of this experiment was to evaluate the effects of repeated inhalation of brevetoxin 2 (PbTx-2) in the spontaneously hypertensive rat (SHR), a rodent model for pulmonary hypertension. Two groups of male SHR were exposed 2 hr/day, 4-5 days/wk for 3 wks to 0 or 250 to 500 ug PbTx-2/m^3. In-life endpoints included evaluation of ECGs, body temperature, and pulmonary function. At sacrifice, lavage fluid was analyzed for indicators of pulmonary toxicity and inflammation. Lung, brain, liver, kidney and spleen were taken for histopathological evaluation. PbTx-2 exposure resulted in small increases in heart rate and minute volume, but no pronounced effects on ECGs. There was a small, statistically significant increase in macrophages in lavage fluid, but no effect on differential cell counts, lactate dehydrogenase activity, or protein concentration. In a parallel experiment, F344 rats were exposed similarly for one week. Changes in lavage fluid were limited to increases in the numbers of macrophages. Results suggest that repeated inhalation exposure to concentrations of brevetoxin several orders of magnitude above that observed in the environment produced minimal effects in rodents. Long term effects on the nervous and immune systems remain to be evaluated. Research funded under NIH P01-ES10594.

148 PHYSICAL CONDITIONING MODULATES CARDIAC VEGF GENE EXPRESSION AND NITRIC OXIDE LEVELS IN NO-DEFICIENT HYPERTENSIVE RATS.

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Many individuals with cardiac diseases undergo periodic physical conditioning with or without medication. Therefore, this study investigated the interaction of physical training and chronic nitric oxide synthase (NOS) inhibitor (Nitro-L-Arginine Methyl Ester, L-NAME) treatment on blood pressure (BP), vascular endothelial growth factor (VEGF) gene expression and nitric oxide (NO) generating system in the heart of the rat. Fisher 344 rats were divided into four groups and treated as follows: 1) sedentary control, 2) Exercise training (ET) for 8 weeks, 3) L-NAME (10 mg/kg, s.c. for 8 weeks) and 4) ET + L-NAME. BP was monitored with tail-cuff method. The animals were sacrificed 24 hr after last treatments and hearts were isolated and analyzed. Physical conditioning significantly increased respiratory exchange ratio (RER), cardiac VEGF mRNA levels, NO level, NOS activity, endothelial eNOS and inducible iNOS protein expression. Training also caused depletion of cardiac malondialdehyde (MDA) levels. Chronic L-NAME administration resulted in depletion of cardiac VEGF mRNA levels, NO level, NOS activity, eNOS, neuronal (nNOS) and iNOS protein expression. Chronic L-NAME administration enhanced cardiac MDA levels. These biochemical changes were accompanied by increases in BP after L-NAME administration. Interaction of training and NOS inhibitor treatment resulted in the normalization of BP and the up-regulation of cardiac VEGF mRNA levels. The data suggest that physical conditioning attenuated the oxidative injury caused by chronic NOS inhibition by up-regulating the cardiac VEGF gene expression and NO levels and lowering the BP in rats(Supported by AHA/IA grant #0051395Z).

149 CONTROL REFERENCE DATA FOR DOPPLER ECHOCARDIOGRAPHY IN HEALTHY CYNOMOLGUS MONKEYS UNDER KETAMINE HYDROCHLORIDE SEDATION.

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In addition to the routine electrocardiogram recording, echocardiography is a useful noninvasive technique that is commonly used to evaluate cardiac structure and function. The cynomolgus monkey is a frequently used nonhuman primate animal model of human cardiovascular disease because of anatomical and physiological similarity. Furthermore, cardiovascular toxicity is a key parameter in toxicity studies providing important information on the health status. However, whilst cynomolgus monkeys are frequently used in cardiovascular research and preclinical toxicity studies, standardized echocardiographic parameters generated from large numbers of healthy animals are not available. In the present study, echocardiographic investigations were undertaken on more than 100 untreated animals in order to obtain normal reference values for this species. The following standard views were determined: (1) Diameters and visual assessment of left ventricular (LV) function in LPQ (long parasternal heart axis), (2) Visual assessment of LV and (right ventricular) RV function in SPQ (short parasternal heart axis), and (3) Diameters and visual assessment of LV and RV function in 4KB (4-chamber view of the heart). In addition to these parameters, pump function was analyzed by heart rate, ejection fraction (EF), beat volume (together with heart rate and body weight for cardiac output determination) and the shortening fraction (FS; as a parameter of pump function) based on wall thickening properties and planimetric measurements ("Areas") during systole and diastole were determined. This historical control data base will allow a reasonable discussion of possible cardiologic findings in preclinical toxicity studies.

150 THE PROTECTIVE EFFECTS OF CHEMICALLY-INDUCED ENDOGENOUS GLUTATHIONE ON PEROXYNITRITE-MEDIATED TOXICITY IN VASCULAR CELLS.

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Sponsor: L. Trombetta.

Accumulating evidence suggests that peroxynitrite generated from the reaction of nitric oxide and superoxide attributes to the pathogenesis of cardiovascular diseases, such as atherosclerosis. Reaction with glutathione (GSH) is believed to be a major detoxification pathway of peroxynitrite in biological systems. This study was undertaken to test the hypothesis that chemically-elevated intracellular GSH may afford protection against peroxynitrite-mediated toxicity in vascular cells. Incubation of rat aortic smooth muscle cells or human aortic endothelial cells with micromolar concentrations of 3H-1, 2-dithiole-3-thione, a unique chemoprotective agent, led to a concentration- and time-dependent elevation of cellular GSH. Increased gene expression of gamma-glutamylcysteine synthetase, the key enzyme involved in GSH biosynthesis, was also observed in D3T-pretreated cells. To examine the protective effects of D3T-induced cellular GSH on peroxynitrite-mediated toxicity, vascular cells were pretreated with D3T for 24 h and then exposed to either purified peroxynitrite or the peroxynitrite-generator, SIN-1. We observed that D3T-pretreatment of vascular cells resulted in a significant protection against purified peroxynitrite- or SIN-1-mediated toxicity, as assessed by MTT reduction and morphological changes. Conversely, depletion of cellular GSH by buthionine sulfoximine (BSO) caused a marked potentiation of purified peroxynitrite- or SIN-1-mediated toxicity. To further demonstrate the critical involvement of GSH induction in D3T cytoprotection, vascular cells were co-treated with BSO to abolish D3T-mediated GSH elevation. Co-treatment of cells with BSO was found to greatly reverse the protective effects of D3T on purified peroxynitrite- or SIN-1-mediated toxicity. Taken together, our results demonstrate for the first time that induction of endogenous GSH by D3T affords marked protection against peroxynitrite-mediated toxicity in vascular cells.

151 MESENTERIC AND PANCREATIC VASCULAR INJURY INDUCED BY FENOLDOPAM IN SPRAGUE-DAWLEY (SD) RATS: EVIDENCE FOR MAST CELL MEDIATED PATHOGENESIS.

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Fenoldopam (FP) has been shown previously to induce mesenteric and splanchnic arterial injury in rats. This toxicity is thought due to excessive vasodilation following sustained dopaminergic receptor stimulation. In the present study adult male SD rats were given a single s. c. injection of 60 (11 rats) or 120 (5 rats) mg/kg FP or saline (10 rats) and euthanized 24 hr later. At necropsy, the entire mesentery of each rat was spread out, divided into three ring-shaped sections, fixed, sectioned and stained with H&E, toluidine blue (for mast cells) and Luna's method (for eosinophils). Vascular alterations were similar to those observed in our previous studies with SK&F 95654. Two categories of vascular injury were again noted: (1) muscular arteries showed medial hemorrhage with smooth muscle cell apoptosis and necrosis and periadventitial inflammation; (2) small vessel (capillaries, venules, arterioles, and small veins) injury was characterized by inflammation, vascular congestion, and venous thrombosis. Endothelial cell activation and injury (denudation, vacuolization, apoptosis and necrosis) were found in all vessels. Degranulation of

mast cells, infiltration of eosinophils, macrophages and neutrophils, and fibrin exudate were present in the perivascular spaces and connective tissues of the mesentery. Dose dependent increases in serum alpha-2-macroglobulin were seen. Granulocytosis but not generalized activation was observed. Rats treated with 60 or 120 mg/kg FP had an average vascular injury score of 3.4 or 3.8 on a scale of 0 (unaffected) to 5 (most severe) with an average of 30% or 53% of mesenteric vessels showing hemorrhage, respectively. The acute phase response, findings of mast cell degranulation, accumulation of numerous eosinophils and widespread edema reinforce our previous hypothesis that a localized pseudoallergic reaction can contribute to drug-induced rat mesenteric vascular injury.

152 VALIDATION FOR QT PROLONGATION IN CONSCIOUS BEAGLE DOGS ADMINISTERED SOTALOL VIA THE ORAL ROUTE.

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The purpose of this study was to evaluate the DSI (Data Sciences, St. Paul, MN) telemetry system in our Testing Facility environment with an agent known to cause QT prolongation. Eight adult beagle dogs (4 /sex) with implanted telemetry transmitters were used for this study. Pretest data was collected for a 24 hour period prior to initiation of dosing. Sotalol was administered orally in capsules in a dose escalation regimen. Doses of sotalol were 0, 8, 16, and 32 mg/kg, respectively with a 2-3 day washout period between each dose. ECG waveform, blood pressure, and heart rate (HR) were collected for 30 seconds intervals. Prior to dosing all parameters were collected every 15 minutes for 2 hours, and every 30 minutes for 24 hours following each dose. QT interval and mean HR were manually measured. The effect of sotalol on QT interval was assessed by several methods including QTcv (Van De Water), QTcr (separate regressions of QT on RR interval), %QTc change from baseline, comparison of absolute QT with similar HR, QT:HR outlier analysis, and the difference between postdose and baseline for QT and each QTc. Data was averaged over 0 to 3 hours, 3 to 6 hours, 6 to 12 hours and 12 to 24 hours. Analysis of covariance for repeated measures was used to test whether treatment effects varied during the above time intervals. Data was also analyzed to test whether the treatment had the same effect in males and females. Treatments were compared to control using Williams test. All levels of sotalol had a significant effect on QT, QTc and HR. QT prolongation started within 1 hour of dose with the peak effect occurring 3 to 4 hours later and lasting about 15 hours. The duration of the effect was dose related though not for the degree of the effect. Treatment effects were not different in between sexes in most parameters except for QTc between 3 to 6 hours. During this time, males had significantly longer QTc compared to females. In conclusion, we have demonstrated the suitability of using DSI telemetry systems to detect changes in QT interval in our Testing Facility environment.

153 THE EFFECT OF THE NONSELECTIVE NOS INHIBITOR, L-NITRO-ARGININE ON THE CANINE PANCREAS.

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Nitric oxide (NO), generated by both constitutive (cNOS) and inducible (iNOS) isoforms of nitric oxide synthase (NOS), is a cell-signaling molecule involved in numerous physiological possesses including gastrointestinal (GI) functions. NO stimulates the release of gastric and pancreatic secretions and increases gastric motility and contractility. We therefore evaluated the effects of L-nitro-arginine (LNA), a NOS inhibitor that preferentially inhibits both constitutive isoforms, on the canine pancreas. LNA was administered orally for 4 weeks at doses of 10 and 30 mg/kg/day (6 female dogs/group). Doses of LNA were selected based on the upper limit of acceptable changes in blood pressure and overt toxicity. To evaluate whether pancreatic effects of LNA were related to the amino acid-like structure an additional group was given L-arginine (L-Arg). Treatment with both doses of LNA resulted in stool changes (decreased, watery, soft, mucoid and/or red-tinged), a reduction of ~40% in heart rate (possible vagal response to increased blood pressure) and elevations in pancreatic enzymes (amylase, lipase, or trypsin-like immunoreactivity (TLI)) in serum. After one week, there was a 2-10X dose-related increase in pancreatic enzymes in both LNA groups, which then declined to ~30% greater than control thereafter. No remarkable histological changes were found in the pancreas after 4 weeks. No effects of treatment with L-Arg were found. The results of this study indicate that the inhibition of constitutive NOS can lead to pancreatic effects, specifically elevations in serum levels of pancreatic enzymes. However, inhibition of constitutive NOS for up to 4 weeks does not result in any observable morphologic changes.

154 VALIDATION OF THE IL 682 CO-OXIMETER FOR EVALUATION OF METHEMOGLOBIN IN THE SPRAGUE-DAWLEY RAT.

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Measurement of methemoglobin (MetHb) can play a valuable role in the determination of adverse effects caused by particular xenobiotics (example: nitrites). Methb decreases the oxygen capacity of the blood, which as a result can exert a dual effect of impairing the supply of oxygen to tissues. The purpose of this study was to validate methods of measuring MetHb levels in the Sprague-Dawley rat using the IL 682 Co-Oximeter System. Untreated rats (Crl:CD(SD)IGS BR) had blood samples collected from the lateral tail vein utilizing 21 gauge needles. Syringes, pretreated with heparin to prevent clotting, were attached to the needles. Exposure of the sample to air was avoided, as exposure to oxygen would decrease the ability to accurately measure MetHb. Evaluation of MetHb was performed on each sample utilizing the IL 682 Co-Oximeter System. An additional set of animals were assigned to two treatments groups; one group treated intraperitoneally with 21 mg/kg of sodium nitrite and one treated intraperitoneally with 42 mg/kg of sodium nitrite. These two groups of animals had blood collected in the same fashion as the untreated animals prior to treatment and approximately two hours post-dose and evaluation of MetHb performed. Mean MetHb values for the untreated rats were 0.7% (range 0.2-1.4%) which are within biological normal ranges for this species. There were no apparent differences noted between sexes. Mean values for the sodium nitrite treated groups at 2 hours post-dose were 5.3% (21 mg/kg) and 32.4% (42 mg/kg). It was concluded that the IL 682 Co-Oximeter System and the procedures utilized for blood collection were an accurate method for determination of methemoglobin in the Sprague-Dawley rat.

155 THE EFFECTS OF CISAPRIDE ON QT INTERVAL IN CONSCIOUS, TELEMETERED MALE BEAGLE DOGS.

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Torsades de pointes (TDP) is a rare but potentially fatal polymorphic ventricular tachycardia associated with prolongation of the QT interval primarily due to drug-induced delayed repolarization. Cisapride, a prokinetic agent, known to prolong action potential duration (APD) in canine purkinje fibers (Gintant et al, 2001) has also been reported to produce transient increases in QTc in anesthetized dogs (Crema et al, 1998). The present study was conducted in order to provide additional information regarding the potential arrhythmogenic effects of cisapride using a conscious model. Four male beagle dogs were surgically implanted with telemetry transducers, for the measurement of arterial blood pressure and lead II ECG. Cisapride, suspended in 1% w/v aqueous methylcellulose, was administered in an ascending dose regimen on days 1, 4, 7, 10 and 14 *via* oral gavage at doses of vehicle, 0.1, 1.0, 2.0 and 4.0 mg/kg, respectively. Measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), heart rate (HR) and the lead II ECG variables (PR interval, RR interval, QRS duration and QT interval) were taken from each animal continuously for 1 hour before and six hours after dosing. Preliminary results indicate that oral administration of cisapride at 0.1, 1.0, 2.0, and 4.0 mg/kg tended to have no effect on arterial blood pressure (systolic, diastolic and mean) or heart rate when compared to vehicle. Furthermore, QTc (Fridericia) tended to increase from baseline to 90 min post dose for the 2.0 mg/kg dose. Data from the completed study and the successful use of this in-vivo assay to investigate QT prolongation will be described.

156 INDUCTION OF 5-LIPOXYGENASE IN VASCULAR ENDOTHELIAL CELLS IN RESPONSE TO ARSENIC EXPOSURE.

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Numerous epidemiological studies have linked arsenic in drinking water to cancer and more recently to cardiovascular disease. Although an association between arsenic exposure and atherosclerosis has been established, the mechanisms are primarily unknown. Atherosclerosis is an inflammatory disease so any agent that enhances the inflammatory state of the vasculature may perpetuate atherosclerosis. The proinflammatory leukotrienes are major products of the 5-lipoxygenase pathway. Patients with atherosclerosis are known to have increased leukotriene production, and a recent study identified the 5-lipoxygenase gene as a major contributor to the development of atherosclerosis in mice. Although 5-lipoxygenase is expressed primarily in leukocytes, there is also evidence for the presence of 5-lipoxygenase in endothelial cells. The goal of this study was to determine the effect of arsenic exposure on 5-lipoxygenase protein expression and leukotriene generation in endothelial cells. Bovine aortic endothelial cells (BAE) were used as a model to measure the induction of 5-lipoxygenase and production of the inflammatory mediator, leukotriene E4 (LTE4). Using Western immunoblots, we showed that 5-lipoxyge-

nase is upregulated in a time-dependent manner in response to arsenic exposure in BAE cells. Densitometry revealed a 3-4 fold increase in 5-lipoxygenase protein over controls after 72 h. LTE4 production was also measured using commercial ELISA kits and showed an increase in response to arsenic exposure concomitantly with induction of the 5-lipoxygenase enzyme. This study links arsenic to an inflammatory pathway that has been associated with atherogenesis and provides insight into the complicated mechanisms of arsenic-induced cardiovascular disease.

157 PEROXYNITRITE GENERATION IN AORTIC ENDOTHELIAL CELLS EXPOSED TO ARSENIC IS INCREASED BY MANGANESE.

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Long-term exposure to arsenic in drinking water has been linked to cancer and other health effects, including cardiovascular disease. Arsenic is found in combination with a range of metals that could influence its toxicity. Manganese is a metal that is typically found in conjunction with arsenic in contaminated groundwater. One possible mechanism for the cardiovascular toxicity of arsenic involves generation of peroxynitrite. Peroxynitrite is a powerful oxidant and a reactive nitrating agent, particularly in the presence of tyrosine residues. Nitrotyrosine, a biological marker for the presence of peroxynitrite, is found in high levels in the plaques of individuals with atherosclerosis. The goal of this study was to examine the effects of manganese on arsenic-induced toxicity and peroxynitrite formation in endothelial cells. The effect of manganese on arsenic toxicity was determined in bovine aortic endothelial (BAE) cells using the MTT assay. Arsenic toxicity was potentiated by the addition of manganese at manganese concentrations that were non-toxic when administered alone. BAE cells were also used to measure the generation of peroxynitrite in the presence of arsenic, manganese and arsenic-manganese mixtures. We have previously shown that arsenic increases peroxynitrite formation in this model. BAE cells were exposed to sodium arsenite, manganese chloride or an arsenite-manganese mixture for 1h. Peroxynitrite was measured using a published assay based on the conversion of hydroethidine to its fluorescent form, ethidium. Individually, arsenic and manganese enhanced peroxynitrite formation by approximately 1.5-fold each over controls at 5 μM arsenic and 25 μM manganese. However, when added together at the same concentrations, peroxynitrite formation was increased 2-fold versus controls. These data suggest that manganese may exacerbate the toxic effects of arsenic on the vascular system.

158 CELL TYPE DEPENDENT SIGNALING WEBS OF OXIDATIVE STRESS.

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Heart disease is a major cause of natural death worldwide. The major cell types in the heart are cardiomyocytes (CMCs) and fibroblasts. While CMCs undergo apoptosis or hypertrophy during heart disease progression, fibroblasts often develop hyperplasia or malfunctions in protein production, causing alterations in the structure and texture of the extracellular matrix. Oxidants have been shown to activate three branches of MAPKs: ERKs, p38 and JNKs. The upstream regulators of oxidant-induced MAPKs have not been identified in CMCs and fibroblasts. We explore the hypothesis that oxidants may transactivate the insulin receptor, EGF receptor and Src tyrosine kinase, leading to activation of MAPKs. Measurements of insulin receptor substrate (IRS)-1 or IRS-2 phosphorylation in CMCs following H_2O_2 treatment yielded negative data. AG1478, a pharmacological inhibitor of EGFR, reduced the activation level of ERK1/2 and JNKs but not p38 in CMCs after oxidative stress. AG1478 did not block any of the three MAPKs in fibroblasts treated with the same dose of H_2O_2 as CMCs. Inhibiting Src family tyrosine kinases with PP2 blocks JNKs but not ERKs or p38 in CMCs. In contrast, PP2 inhibits ERKs and JNKs but not p38 in fibroblasts. Therefore, it appears that while EGFR-like signals may be activated by oxidants and contribute to ERK1/2 or JNK activation in CMCs, the Src pathways contribute to activation of JNKs in CMCs and to activation of ERKs and JNKs in fibroblasts. Neither EGFR nor Src pathways contribute to p38 activation by oxidants in CMCs or fibroblasts. In conclusion, although ERKs, p38 and JNKs are activated by many types of stress, the upstream regulators of these pathways differ depending on cell type.

159 cDNA SEQUENCE OF CHICK BETA-1-ADRENERGIC RECEPTOR MAY EFFECT RESPONSES TO CARDIOTOXIC XENOBIOTICS.

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It has been known for over three decades that the avian cardiovascular system is sensitive to halogenated aromatic hydrocarbon toxicity. Recently, research has demonstrated that 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (Toxicol Appl Pharmacol 167:210) inhibits the ability of isoproterenol, a β-adrenergic receptor (β-AR) agonist, to increase heart rate of day 10 and 12 chick embryos. This reduced response to β-AR stimulation may be mediated at the receptor level. Thus, we have cloned a nearly complete cDNA sequence of the chick β_1-AR by routine RT-PCR and RACE. One-day old chick heart total RNA was isolated, treated with RNase-free DNase, and used to synthesize cDNA. Initial PCR primers were designed based on turkey β_1-AR cDNA sequence (GenBank M14379) and subsequent primers were based on chick β_1-AR cDNA sequence cloned in our laboratory. The cDNA sequence of the chick β_1-AR is highly conserved with turkey, sharing 96% amino acid identity. In addition, the membrane spanning regions and short interspan loops of the chick β_1-AR shares 81-83% amino acid identity with the corresponding regions of the mouse, rat or human β_1-AR. However, the turkey β_1-AR has an alternative splice variant that is not found in mammals. The variant encodes a carboxyl-terminus with 59 additional amino acids that block agonist-promoted endocytosis and down-regulation of the β_1-AR. Expression of the variant is age- and tissue-specific providing avian species with an additional mechanism to regulate receptor levels following agonist exposure. Given the importance of β_1-AR regulation in progression and severity of cardiovascular disease, it is possible that differential regulation of β_1-AR between avian and non-avian species could result in varying sensitivity to cardiotoxic chemicals. Future research will continue to investigate the mechanisms by which developmental TCDD exposure decreases the response of the chick embryo heart to stimulation by β_1-AR agonist. Supported by NIH R15 ES011806.

160 ARE THERE SEX DIFFERENCES IN ACTION POTENTIAL DURATION RECORDED FROM CANINE PURKINJE FIBRES?

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Two-thirds of the cases of drug-induced torsades de pointes occur in women. This gender-related risk of arrhythmia is associated with a longer corrected QT interval at baseline and a greater response to IKr blockers (Drici & Clement, Drug Saf., 2001, 24, 575). Sex differences in rabbit Purkinje fibre action potential have been reported by Lu *et al.* (J.Cardiovasc. Pharmacology, 2000, 36, 132). However, in the conscious dog this difference was not evident, QT interval being similar: 206 vs 203 ms in males and females respectively (Osborne & Leach, Fd. Cosmet.Toxicol., 1971, 9, 857); 233 vs 230 ms in males and females respectively (Quintiles telemetry data). Action potentials were recorded from Purkinje fibres isolated from the hearts of both male and female Beagle dogs according to Patmore *et al.* (Eur. J. Pharmacology, 2000, 406, 449). Values were compared to establish if there were baseline gender differences. Data are shown below for action potential duration (APD) recorded at 90% repolarization at stimulation frequencies of 1 and 0.5Hz. These values are from an extensive database of action potential parameters. No gender related differences in APD were evident. Exposure of fibres to 50 μM dl-sotalol resulted in comparable increases in APD indicating that the sensitivity of male and female dog Purkinje fibres to IKr blockers is not different. These data contrast those obtained for rabbit Purkinje fibres by Lu *et al.* but are consistent with a lack of difference in QT interval measured in conscious male and female Beagle dogs.

APD90	1 Hz		0.5 Hz	
	Male	Female	Male	Female
Mean at baseline	275.5	275.6	3[illegible]2.2	340.1
95% confidence limits	270.8-280.3	269.8-275.7	316.1-349.3	305.1-414.0
n	328	594	310	569
Mean % increase with 50 μM dl-sotalol	46.9	44.8	66.1	62.8
95% confidence limits	44.3-49.5	42.9-46.7	64.6-70.6	59.5-66.1
n	130	214	137	203

161 ENHANCEMENT OF NO RELEASE FROM S-NITROSOALBUMIN BY FATTY ACIDS: RELEVANCE TO OXIDATIVE/NITROSATIVE STRESS OF PREECLAMPSIA.

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S-nitrosothiols (RSNO), mainly S-nitrosoalbumin (NO-Alb), are potent plasma vasodilators whose action is associated with NO release. Release of NO requires the presence of a redox-active transition metal (copper) and a reductant (ascorbate).

Oxidative stress-induced ascorbate deficiency might result in decreased rates of decomposition of RSNO in disease conditions e.g., in preeclampsia, diabetes. Because free fatty acids (FFAs) affect redox activity of albumin-bound Cu, we studied the role of FFAs and ascorbate in the release of NO from NO-Alb in the presence of Cu/Alb using NO-sensitive electrode. No release of NO occurred in PBS solution. Cu/Alb (0.3:1 mole/mole) slightly stimulated decomposition of NO-Alb and ascorbate caused a strong concentration-dependent increase of NO release. Albumin/FFA complexes (oleic acid, linoleic acid, 1-6 mole/mole albumin) also increased the rate of ascorbate-dependent NO-Alb decomposition, particularly at FFA/albumin ratios >2-3. Thus two major factors - ascorbate and FFAs - regulate stability of NO-alb in plasma. To assess the relevance of these results to vasoactive properties of NO-Alb, the relaxation responses of isolated phenylephrine-preconstricted mouse mesenteric arteries (incubated in 1% plasma from women with normal or preeclamptic pregnancies) after exposure to 5.8 μM albumin with 0.5 μM NO-Alb were tested. To eliminate differences in the endogenous levels of ascorbate, the samples were spiked with exogenous ascorbate. Preeclamptic plasma that contains elevated levels of albumin-bound FFAs (so-called cytotoxic albumin with pI =4.8) caused a greater relaxation response (45.3±6.3%) than normal pregnancy plasma (22.7±5.8%, p=0.02). Thus enrichment of albumin with FFAs can enhance decomposition of NO-Alb if sufficient ascorbate is available in plasma. Supported by NIH HL64145.

162 OPENING OF MITOCHONDRIAL PERMEABILITY PORES CAUSES ISCHEMIA/REPERFUSION-INDUCED KILLING OF CULTURED ADULT RAT MYOCYTES.

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BACKGROUND: Mildly acidic pH is protective in toxic and hypoxic injury but the restoration of normal pH is an independent factor promoting cell death after reperfusion. Studies suggest that onset of the mitochondrial permeability transition (MPT) contributes to ischemic damage to heart and hypoxic injury to cardiac myocytes. The **AIM** of this study was to test the hypothesis that onset of the MPT underlies the pH-dependency of ischemia/reperfusion injury. **METHODS:** Overnight cultured adult rat myocytes were incubated in anoxic Krebs-Ringer-HEPES (KRH) buffer at pH 6.2 for 3h to simulate ischemia. Reperfusion was simulated by reoxygenating cells at pH 6.2 or 7.4 for 2h. Some myocytes were treated with 1.0μM cyclosporin A (CsA), a MPT blocker, 20min prior to reperfusion. Cell viability was determined by propidium iodide fluorometry and LDH release. To monitor mitochondrial Ca^{2+} and reactive oxygen species (ROS), myocytes were loaded with 2μM Rhod-2 and 10μM chloromethyldichlorofluorescin (DCF) diacetate for 20min at 37°C prior to reperfusion, respectively. Some cells were loaded with 1μM calcein for 20min at room temperature before ischemia to monitor onset of the MPT. DCF fluorescence was also assessed by a multiwell fluorometry. Confocal images of Rhod-2, calcein, DCF fluorescence were collected with a Zeiss LSM 410 microscope. **RESULTS:** Reoxygenation at pH 7.4 decreased cell viability to 40% after 2h (n=4). In contrast, reoxygenation at pH 6.2 or with CsA at pH 7.4 prevented cell killing. Confocal images of DCF and Rhod-2 revealed that mitochondrial ROS generation increased after reperfusion at pH 7.4 whereas mitochondrial Ca^{2+} remained unchanged. CsA did not block increased ROS formation after reperfusion, as measured by DCF fluorometry. The MPT occurred within 5min of reperfusion as assessed by confocal microscopy from the redistribution of mitochondrial calcein into cytosol, an event that was inhibited by CsA. **CONCLUSION:** The MPT is a key mechanism inducing pH-dependent reperfusion injury to myocytes. Mitochondrial ROS generation may also help trigger the MPT after reperfusion.

163 UPREGULATION OF ENDOTHELIN SYSTEM IS INVOLVED IN NICOTINE-INDUCED CARDIOTOXICITY IN RATS WITH ACUTE MYOCARDIAL INFARCTION.

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The adverse effects of nicotine at the exposure level found in mild cigarette smokers on the pre-existing ischemic infarction myocardium are clinically concerned, but have received litter attention experimentally. Studies have shown that smokers had higher plasma concentrations of endothelins (ETs) than non-smokers, and ET elevation is implicated in heart failure in both humans and animal models. This study was undertaken to test the hypothesis that compromised heart has higher sensitivity to nicotine toxicity due to upregulation of the ET system. Adult rats weighing about 200 g were subjected to a surgery procedure of left coronary artery ligation to induce myocardial infarction (MI). A sham surgery was applied to control groups. Twenty-four hours after the surgery, a dose of 0.8 mg/kg nicotine, which has been shown to produce plasma concentrations of nicotine comparable to that found in mild cigarette smokers, was injected through tail vein in rats with acute MI along with sham-operated controls. The results showed that plasma total ET concentrations, determined by an ELISA assay, were significantly elevated in the nicotine-treated rats and ET receptor density, measured by an immunohistochemical assay, was markedly elevated in the MI heart. Importantly, a ventricular arrhythmia, in the form of premature ventricular complexes, occurred in the MI heart and nicotine significantly increased this incidence. Myocardial dysfunction as measured by an in situ cardiac performance analysis was also dramatically worsened in the MI rats after nicotine exposure. These results thus demonstrate that upregulation of ET system plays an important role in nicotine-induced cardiotoxicity and overexpression of ET receptors in the ischemic myocardium would make the heart more susceptible to the deteriorating effect of subsequent bursts of ETs by exposure to nicotine. (Supported in part by NIH grants HL 59225 and HL63760).

164 THE UTILITY OF THE HERG BINDING ASSAY TO ASSESS QT PROLONGATION AND A STRATEGY FOR THE DISCOVERY PROCESS.

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Some drugs can cause QT prolongation, arrhythmias and occasionally torsades de pointes in humans. Drugs elicit QT prolongation by blocking the hERG channel carrying the IKr current.In vitro assays are currently utilized in preclinical studies to assess a compounds potential to interact with the hERG channel, the hERG binding assay being one. The objective was to test the utility of the hERG binding assay as a low-cost, high throughput screen to assess compounds with a potential to prolong QT *in vivo*. A set of 207marketed compounds were selected at random based on availability and purity. Membranes from HEK-293 cells stably transfected with hERG cDNA(from the University of Wisconsin, Zhou et al, 1998)were prepared and incubated with compounds ranging from 0.0016 uM to 10uM and ligand (3H-Dofetilide, 4 nM) in duplicate 96-well plates for 80 minutes at 22C. The percent inhibition of ligand uptake by the test compound was assessed by counting radioactivity in a Packard Topcount and an inhibiton Ki value determined. Of the 207 compounds, 181 (87%) were weak binders of the hERG channel (Ki>5uM) and except for four, did not have reports of QT prolongation in pateints (PDR). The four compounds were associated with rare incidences of QT prolongation (PDR) and thus false negatives (2%) in the hERG binding assay. Eleven of the 207 compounds were potent hERG binders (Ki <1 uM) and were associated with adverse QT prolongation problems in patients. The 14 compounds that were moderate binders (Ki's of 1 -5 uM) were associated with QT prolongation (PDR), and had other complexing factors such as other ion channel activity. Verapamil is a false positive in the assay as it also is a Ca++ channel blocker negating the hERG binding effects *in vivo*. Thus, with a concordance rate of >96%, the hERG binding assay is a useful tool as a first level high throughput screen to assess QT issues early in discovery and reduce attrition in development due to QT issues. A testing strategy was developed based on these results.

165 CYCLOSPORIN A PROTECTION AGAINST pH-DEPENDENT ISCHEMIA/REPERFUSION INJURY TO NEONATAL MYOCYTES: ROLE OF Ca^{2+} AND GLYCOLYSIS.

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In cardiac ischemia, ATP hydrolysis and lactic acid production by glycolysis cause a naturally occurring acidosis that protects against onset of necrotic cell death. pH protection is lost after reperfusion as pH returns to physiologic levels. Here, our aim was to assess the ability of cyclosporin A (CsA), an inhibitor of the mitochondrial permeability transition (MPT), to prevent pH-dependent ischemia/reperfusion (I/R) injury in isolated neonatal rat cardiomyocytes. Myocytes (4 to 8 days in culture) were exposed to 4 h of anoxia at pH 6.2 in Krebs-Ringers-HEPES (KRH) buffer to simulate ischemia. After anoxia, myocytes were re-oxygenated with KRH at pH 7.4 or 6.2 to simulate reperfusion. Cell killing was monitored by propidium iodide fluorometry using a fluorescence plate reader. At 1 mM Ca^{2+}, cell killing did not increase during ischemia but progressively increased to 47 ± 7% during 2 h of reperfusion. Reperfusion-induced killing was strongly dependent on Ca^{2+}, being nearly negligible with 0.2 mM Ca^{2+} and maximal with 1 mM Ca^{2+}. CsA blocked most I/R killing of myocytes in 0.5 to 1 mM Ca^{2+} but was only weakly effective at 1.5 and 2 mM Ca^{2+}, although reperfusion at pH 6.2 completely prevented cell killing at 2 mM Ca^{2+}. However, when myocytes were reperfused with CsA plus 20 mM glucose during reperfusion, protection against cell killing became virtually complete at all Ca^{2+} concentrations examined. This enhanced protection was lost if 2-deoxyglucose was used during the ischemic phase only to inhibit glycolysis. These results suggest that a CsA-sensitive MPT contributes to pH-dependent killing in

this model for I/R injury to neonatal cardiomyocytes, at least at $Ca^{2+} \leq 1$ mM. At higher Ca^{2+}, an unregulated, CsA-insensitive MPT may be occurring. Glucose may add to the protection afforded by CsA by decreasing intracellular ADP and Pi, compounds that promote MPT onset.

166 CONSIDERATIONS FOR ANESTHETIZED CARDIOVASCULAR SAFETY PHARMACOLOGY STUDIES: EFFECT OF ISOFLURANE ANESTHESIA ON HEART RATE AND QT INTERVAL IN THE CYNOMOLGUS MONKEY.

M. Vézina, C. Copeman and P. Shaver-Walker. *Infusion, Pharmacology and Neurotoxicology, CTBR, Senneville, QC, Canada.* Sponsor: D. Jones.

Isoflurane is a common anesthetic used in preclinical laboratories due to its relative safety and rapid recovery time. Though the current ICH S7a guidelines recommend use of a conscious model for cardiovascular safety pharmacology, there are instances where an anesthetized model is appropriate. In this comparison, systemic blood pressure and ECG (modified lead II) were obtained from 5 cynomolgus monkeys on 2 occasions using a DSI telemetry system or Schiller ECG recorder. On the first occasion, unrestrained animals were monitored in the conscious state for 24 hours and ECGs recorded every 30 minutes. On the second occasion, the same animals were anesthetized with Isoflurane/oxygen and ECGs were recorded every 15 minutes for 2 hours. As expected, heart rates (HR) were reduced and QT interval prolonged in the anesthetized state compared to the conscious state. However, the heart rate reductions and QT interval lengthening were not static during the 2 - hour anesthetized state. Over the course of 2 hours HR progressively declined by approximately 25% and QT progressively increased by 21%. The magnitude of these changes was relatively consistent between animals. This anesthetic-related effect can impact directly on the outcome of a study and should be considered when designing and interpreting anesthetized cardiovascular safety pharmacology studies. Using the anesthetized model over the course of several hours can lead to progressive cardiac depression that may obscure or enhance an effect on the QT interval, or the risk of an adverse reaction to a test material.

167 HOMOCYSTEINE INDUCES PROLIFERATION, STIMULATION OF ERK1/2 AND CALCIUM INFLUX IN VSMC, WHICH ARE INHIBITED BY THE NMDA RECEPTOR ANTAGONIST MK801.

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Homocysteine(HYC) is recognized as an independent risk factor for the development of atherosclerosis. However, the mechanism by which HYC acts remains unknown and controversial. Recent reports have suggested that the NMDA receptor may play a pivotal role in homocysteine-induced vascular effects. The current investigation was undertaken to better define the relationship between NMDA receptors and homocysteine in cultured vascular smooth muscle cells (VSMC). In these experiments VSMC were obtained from the thoracic aorta of male New Zealand White rabbits (2-4 months of age). Smooth muscle cells were cultured in supplemented media (M199 with Earle salts with L-glutamine and 10% FBS), which was changed every other day, and the cells were maintained at 37°C and 5% CO2. Initially it was determined that the NMDA receptors were present on VSMC. It was then demonstrated that HYC induced the proliferation of these cells, in a dose dependent manner. Experiments that were performed in the presence of MK801, an antagonist of the NMDA receptor, demonstrated a significant reduction in the amount of proliferation. One transduction pathway that mediates mitogenic activity is the mitogen-activated protein kinase (MAP-kinase) pathway. Therefore, we examine whether HYC could stimulate ERK1/2. VSMC were exposed to various concentrations of HYC (0-50uM) with or without 10uM MK801 for various durations (2, 30, 60 seconds, or 2, 5, 10 minutes). These experiments demonstrated that every concentration of HYC tested stimulated the phosphorylation of ERK1/2 at all time points. The activation of ERK1/2 was inhibited by PD98059, a specific inhibitor of ERK1/2 phosphorylation. Similarly, the phosphorylation of ERK1/2 was inhibited by MK801. HYC also stimulated an influx of Ca+2 in VSMC, which was inhibited by MK801. Therefore, HYC induces proliferation of VSMC *via* the MAP-kinase pathway, which appears to be mediated by NMDA receptors. Support for this research was provided by NHLBI grant # 5RO1HL58969-03

168 BACKGROUND HEART RATE AND QT INTERVAL DATA IN THE ANESTHETIZED BEAGLE DOG.

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The use of an anesthetized model for cardiovascular safety pharmacology can lead to confounding results. For this reason, the ICH S7a guideline thus suggests the use of a conscious model, however, if a test material is intended to be administered under anesthetic, or severe clinical signs prevent the achievement of a suitable safety factor in the conscious model, the anesthetized model can be considered a suitable alternative. Typically, the anesthetized model requires the animals to be under anesthetic for a considerable amount of time to allow for instrumentation, stabilization and several doses of control and test material (eg up to 12 hours). This background data represents 8 beagle dogs that had been under Isoflurane/oxygen anesthesia for approximately 6 hours prior to 4 further hours of monitoring. ECGs were obtained at 15-minute intervals for the first hour, followed by 30 minute intervals for the remaining 3 hours. The effects on heart rate and QT interval over the 4 hour monitoring period were quite variable among dogs. Some individuals exhibited little cardiac depression and remained stable - even comparable to conscious dogs but the majority exhibited varying magnitudes of slowly progressive heart rate reduction and QT interval prolongation. The impact of long-term anesthesia on these critical safety parameters should be considered when choosing a conscious or anesthetized model and also when designing and interpreting data from anesthetized dogs.

169 MECHANISMS OF ARSENIC TOXICITY IN VASCULAR DEVELOPMENT.

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Arsenic is an abundant toxin in ground water and soil around areas with extractive industries. Exposure to arsenic is linked to several developmental defects in the nervous system and limbs, pregnancy complications and miscarriage. Vascular defects are often the underlying basis for limb deformations and developmental anomalies in other systems and vascular abnormalities often form the basis for placentation defects linked to miscarriage. The overall goal of this project was to define the developmental mechanisms for how arsenic exposure causes developmental defects and miscarriage, and to characterize the molecular mechanisms for arsenic mediated alteration of endothelial cell physiology. We hypothesize that arsenite toxicity causes defects in vascular development, predisposing the embryo to other developmental anomalies and miscarriage. The aims are: 1 Determine how arsenite exposure affects angiogenesis *in vitro*. Cultured bovine aortic endothelial (BAE) cells on Matrigel were exposed to 0, 10 um, 20 um, 50 um, 100 um arsenite for 7 days. The data suggest that increasing concentrations of arsenite inhibits angiogenesis *in vitro*. 2. Characterize the effects of arsenite exposure on embryonic mice and vascular development. Timed-pregnant FVB/NJ mice were exposed to 1 ppm, 75 ppm and 150 ppm arsenite in their drinking water for duration of pregnancy and until weaning (21 days). Litter size was recorded and pups were weighed weekly. The data suggest that there were no significant differences observed in either litter size or pup weight for arsenite exposures of 1 ppm. However, there were significant differences for 75 ppm exposures. Arsenite exposures of 150 ppm were toxic to pregnant females, but not control (non-pregnant) females; suggesting that pregnancy may cause increased sensitivity to arsenic exposure.

170 TELEMETRY MONITORING OF CARDIOVASCULAR AND RESPIRATORY FUNCTION IN MALE CYNOMOLGUS MONKEYS.

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A primate telemetry system (Open-A.R.T./Ponemah: DSI/Gould) was evaluated and compared to conventional systems for monitoring cardiovascular and respiratory function in male cynomolgus monkeys surgically implanted with a telemetry transmitter (TL11M2-D70-PCT). The animals were sedated, surgically prepared and maintained on isoflurane gas anesthesia. A calibrated transducer was used for direct blood pressure and heart rate comparisons. The transducer catheter was inserted through the contralateral femoral artery to approximately the same location in the dorsal aorta as the telemetry catheter. A Vetronics VitalScan system was used for ECG and temperature monitoring. All ECGs were over-read. A trained observer performed direct respiratory count. Each animal was subjected to the following stimuli: Elevated temperature, mechanical ventilation, epinephrine, Dopram V (R) and sodium pentobarbital. Parameters were evaluated before and after the various manipulations. Telemetry files were analyzed for systolic and diastolic blood pressure, heart rate, respiratory rate, temperature and ECG parameters. Blood pressure comparison revealed a high correlation coefficient (r=0.99) for both systolic and diastolic blood pressure with pressures fluctuating from 196 to 31 mmHg following epinephrine or sodium pentobarbital respectively. Heart rate was directly correlated to the transducer pulses (r=0.99). Printed ECG waves evaluated by a veterinary cardiologist revealed a high degree of accuracy on both systems used. All ECG parameters were highly correlated (r=0.90 to 1.00) to the manual measurements. The telemetry system accurately measured mechanical ventilation, hyperventilation, apnea, shallow respiration and transitory increase in respiratory rate following Dopram V (R) infusion. The overall correlation coefficient for respiration was r=0.95. In conclusion, the telemetry system was able to monitor and accurately report cardiovascular and respiratory parameters over the expected range of physiological values.

171 CARDIOTOXICITY STUDY OF NSC-638850 (UCN-01) AND CYTOSTAR (ARA-C) GIVEN ALONE OR IN COMBINATION TO BEAGLE DOGS.

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Combination chemotherapy with multiple drugs is commonly used in cancer therapy. This study evaluated the cardiotoxicity of the chemotherapeutics UCN-01 and Ara-C given individually or in combination. Pairs of dogs were infused intravenously for up to 96 hours with vehicle, 1.2 mg/kg UCN-01, 5.0 mg/kg Ara-C or combinations of UCN-01/Ara-C at 1.2/2.5 mg/kg, and 1.2/5.0 mg/kg. Caridiotoxicity parameters evaluated included blood pressure, heart rate, ECGs, body temperatures, clinical pathology, and histopathology. All but one of the dogs exposed to UCN-01 alone or in combination were euthanized in a moribund condition. Clinical signs included lethargy, thin, pallor and mucoid feces. Body temperatures were increased in all animals receiving UCN-01. Persistent post-exposure cardiovascular evaluations in animals exposed to UCN-01 or UCN-01/Ara-C included increased heart rate, attributed to a decrease in blood pressure, and decreased systemic blood pressures, possibly due to a negative inotropic effect, a decrease in preload or vascular resistance, venodilation or a combination thereof. ECGs of several animals exposed to UCN-01/Ara-C exhibited J-point depression, which has attributed to subendocardial changes noted in the ventricular free wall. The heart rate changes and decreased systemic blood pressure were not seen in the Ara-C group. No changes in ECGs were seen in the animals exposed to either the Ara-C or UCN-01 group. Histopathology revealed UCN-01-related necrosis and inflammation of veins, injection sites, and medium-sized arteries, which were possibly related to caustic action. Additional UCN-01-associated lesions included depletion of gut-associated lymphoid tissue, atrophy of lymph nodes, and infiltration of neutrophils in the mantle zone of splenic lymphoid follicles. Conducted under contract N01-CM-87028, Division of Cancer Treatment of the National Cancer Institute.

172 ALLYLAMINE INDUCES VASOSPASM IN HUMAN CORONARY ARTERY BYPASS GRAFT BLOOD VESSELS *IN VITRO*: ROLE OF SEMICARBAZIDE-SENSITIVE AMINE OXIDASE.

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Allylamine (AA) is a cardiovascular toxicant that produces rat coronary artery vasospasm *in vitro*. To test the applicability of this model for human vasospasm, we exposed uncontracted and norepinephrine-precontracted (NE, 1 μM) human coronary artery bypass graft (CABG) blood vessels (internal mammary artery, IMA; radial artery, RA; saphenous vein, SV) to AA (1-1, 000 μM) *in vitro*. AA (1-100 μM) stimulated concentration dependent relaxations in precontracted IMA and RA or contractions in precontracted SV, but induced vasospasm at 1 mM in all three blood vessels. Vasospasm was defined as a sustained hypercontracture with intractability (inability to relax). About 5 min after addition of 1 mM AA, vessel tension increased rapidly and at times exceeded 300% of the NE-precontraction tension. Vasospasm was intractable as evidenced by significant maintenance of NE-induced tension following 3 buffer changes over >30 min. Vasospasm and intractability were both significantly blocked by the pretreatment of IMA with 1 mM semicarbazide implicating semicarbazide-sensitive amine oxidase (SSAO) enzyme activity in AA-induced vasospasm. The presence of CABG blood vessel SSAO activity was confirmed by radiometric assay. Naïve IMA were also exposed to either AA, benzylamine (BZA) or methylamine (MA) for comparison. Both BZA and MA are known SSAO substrates and each produced concentration dependent relaxations in NE-precontracted IMA without incidence of vasospasm or intractability. Thus, AA produces vasospasm in human CABG blood vessels *via* an SSAO-dependent pathway, and acrolein is a likely candidate for mediating vasospasm. This work supported by the ORSP at the UWEC and NIEHS #1 R15 ES011141-01.

173 INHIBITION OF PEROXYNITRITE-INDUCED DAMAGE IS INVOLVED IN METALLOTHIONEIN PREVENTION OF DIABETIC CARDIOTOXICITY.

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Previous studies have shown that oxidative stress caused by superoxide accumulation and peroxynitrite-induced injury is a major cause of diabetic cardiotoxicity, and metallothionein (MT) protects the heart from various oxidative injuries. In the present study, cardiac-specific MT-overexpressing transgenic (MT-TG) mice were used to investigate the role of MT in prevention of diabetic cardiotoxicity. Diabetes was induced by streptozotocin (STZ). Both MT-TG and wild-type (WT) mice were induced the same diabetic manifestations including increased blood glucose, glycated hemoglobin (HbA1), serum lipid peroxidation, increased renal weight, and decreased body-weight gain. However, serum creatine phosphokinase (CPK) activity, an indicator of cardiotoxicity, was increased in the WT diabetic mice, not in the MT-TG diabetic mice. Myocardial pathological changes examined by light and electron microscopy, along with cardiac dysfunction assessed by cardiac hemodynamic analysis, were observed in the WT diabetic mice. These cardiac pathological and functional changes were almost completely inhibited in the MT-TG diabetic mice. Correspondingly, peroxynitrite-induced damage detected by 3-nitrotyrosine and the accumulation of superoxide also significantly increased in the WT-diabetic hearts, but not in the MT-TG diabetic hearts. Furthermore, *in vitro* studies have shown that MT directly interacts with peroxynitrite and prevents peroxynitrite-induced lipid and protein damage. These results thus demonstrated that MT prevents the early-phase diabetic cardiotoxicity most likely by suppression of oxidative damage in the myocardium. (Supported by NIH, ADA, Philip Morris, Jewish Hospital Research Foundation and University of Louisville School Medicine)

174 APPLICATION OF REACTION NETWORK MODELING TO A PRIORITY CARCINOGENIC ENVIRONMENTAL POLLUTANT, BENZO(a)PYRENE.

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Reaction Network Modeling (RNM) is a new approach for predicting the complex biotransformation pathways of xenobiotics. The reaction network is automatically generated based on the chemical structures of the parent compound and reaction rules. Graph theory is used to encode chemical structures and reaction rules at the molecular level. Benzo[a]pyrene (BaP) was used as the substrate for the first application of RNM to biochemical reactions since it is a human carcinogen as well as an ubiquitous environmental pollutant. The reactions used in this model include epoxidation of double bonds, epoxide hydrolysis, NIH shift of arene oxides to phenols, and oxidation of phenols to quinones. RNM provided good predictions for the time course profiles of BaP metabolites from data reported by Gautier et al. (*Chem Res Toxicol*, 1996, *9*:418-425). The rate constants of BaP RNM were further measured by biotransformation studies using purified human cytochrome P450 1A1 and epoxide hydrolase. HPLC methods were used to analyze 12 BaP metabolites including dihydrodiols, phenols, tetrols, and quinones with average detection limit in the nM range. The rate constants of BaP biotransformation measured by experiments were then used to establish quantitative structure/reactivity correlations (QSRCs). When RNM is applied to other chemicals, QSRCs can be used to predict their rate constants based on quantum chemistry. The top candidates for further application of RNM are the air pollutants benz[a]anthracene and benzo[b]fluoranthene. From there, RNM could be used to predict the complex reaction networks arising from exposure to mixtures of polycyclic aromatic hydrocarbon air pollutants that result from incomplete combustion of petroleum fuels. This work was supported by NIEHS Grant # R01 ES09655.

175 A REACTION NETWORK MODEL FOR CYP2E1-MEDIATED METABOLISM OF TOXICANTS.

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One of the principal catalysts in the Phase I metabolism of toxicants is cytochrome P450-2E1 (CYP2E1). Substrates for this isozyme include low molecular weight, hydrophobic and carcinogenic species such as benzene, chloroform, carbon tetrachloride, vinyl chloride, and trichloroethylene. Metabolism of chemical substrates and substrate mixtures often involves complex reactions in which the species associated with one reaction are constituents of numerous other reactions. This interdependent, coupled set of reactions can be thought of as a 'reaction network', and can occur for even relatively simple systems. The objective of this project is to develop a computer-based biochemical reaction network modeling framework that can create detailed and accurate predictions of the interlinked networks and kinetics resulting from CYP2E1-mediated metabolism of both pure species and chemical mixtures. To carry out this task, the modeling tool comprises a number of software modules, each of which is responsible for a separate 'phase' of the simulation. These phases

include testing of substrate binding feasibility, performing virtual chemistry to predict intermediate and product species, formulating reaction rate equations, constructing the network topology, and computing the reaction kinetics and time-dependent species concentrations. To date, this framework has predicted the reaction networks for the metabolism of several of the compounds on the CERCLA Priority List of Hazardous Substances that are catalyzed by CYP2E1. When fully realized, we expect this modeling methodology to be an important addition to the toolkit for investigators seeking to quantify the impact of chemicals and chemical mixtures on human health. This work was supported by a Mentored Quantitative Research Career Development Award to B. Reisfeld from the National Institutes of Health (NIEHS K25 ES11146-02).

176 A MATHEMATICAL MODEL TO DETERMINE *IN VITRO* CLEARANCE IN THE SANDWICH RAT HEPATOCYTE CULTURE.

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For risk assessment it is important to have knowledge of the kinetics of a compound. *In vitro* kinetics can be extrapolated to *in vivo*kinetics using physiologically based pharmacokinetic models. To determine the *in vitro* clearance of slowly metabolized compounds, long-term systems such as the sandwich hepatocyte culture are preferred over the more commonly used short-term systems, such as microsomes, liver slices, hepatocyte suspensions and primary cultures. Since the hepatocytes in a sandwich culture are located between two layers of collagen, the upper collagen layer will form a transport barrier between medium and cells. To determine *in vitro* clearance, these molecular diffusion coefficients as well as protein binding should be taken into account. A mathematical model was developed in which these processes were incorporated using a mechanistic approach. The 'non-stirred sandwich model' was evaluated using the slowly metabolized compound tolbutamide as a reference. Tolbutamide was added to the sandwich cultures; subsequently the decrease of tolbutamide and the increase of its metabolites were measured over time. Molecular diffusion coefficients and protein binding were determined in separate experiments. Using the non-stirred model *in vitro* clearance was calculated to be 2.4 µl/min. This finding was in accordance with experiments in which the clearance of tolbutamide was determined in liver slices using a similar approach. In conclusion, the *in vitro* clearance of tolbutamide can be determined in sandwich hepatocyte cultures using the mathematical approach described above.

177 PARAMETERIZATON OF A BIOLOGICALLY BASED KINETIC (BBK) MODEL OF THE ISOLATED PERFUSED RAT LIVER (IPRL): BROMOSULPHOPHTHALEIN (BSP) AND CADMIUM (CD) KINETICS.

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A previously developed BBK model (Foy et al., Toxicol.Sci. 50:20-29, 1999) was parameterized with experimental studies of clearance for BSP, the BSP glutathione conjugate (BSP-GSH), or Cd, in the IPRL. The goal of this work is to provide a mathematical structure for exploring the mechanisms governing the kinetics of water soluble compounds, using the IPRL and *in vitro* data, to make predictions in the whole animal system. To parameterize the model, *in vitro* data were collected for the binding and S-9 metabolism of BSP to BSP-GSH. Proper incorporation of *in vitro* metabolic data into the IPRL BBK analysis required the determination of albumin binding of BSP. Also, non-enzymatic conjugation of BSP contributed significantly to the overall metabolism of BSP, and was incorporated into the enzymatic analysis. IPRL studies were conducted with BSP clearance under various physiological conditions, including a range of starting concentrations. To experimentally simulate choleostasis, the bile duct was ligated and the effects on BSP kinetics in the perfusate were followed over time. Cd kinetics in the IPRL were studied at two different albumin concentrations, 0.25% and 4%, to modulate the levels of free Cd available in *in vitro* and *in vivo* experiments. Starting concentrations for these studies ranged from 10 to 1000 µM, depending on the particular albumin concentration being employed. Comparison of the calculated free Cd in the perfusate and the onset and degree of cessation of bile flow, provided the basis for understanding critical mechanisms of Cd kinetics for BBK model development. IPRL kinetic studies starting with the BSP-GSH conjugate were conducted to allow for BBK model parameterization of the conjugate for better tracking of conversion of BSP parent to the metabolite. Parameterization of the BBK model for BSP and Cd allowed analysis of other IPRL studies with these compounds and provided verification of the general model structure.

178 A NONSTEADY STATE MODEL FOR THE TIGHT-BINDING INHIBITION OF THYMIDYLATE SYNTHETASE BY 5-FLUOROURACIL.

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5-Fluorouracil (5-FU) is a widely used chemotherapeutic drug and teratogen that was chosen as a prototypic toxicant to construct a biologically based dose-response (BBDR) model (Setzer et al., 2001). Part of the BBDR model simulates the inhibition of thymidylate synthetase (TS), an essential enzyme for DNA synthesis. TS methylates deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP). A metabolite of 5-FU, fluorodeoxyuridylate (FdUMP), inhibits TS by forming a tight-binding complex. The resulting low level of dTMP leads to adverse effects on cell growth and morphology. The FdUMP/TS inhibition model needed to: 1) account for an observed 3-5 hour delay between peak 5-FU blood concentration and peak TS inhibition; and 2) simulate the recovery of TS activity that occurs within 24-48 hours following a bolus 5-FU dose. A steady state model for a tight-binding inhibitor provided an initial simulation of both the delay in peak inhibition and the recovery of TS activity. The steady state model simulates the competition between FdUMP and the endogenous dUMP substrate for free TS binding sites. Nonsteady state conditions, however, result from a constitutive increase in TS and dUMP as well as potential up-regulation of TS. A nonsteady state model was developed that provided a better fit of the data. The nonsteady state model requires considerably more kinetic data to develop the microconstants for the different reaction rates, and the parameters for the constitutive increases in substrate and TS levels. The nonsteady state model, however, offers more heuristic value and insight into how alterations in enzyme and natural substrate levels affect the apparent rate of enzyme inhibition and recovery. The apparent concentration-time curve for the toxic metabolite, FdUMP, also depends upon dUMP and TS levels. Consideration of these variables leads to a better understanding of the mechanisms underlying nonlinear dose-response curves and thresholds for adverse effects. [This abstract does not necessarily reflect EPA policy.]

179 EVALUATION OF INTERSPECIES VARIABILITY DURING NEOCORTICAL NEUROGENESIS USING BIOLOGICALLY BASED COMPUTATIONAL MODELS.

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From an evolutionary perspective, neocortical expansion is a hallmark of human speciation which has been functionally linked to the social and intellectual evolution of humans. We are investigating approaches for evaluating cross-species differences in neocortical development and its responsiveness to environmental injury. Neocortical neurogenesis is a critical sensitive window for numerous human neurodevelopmental toxicants including radiation, methyl mercury, and ethanol. The final number of neocortical neurons generated depends on several factors including the number of founder cells at the beginning of neurogenesis, the rate of cell division, the duration of neurogenesis, the modes of cell division (proliferative or terminal), and finally the extent of cell death. Here we build on a previously developed rodent neocortical neurogenesis computational model to quantitate the differences in rodent versus primate (rhesus monkey) neurogenesis to build our novel primate neocortical model. The genesis of neurons for the rodent neocortex takes approximately 6 days and 11 cell cycles, primate neocortical neurogenesis can span 60 days and 28 division cycles. Furthermore, while the rodent cell cycle lengthens as neurogenesis progresses, the cell cycle length decreases with time in the rhesus monkey. Simulations suggest the large increase in duration and total neuron output of neocortical neurogenesis in the primate model can increase the suseptibility of the primate neocortex to neurodevelopmental toxicants effecting neurogenesis. Adding mechanistic data on ethanol induced cell cycle inhibition into our primate neocortical neurogenesis models shows a greater deficit in total neuron number is predicted in the adult primate neocortex compared with the rodent neocortex. These preliminary results highlight the important role of computational modeling in quantitating interspecies differences in toxicodynamic processes.

180 EFFECTS OF DIFFERENCES IN NASAL ANATOMY ON AIRFLOW DISTRIBUTION: A COMPARISON OF THREE INDIVIDUALS.

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Differences in nasal anatomy and respiratory airflow patterns between different human individuals may cause significant differences in the regional dose of inhaled gases in the nasal passages and subsequently the lung. For water-soluble, reactive gases, nasal surface area and volume may be predictors of nasal scrubbing efficiency,

which can be estimated using computational fluid dynamics (CFD) models. To construct such models, MRI scans of the head were digitized for a number of individuals in a study led by Dr. Ray Guilmette and the Lovelace Respiratory Research Institute, and nasal surface area and volume were calculated. Three individuals were selected whose ratio of nasal surface area to volume (SAVR) represented low and high values: Subject A = 0.74, Subject 14 = 0. 94, and Subject 12 = 1.03. Nasal CFD models for these individuals were constructed by a semi-automated process that provides input to a commercial mesh generator (Gambit, Fluent, Inc., Lebanon, NH). Steady-state inspiratory airflow at 7.5 L/min was calculated using commercial CFD software (FIDAP, Fluent, Inc., Lebanon, NH). Ventral, middle, and dorsal flow allocation percentages were computed for each subject. Flow in Subject 12 favored ventral over middle (48% versus 34%) whereas flow in Subjects A and 14 favored middle over ventral (55% and 58% versus 37% and 39%, respectively). In all three cases, dorsal flow was the lowest of the three regions. More swirling flow in both the vestibule and the nasopharynx was evident in Subjects A and 14 than in Subject 12. These simulations indicate that there are interindividual differences in bulk airflow patterns in the nose. Concurrent studies are focusing on the effects of inhaled gas uptake based on these flow simulations. These studies will test the dependence of potential regional dose differences and nasal uptake efficiencies on SAVR.

181 EFFECTS OF DIFFERENCES IN NASAL ANATOMY ON UPTAKE OF INHALED GASES: A COMPARISON OF THREE INDIVIDUALS.

J. S. Kimbell[1], R. A. Segal[1], D. L. Kalisak[1], R. B. Richardson[1] and G. M. Kepler[2]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC and [2]Consultant, Chapel Hill, NC.*

Differences in nasal anatomy, airflow, and patterns of uptake and deposition among different human individuals may cause significant differences in the regional dose of inhaled gases, vapors, and particles in the nasal passages and subsequently the lung. Computational fluid dynamics (CFD) models can quantify the contribution of anatomical variation to risk assessment uncertainty and reduce the reliance on default uncertainty values. For water-soluble, reactive gases, nasal surface area and volume may be predictors of nasal scrubbing efficiency. To test this hypothesis, MRI scans of the head were digitized for a number of adult individuals by Dr. Ray Guilmette and the Lovelace Respiratory Research Institute, and nasal surface area and volume were calculated. Three subjects were selected whose ratio of nasal surface area to volume (SAVR) represented a range of values: 0.74, 0.94, and 1.03 for Subjects A (male), 14 (male), and 12 (female), respectively. Anatomically-realistic CFD models for the nasal passages of these individuals were constructed by a semi-automated process that provided input to a commercial mesh generator (Gambit, Fluent, Inc., Lebanon, NH). Steady-state inspiratory airflow at 7.5 L/min and inhaled gas uptake were simulated for a reactive, water-soluble, low-molecular-weight gas using commercial CFD software (FIDAP, Fluent, Inc., Lebanon, NH). Nasal uptake efficiency was estimated to be 95, 97 and 98% for the highest, middle, and lowest SAVR individuals, respectively, indicating that overall uptake was not highly sensitive to SAVR. Uptake efficiency in the nasal vestibule was higher in Subjects A and 14 than 12, and higher in the turbinate region in Subject 12 than Subjects A and 14, suggesting that SAVR should be calculated regionally. These studies and their extension to additional individuals form a basis for developing an empirical means of characterizing populations that may be at a higher risk for developing respiratory tract effects than the general population.

182 BUILDING BBDR MODELS FOR CELL SIGNALING PATHWAYS USING TRANSGENIC ANIMAL MODELS.

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We have developed a Biologically Based Dose-Response (BBDR) model utilizing stochastic processes to describe the interaction of p21 and contaminant exposure to methylmercury (MeHg) during fetal development. The model was developed using data from a previously published study (Mendoza, et al., TAP 178(2), 117-125, 2002) of transgenic mouse fetal fibroblasts, p21(+/+), p21(+/-), and p21(-/-), collected at gestation day 14 and cultured for 3-4 passages before exposure. The BBDR model examines the role of p21 cell signaling pathways in cell cycle checkpoints, and how the fraction of cells in different parts of the cell cycle are altered by doses of 0, 2, 4 and 6 μM MeHg at 24 and 48 hours after exposure. Multiple passages through the cell cycle after exposure to MeHg were measured by flow cytometry using double labeling with Hoescht 33258 and ethidium bromide. Cytoxicity was quantified using an LDH assay. The cell cycle is regulated by a number of proteins that have redundant and cross-talk pathways. Using a BBDR model of transgenic p21 animals allowed us to bound of the influence of p21 pathways on the the MeHg induced cell cycle inhibition. The results show that p21 alters the dynamics of MeHg inhibition of the cell cycle. After exposure to 6 μM MeHg the p21 null (-/-) cells had 85% lower rates of inhibition than the p21(+/+) cells and 65 % lower than the p21(+/-) cells at G1 checkpoints. Lower doses of MeHg had intermediate rates of inhibition in a dose dependent manner. However p21 status had no effect on G2/M accumulation induced by MeHg. Acknowledgement: this work was funded by: USEPA & NIEHS grants R 826886-01 and 5 P01 ES09601-02, Center for Child Environmental Health Risk's Research; NIEHS grant 1 R01 ES10613-01, Cell Cycle Regulation in Metal Developmental Toxicity; and NIEHS grant 5 P30 ES07033, Center for Ecogenetics and Environmental Health.

183 TUMOR INCIDENCE IN HUMANS AND RODENTS: EFFORTS TO RECONCILE THE OBSERVED DATA WITH BBDR MODEL PREDICTIONS.

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Because human carcinogenic potency is most often estimated from animal bioassay data, understanding potential species differences in the carcinogenic process is a critical need in risk assessment. We compared human tumor incidence data from the Surveillance, Epidemiology and End Results (SEER) database of the National Cancer Institute to the incidence of spontaneous tumors in control animals reported in the Toxicology Database Management System (1974 to 1995) of the National Toxicology Program. Tumors strongly associated with environmental or lifestyle factors (e.g., lung or skin tumors) were not included. Rates of fatal tumors, which occurred prior to the conclusion of the rodent studies, were compared with lifetime tumor risks reported in humans, which are calculated as the primary cause of disease, death or disability. The tumor incidence in humans and laboratory rodents was found to be relatively similar. For example, the background lifetime probability of being diagnosed with a liver tumor in the US during 1997-1999 was approximately 0.6% whereas the corresponding probabilities in rats and mice were approximately 2% and 34%, respectively. The cross-species similarity was also observed for other tumor sites. These results are inconsistent with the results of biologically-based tumor incidence models. For example, when the approach of Kopp-Schneider et al. (1994) is used to predict tumor incidence in mice, rats and humans, the predicted lifetime probability of liver tumors in humans exceeds that in rodents by a factor of over 1 million. Previous work we have conducted suggests that model inputs (e.g., rates of cell division, death and mutation) are similar across species and would not reconcile model predictions with observed tumor incidences. We therefore consider information on other species-specific differences (e.g., telomere length, DNA methylation) which are not explicitly incorporated in most BBDR models but may be of use for explaining the observed inter-species patterns in tumor incidence.

184 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP (QSAR) EVALUATIONS OF THE PHARMACOLOGY AND TOXICOLOGY OF HISTONE DEACETYLASE INHIBITORS.

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Histone deacetylation is a fundamental step in cancer cell division. Thus histone deacetylase inhibitors (HDAIs) are potential targets for cancer therapy. In order to gain more information on the correlation betweeen chemical structure and pharmacological and toxicological molecular mechanisms, experimental data were analysed by means of QSARISTM (SciVision). In this study data from 95 HDAIs were analysed concerning the pharmacological activities, HDA-enzymatic activity, p21 activation as well as growth inhibition of the tumour cell lines HTC116 and H1299. The impairment of the spontaneous frequency of contraction of rat cardiomyocytes and the ability of HDAIs to inhibit the Angiotensin II-induced contraction of rat thoracic aortic rings (RTAR) were used as *in vitro* toxicological endpoints. A maximum of 227 molecular 2D descriptors (e.g., topological and electrotoplogical indices) descriptors were employed with the generic algorithm function (GAF). All QSAR models were done *via* Multi-linear Regression (MLR) fitting except one, where Partial Least Squares (PLS) was employed. The resultant R2 values for four bioactivity parameters (HDA-inhibition, p21 activitation, RTAR IC25, and growth inhibiton of HCT116 cells) ranged from 0.75 to 93 with corresponding Q2 values of 0.69 and 0.9 respectively. Likewise, the analysis pinpointed important E-State atom types (9) as well important bulk descriptors (e.g. LogP). Use of an E-State similarity search based on one compound with high HDAI activity/low toxicity identified a grouping of four similar compounds with close similarity (i.e., cosine coefficient ranged from 0.91 to 0.98) as opposed to reduced similarity of four other compounds (0.74 to 0.88) with high toxicity and high activity. The results of the present study suggest that QSARISTM is a powerfull tool to analyse pharmacological-toxicological *in vitro* data in order to gain more insight into the underlying molecular mechanisms of action.

185 QUANTITATIVE MECHANISTIC MODELING OF MIXTURE INTERACTIONS: AN APPROACH FOR MANGANESE (Mn) AND ORGANOPHOSPHATES (OPs).

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Traditional approaches to analyzing health effects of chemical mixtures often involve the prediction of interactions between more than one environmentally relevant chemical and their combined impact vis a vis exposure to the individual chemicals. These approaches center on dose-response analysis and lead to the development of such concepts as dose or response additivity, synergism and antagonism. While useful, such approaches have limitations, as discussed in the recent NIOSH/NORA Mixed Exposures Research Agenda (HHS/CDC, Draft July 2002). We propose an alternative approach beginning with the analysis of a specific multifactorial disease (such as gulf war syndrome or vCJD) of complex etiology with significant environmental or occupational influence. Key environmental components can be identified by from epidemiological studies, cluster analyses, and many other rapidly developing bioinformatics tools. An existing hypothesis for the ontogeny of BSE and vCJD is based on exposure to high levels of OP pesticides and Mn, together with low endogenous copper and other co-factors, which may lead to conformation changes in specific brain proteins (Purdey, Medical Hypotheses 57, 29-45, 2001). This model qualitatively explains a number of epidemiological observations, and is supported by *in vitro* studies (Brown et al., EMBO Journal 19, 1180, 2000). However, to further validate this model and to apply it to risk assessment, target site dosimetry of key components and chemical interactions must be quantitatively predicted. Only then can testable extrapolations to other exposure scenarios be made. Therefore, we have combined previously developed PBPK models for Mn and OPs in rodents and humans (Andersen et al., NeuroToxicology, 20, 161, 1999; Gearhart et al., Environ Health Perspect, 102 (Suppl 11), 51, 1994) and modified them to account for chemical interactions and multiple exposure scenarios. By including chemical and mechanistic information, these models can provide quantitative predictions of target site dosimetry and potential combined health effects.

186 FROM CONCENTRATION TO DOSE: FACTORS INFLUENCING AIRBORNE PARTICULATE MATTER DEPOSITION IN HUMANS AND THE RAT-HUMAN DOSE EXTRAPOLATION.

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The Multiple Path Particle Dosimetry model (MPPD) allows calculation of PM deposition fractions and exposure doses for humans. Human regional and lobar depositions as well as alveolar deposition distributions for different particle sizes were calculated for an individual stochastic adult human lung. For coarse particles (>2.5 um) tracheobronchial and thoracic deposition fractions and dose as well as the doses per alveolus are significantly larger for children (<15 yr.) than for adults. Pulmonary exposure doses per unit surface area increase nearly linear between the ages of 20 and 80 years by approximately 30%. Pulmonary deposition fraction of ultrafine particles (<0. um) increases and of coarse particles decreases from rest to light exercise. Thoracic exposure dose increases with increasing physical exertion. The estimation of inhaled particulate matter (PM) dose and regional deposition patterns in the lungs is not only based on exposure concentrations, but also on physical characteristics of the particles, morphometry and breathing parameters is an essential part of the source-effect chain for risk assessment.

187 SEASONAL EFFECTS OF ULTRAFINE, FINE, AND COARSE PARTICULATE MATTER(PM) ON HUMAN PRIMARY AIRWAY EPITHELIAL CELLS.

L. Dailey and R. B. Devlin. *ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC.*

Exposure of humans to PM results in increased mortality and morbidity. Recent toxicology studies have shown a number of pathophysiological pulmonary and cardiovascular changes following exposure to fine PM. However, the relative potency of ultrafine, fine, and coarse PM has not yet been characterized, nor is it well understood if PM emitted at different seasons of the year has different potencies. We have been collecting ultrafine, fine, and coarse ambient PM in Chapel Hill for the past year using the ChemVol High Volume Cascade Impactor developed by the Harvard School of Public Health. Samples were pooled into monthly batches to allow us to examine seasonal differences in PM effects on human airway epithelial cells. The fractions tested included coarse (2.5-10 microns), fine (0.1-2.5 microns), and ultrafine (<0.1micron). This abstract presents results from PM collected in October 2001 and April 2002. Human primary airway cells were exposed to equal masses (0, 25, 50, 100, and 250 ug/ml) of each PM fraction for 2 and 24hrs. Changes in expression of IL8, COX2 and heme oxygenase-1(HHOX1) genes were assessed at each time point. Exposure to October fine and ultrafine PM resulted in 5x and 9x greater increases in HHOX1 expression, respectively, compared with the corresponding April fractions; coarse PM from either month had a minimal effect on HHOX1 expression. In contrast, exposure to coarse PM collected in both October and April resulted in a 10 fold increase in COX2 expression while fine and ultrafine PM stimulated minimal expression of COX2. Increases in IL8 paralleled those seen for COX2. Detailed chemical analysis of each fraction will allow us to determine if the differential responses we have seen are associated with specific components present in each fraction. These data will provide useful information to the EPA on health effects associated with specific size fractions of PM. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

188 AIRBORNE COARSE PARTICLES OF THE SAN JOAQUIN/SACRAMENTO VALLEY INDUCE INFLAMMATION IN THE LUNGS OF RATS.

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Epidemiological studies have suggested particle size is an important determinant in adverse health effects. Particles less than 2.5 μm in diameter are considered to be the most deleterious, while particles with a mean aerodynamic diameter between 2.5 and 10 μm, referred to as coarse particles, are thought to produce less injury to the cardiopulmonary systems. Since the dominant PM fraction in the San Joaquin/Sacramento Valley is coarse particles, especially in the summer and fall seasons, we wished to examine the effects of exposure to ambient particles in the coarse mode on the lungs of healthy adult rats. Rats were exposed in Fresno, CA and Davis, CA to filtered air or the coarse fraction of PM enhanced approximately 40-fold over ambient levels with a particle concentrator system. Exposure to these particles was for 4 hours per day for 3 consecutive days. The mean mass of concentrated particles ranged from 0.2 to 1.1 mg/cubic meter. PM was composed primarily of nitrate, metals, sulfate, and organic carbon. Bronchoalveolar lavage (BAL) was performed on each group of rats following exposure. Total cells in BAL from rats exposed to concentrated PM were significantly increased during 1 of 2 weeks in Fresno and 2 of 2 weeks in Davis, compared to rats exposed to filtered air ($P<0.05$). BAL macrophages were increased during 1 of 2 weeks in Fresno and 2 of 2 weeks in Davis, neutrophils increased during all 4 weeks and lymphocytes during 2 of 2 weeks in Davis. The increase in macrophages, neutrophils, and lymphocytes in rats exposed to coarse PM indicates a significant inflammatory response with short-term exposure. These observations suggest that additional studies to elucidate potential mechanisms for possible health effects of coarse particles are warranted. Research supported by USEPA 827995 and NIEHS ES05707.

189 REGIONAL AND LOBAR DEPOSITION OF FINE AND COARSE PARTICLES IN THE LUNGS OF RATS AND MICE.

B. A. Wong, E. W. Tewksbury, J. T. Kelly and B. Asgharian. *CIIT Centers for Health Research, Research Triangle Park, NC.* Sponsor: O. Moss.

Inhalation toxicology studies have used various strains of rats as a surrogate for humans. Recently, attention has been focused on the use of mice for mechanistic inhalation studies because of the availability of transgenic mouse strains. There are always uncertainties for a given strain of animal regarding an appropriate exposure level to yield comparable doses in humans. Interspecies dosimetric comparison can help in reducing the uncertainties and improve risk assessment in humans from exposure to inhaled pollutants. Deposition of fine and coarse particles in the lungs of rats and mice was measured in this study. A condensation monodisperse aerosol generator was used to produce uniformly sized particles containing radiolabeled iron chloride ($^{59}FeCl_3$). Long-Evans rats and B6C3F1 mice were exposed in separate studies to radiolabeled monodisperse particles of different sizes using a nose-only exposure tower. Exposure concentration and animals' breathing parameters were measured and used in deposition calculations. The exposure durations were 10 to 15 min to reduce the impact of clearance processes. Deposition fractions in the head, lung lobar, and total lung were found by comparing measured deposition with that on a filter sampling from the Cannon nose-only tower. The results showed that total deposition fraction increased with particle size for both animals and reached a maximum value for particles 2 to 3 μm in size. For particles larger than 3 μm, total deposition fraction decreased with particle size, which stemmed mainly from the inability of the particles to enter the respiratory tract of the animal on inhalation (inhalability). Deposition fractions in mice were found to be larger than those in rats. By establishing the database for particle deposition in rats and mice in this study and linking them to responses from other studies, various dosimetrics can be developed for use in establishing safe exposure limits for humans. μ

190 THE COMPARATIVE TOXICITY STUDY OF KOREAN URBAN PM 2.5 AND PM 10 IN RAT LUNG EPITHELIAL CELLS.

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Air pollutants have long been recognized as a major problem for human health. A number of epidemiologic studies have been reported that elevated as well as ambient levels of PM10 were associated with increased frequencies of chronic cough, bronchitis, chest illness, hospital admissions, and mortality. Recent evaluation of the contribution of PM 2.5 to health outcomes has suggested that fine particles may be more closely associated with mortality and adverse respiratory health effects than particles of larger size. It has been hypothesized that PM 2.5 may cause more toxicity than PM 10 mediated through diverse mechanisms. This study was performed to find the toxic mechanism of especially Korean urban PM 2.5-induced toxicity in rat lung epithelial cell, and evaluate the risk of the exposure of PM 2.5. In the group treated with PM 2.5, the cytotoxicity by MTT assay was higher than in the group treated with PM 10. Also, in the group treated PM 2.5, the malondialdehyde was more produced than that in the same concentration of the group treated with PM10. In pBR322 DNA treated with 25 μg/ml of PM2.5, single strand breakage of DNA was higher than the DNA treated with 25 μg/ml of PM2.5. In electro mobility gel shift assay, both particles induced NF-kB activation, but only PM2.5-treated group induced AP-1 activation in rat lung epithelial cell significantly. These data suggest that PM2.5 rather than PM10 may induce more damage of DNA, oxidative damage and alteration of signal tranduction in NF-kB and AP-1 in rat lung epithelial cell and, and this may be associated with PM2.5-induced toxicity. Our data can be applicable for the risk assessment of PM2.5. Further PM2.5 related study for mechanism at the molecular level should be performed. Supported by Korean Deparment of Environment Grant

191 EVALUATION OF INORGANIC SOLUBLE SPECIES CONTENT IN PM10 FROM MEXICO CITY AND THEIR CYTOTOXICITY IN HUMAN BRONCHOEPITHELIUM CELLS.

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The debate over the scientific evidence for an underlying cause linking airborne particle levels to adverse health effects has intensified in major urban centers in recent years. However there are still uncertainties about the exact chemical and physical properties causative of the associated health effects and their toxicity mechanisms. In Mexico City the persistent high mass concentration of PM10 in the air has intensified research to elucidate the role of their chemical composition. Among the chemical components of PM10 inorganic species are important due to their high toxicity. The purpose of this study is to investigate if the water-leachable inorganic species contribute to the cytotoxic effects of PM10 in human bronchial epithelial cells (BEAS-2B). PM10 samples were collected over 24 hr from July to December 2000 from four different monitoring network stations from Mexico City (northern, central and sourthern regions). PM10 were suspended in deionized water, sonicated for 15 min, the soluble fraction was analyzed by HPLC and AAS to determinate anions (chloride, nitrate and sulfate), cations (ammonia, sodium and potassium) and ionizable transition metals. Trends in the concentration levels for soluble ions in the PM10 showed high amounts of sulfate (13.7-15.8ug/m3) and ammonia (2.4-3.5ug/m3) for the four regions in December. We used the soluble fraction of the highest concentration for colorimetric cytotoxicity assay (MTT method). Preliminary results showed that 48 hr *in vitro* exposure to 2.7-4.07ug/cm2 of NH4 and 15.8-18.3ug/cm2 of SO4 from the four regions of Mexico City produced 15% cell death associated to inorganic species. These data suggest that inorganic soluble constituents from PM10 could be responsible for the cytoxicity, yet additional information at a molecular level is needed and is currently in progress. (Study supported by the Financial Research Training Program from CIIEMAD/COFFA-IPN and CINVESTAV-IPN).

192 CONCENTRATED AMBIENT PARTICULATE STUDIES IN HEALTHY AND COMPROMISED RODENTS.

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Epidemiological studies have reported a consistent association between exposure to higher concentrations of ambient particulate matter (PM) and excess cardiopulmonary-related morbidity and mortality. To further examine this phenomenon, we exposed various rodent models of cardiopulmonary dysfunction to Concentrated Ambient Particulates (CAPs) and monitored changes in indices of cardiopulmonary function and damage. Animal models used included young (3 mo) Spontaneously Hypertensive (Y-SH), old (11 mo) Spontaneously Hypertensive (O-SH), and healthy (SD) and monocrotaline-treated Sprague-Dawley (MCT-SD) rats. Subsets of animals were implanted with radiotelemeters to monitor (5-min intervals x 24h/d) electrocardiogram (ECG), heart rate (HR), systemic blood pressure (BP), and core temperature (T_{co}). Exposure protocols for Y-SH rats were either Continuous (4h/d x 2–3d/wk x 11wk) or Intermittent (4h/d x 1d/wk x 11wk) while SD, MCT-SD, and O-SH were exposed 4h/d x 2–3d/wk x 1wk. Pulmonary function tests (Buxco Electronics) were performed on all animals before, during, and after exposures. At the termination of the study, animals underwent bronchoalveolar lavage (BAL) and the BAL fluid was examined for biochemical indices of pulmonary damage and inflammation. Heart and lung tissues were harvested for histological and morphological analyses. PM exposure concentrations ranged from 135–1600μg/m^3. In general, despite the variety of exposure protocols and compromised animal models used, these studies demonstrated minimal adverse effects on cardiopulmonary and thermoregulatory function in cardiopulmonary-compromised rats after exposure to CAPs. Furthermore, these studies underscore the inherent complexities of conducting toxicology studies using "real-world" exposure atmospheres. (Abstract does not represent USEPA policy. This research was supported in part by EPA CT826513.)

193 EFFECTS OF EXPOSURE TO CONCENTRATED AMBIENT PARTICULATES ON INDICES OF CARDIOPULMONARY AND THERMOREGULATORY FUNCTION IN HEALTHY AND MONOCROTALINE-TREATED SPRAGUE-DAWLEY RATS.

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Numerous epidemiological studies have reported a positive association between levels of ambient particulate matter (PM) and morbidity and mortality, noting higher correlations in persons with cardiopulmonary disease. Supporting data from animal studies are sparse and generally derived from exposures to large doses/concentrations of highly toxic PM surrogates. To investigate this phenomenon using a more environmental PM, we exposed healthy (SAL) and monocrotaline (MCT)-treated Sprague-Dawley (SD) rats to Concentrated Ambient Particulates (CAPs); paired groups were exposed to filtered air (AIR). Rats were implanted with radiotelemeters to monitor electrocardiogram (ECG), heart rate (HR), and core temperature (T_{co}). Animals were divided into four groups (SAL/AIR; MCT/AIR; SAL/CAPs; MCT/CAPs) and exposed (4h/d x 3d) to RTP CAPs. Telemetered rats were monitored continuously (4d preexposure-1d postexposure) and ventilatory function was examined daily (10 min; 1d preexposure-1d postexposure). At 24h postexposure, rats underwent bronchoalveolar lavage (BAL) to quantify biochemical indices of pulmonary injury and inflammation. On exposure Day 1, increases in HR (≈50 bpm) and decreases in T_{co} (≈1.0°C) were observed in all CAPs animals. Similar decreases in T_{co} occurred on Day 2. These effects do not appear to be associated with PM concentration *per se* but may be related to differences in composition. Protein and albumin in BAL fluid were elevated for rats receiving CAPs and appear exacerbated in MCT-pretreated animals. The above effects, while mild compared to those of previous studies that exposed MCT-SD to Residual Oil Fly Ash PM, nevertheless demonstrate a small toxic response in compromised rats following exposure to CAPs that, when projected over larger subject numbers, may help explain some of the reported epidemiological findings. (Abstract does not represent USEPA policy. This research was supported in part by EPA CT826513.)

194 CONSISTENT INFLAMMATORY RESPONSE FOLLOWING EXPOSURE TO CONCENTRATED AMBIENT PARTICLES (CAPs) DURING FALL SEASON IN WISTAR-KYOTO RATS.

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Animal studies involving CAPs exposures have demonstrated variable pulmonary effects due likely, in part, to the animal model employed and the composition of ambient particulate matter (PM). To determine the consistency of CAPs response with repeated studies, we exposed male Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) rats to CAPs in Research Triangle Park, NC (4h/d x 2 consecutive days) and measured pulmonary inflammation, cytokine levels, and changes in plasma fibrinogen and white blood cells. Seven replicates were conducted using the same exposure protocol during the period from August 27-October 24, 2001. The CAPS concentrations ranged from 145-2758 mg/m3, with the highest concentration achieved for the August 27-28 exposure period. After the first week of expo-

sure, the atmospheric temperature dropped significantly; the CAPs concentration also dropped significantly and both parameters remained low for the remainder of the study. There were consistent and significant changes in inflammatory cells in bronchoalveolar lavage fluid (BALF). All seven exposures resulted in significant decreases in total lavageable cells, and increases in neutrophils in WKY rats. The smallest increase was noted during the first week of exposure. The biochemical measures of BALF such as protein, albumin, lactate dehydrogenase and cytokines were variable. A small but consistent plasma fibrinogen increase was apparent only in SH rats at all exposures except the first. Plasma fibrinogen increases were not as consistent in WKY rats despite a significant inflammatory response in all replicates. This study demonstrates the potential variability between animal models and indicates that the season, and likely the composition of the atmospheric PM, may have a greater influence on CAPs-induced health effects than previously reported. (This abstract does not reflect USEPA policy).

195 DIFFERENTIAL PULMONARY INFLAMMATION AND *IN VITRO* CYTOTOXICITY BY SIZE FRACTIONATED PARTICLES COLLECTED FROM COMBUSTED COAL EMISSIONS.

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Exposure to industrial and urban particulate matter (PM) has been associated with adverse health effects in humans. In the present study we examined pulmonary inflammatory responses in mice after intratracheal instillation of 25 or 100 micrograms of ultrafine, (<0.2uM), fine <2.5 uM) and coarse (2.5-10 uM) coal fly ash from a combusted Montana coal, and fine and coarse fractions from a combusted West Kentucky coal. CD1 mice (60 days old) were intratracheally instilled with either saline or saline suspensions of the different fractions of the coal emissions and the responses were compared to endotoxin as a positive control. After 18 hours, lungs were lavaged and bronchoalveolar fluid (BALF) was assessed for cellular influx. Biochemical markers including protein and albumin content, lactate dehydrogenase (LDH) and N-acetyl glucosaminidase (NAG) activity and pro-inflammatory cytokines (TNF-a, MIP-2 and IL-6) were also analyzed. The results show that on an equal mass basis, the Montana ultrafine particles induced a higher degree of neutrophil inflammation, NAG and cytokine levels than the fine or coarse particles. The Western Kentucky fine particles caused a moderate degree of inflammation and increased levels of protein content in BALF that was higher than the Montana fine particles. Coarse particles did not produce any significant effects. *In vitro* experiments with rat alveolar macrophages showed a similar ranking of potency with the Montana UF displaying the highest amount of cytotoxicity. In summary the Fine and Ultrafine fractions of CFA are capable of generating inflammation and lung injury. These effects were inversely related to particle size and were also associated with increased sulfur content and higher levels of trace elements. This abstract does not reflect EPA policy.

196 SPONTANEOUSLY HYPERTENSIVE RATS ARE SUSCEPTIBLE TO MICROVASCULAR THROMBOSIS IN RESPONSE TO PARTICULATE MATTER EXPOSURE.

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Inhalation of environmental particulate matter (PM) is associated with cardiovascular (CV) health effects. These effects may be augmented by PM-induced thrombus formation in the vasculature. Tissue factor (TF) activation of coagulation, and reduction in thrombododulin (TM)-mediated inhibition of thrombin formation, may enhance thrombosis. The persistence of thrombi is regulated by fibrinolysis which is initiated by tissue plasminogen activator (tPA), and inhibited by plasminogen activator inhibitor-1 (PAI-1). To determine the role of thrombosis in PM-induced injury, we intratracheally (IT) instilled 3.3mg/kg oil combustion-derived PM into male Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) rats. Pulmonary inflammation, changes in plasma fibrinogen, white blood cells (WBC), and expression of TF, TM, tPA, and PAI-1 were analyzed 24 h post IT. The combustion PM used in this study contained primarily zinc and nickel but not vanadium. Significant increases in BALF neutrophils and total cells occurred in the particle exposed WKY and SH rats, however, the SH rats showed greater increases than WKY rats. While BALF protein and LDH levels were increased with all particles versus control exposures, SH rats showed a greater increase. Plasma fibrinogen was significantly increased only in SH rats exposed to particles. Furthermore, the lung mRNA expression of TF was significantly increased, while TM and tPA mRNA decreased only in SH rats exposed to particles. TM protein was decreased in the SH rats following particle exposure. These data suggest that altered coagulation and fibrinolysis may play a critical role in PM-induced CV effects in susceptible individuals. This abstract does not reflect USEPA policy. Supported in part by #CR829522 between EPA and UNC.

197 EFFECT OF EXPOSURE TO PARTICULATE MATTER AND DIESEL EXHAUST PARTICLES ON THE FREQUENCY OF DNA DELETIONS *IN VIVO* IN MICE.

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Particulate Matter (PM) and diesel exhaust particles (DEP) contain a staggering number of known carcinogens and there is some epidemiological indication that exposure to PM causes cancer in people. However, the increased risk is close to the limits of detection of classical epidemiology. For this reason, and to contribute to a mechanistic understanding of the effect of PM and DEP additional tools of investigation are required, such as animal studies based on biomarkers of genotoxicity. We have exposed animals to DEP under various exposure scenarios. As a cancer-related early genotoxic effect, we determined the frequency of DNA deletions on the offspring of exposed C57BL/6J-pun/pun mice. DNA deletions are scored as homologous recombination events between two copies of the partially duplicated pigmentation gene p (pun mutation) resulting in a deletion of one of the two copies. The deletion reconstitutes the wild type p gene visualized as black spots on the gray fur or patches of pigmented cells on the translucent retinal pigment epithelium. The frequency of deletions is measured as percentage of animals with fur spots or number of eye spots per retinal pigment epithelium. Preliminary data from mice exposed to DEP (500 mg/kg) by a single i.p. injection at 10.5 dpc, indicated that the offspring have a significantly higher frequency of eye spots (6.9±2.2; n=40) than those from unexposed mice (5.9±2.0; n=36) spots per retinal pigment epithelium ($P<0.05$). The fur spot assay did not reach statistical significance probably due to low number of animals analyzed. We have also launched a study on the effects of PM of concentrated urban air of the Los Angles basin, in collaboration with the UCLA Southern California Particle Center and Supersite. This study is supported by grants from the UC Toxic Substances Research and Teaching Program.

198 CARDIOVASCULAR EFFECTS OF DIESEL EXHAUST INHALATION IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS.

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Particulate matter air pollution has been associated with increases in cardiovascular mortality in several epidemiological studies. The present study examined cardiac outcomes of exposure to diesel exhaust (DE) in a rodent model of systemic hypertension. SH rats (3 mo old, 6 males and 6 females/group) were exposed to either filtered air or one of four levels of DE (30, 100, 300, and 1, 000 μg particles/m^3, generated by a Cummins B 5.9L engine) for 7 d. Electrocardiographic (ECG) measurements were obtained by radiotelemetry (Data Sciences, Intl) at 15 min intervals from 3 d prior to exposure and ending 4 d following the cessation of exposure. Hearts were isolated following the monitoring period and fixed for morphological examination. Control rats displayed a reduction in daytime heart rate (HR) from the beginning of the protocol, while exposed rats maintained a significantly elevated HR throughout the exposure period. Daytime HR values for control rats averaged 265±5 bpm (mean±se), while values for exposed rats averaged 290±7 bpm. This difference persisted during the evening, but was not observed during the pre- or post-exposure periods. The P-Q interval, an index of AV node sensitivity, was significantly prolonged among exposed animals. No other significant ECG component alteration or arrhythmia induction was observed and no significant pathology was seen in hearts. However, increased HR with prolongation of AV-node activation (P-Q interval) represents a possible substrate for several major ventricular arrhythmias. These results concur with previous reports implicating particulate effects on the pacemaking system of rats, potentially involving alteration of the autonomic nervous system. Supported by the National Environmental Respiratory Center with support from multiple government and industry sponsors, including the USEPA. This abstract does not represent the views of any sponsor.

199 COMPARISON OF PULMONARY RESPONSES TO AUTOMOBILE-GENERATED AND NIST STANDARD REFERENCE MATERIAL DIESEL PARTICULATE EMISSIONS IN MICE.

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Studies have shown that diesel exhaust particles (DEP) worsen respiratory diseases including allergic asthma. The adjuvant effects of DEP in the airways have been widely reported; however, the precise mechanisms of these effects remain ill defined. It is known that the composition of DEP may change with variations in conditions under which they are generated or collected. We hypothesized in this study that two different DEP samples, generated and collected under different condi-

tions, would produce different levels of pulmonary toxicity. CD-1 mice were intratracheally (IT) exposed to 25 or 100 μg of either automobile-generated DEP (A-DEP: dilution tunnel collection, from a 4 cylinder Isuzu engine) or standard reference material DEP (SRM-DEP: NIST SRM 2975, from a forklift) or saline, and brochoalveolar lavage (BAL) was performed 4 and 18 hr later. Both samples produced increased total inflammatory cells with only a moderate increase in microalbumin and total protein in the BAL fluid. SRM-DEP produced 4-5 fold greater increase in neutrophil influx and greater depletion of total antioxidant capacity in the BAL fluid than A-DEP. Both the low and the high doses of A-DEP increased MIP-2 and IL-6, while only the high dose of SRM-DEP increased levels of these proinflammatory cytokines. By contrast, TNFα and N-acetyl glucosamine were significantly increased only by the A-DEP. Percentages of extractable organic material (hydrocarbons) in the two DEP samples were determined by dichloromethane extraction followed by gravimetric analyses. A-DEP was composed of 60-71% hydrocarbons, while SRM-DEP was composed of 1.6-6% hydrocarbons. We suggest that the observed differences in proinflammatory profiles are related to the disparate amounts of organic chemical constituents in the two DEP samples. (Supported by NIH grant # ES11245-01) (This abstract does not reflect EPA policy.)

200 BAX EXPRESSION AND NITRIC OXIDE PRODUCTION IN MACROPHAGES J774A.1 AS BIOMARKERS OF GASOLINE EMISSION TOXICITY.

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Extensive evidence supports that short-term exposure to elevated levels of atmospheric pollutants is associated with respiratory diseases. Although the known toxic effects of atmospheric pollutants results from the synergistic contribution between primary and secondary pollutants, in this study we investigate the contribution of primary pollutants from gasoline emission using an *in vitro* cell line model. We evaluate cytotoxic capability, Bcl-2/Bax ratio and p53 protein expression as cellular biomarkers of toxicity of vehicle emission extracts (VEE), on non-activated J774A.1 murine macrophages. In addition we evaluated the effect on IFNγ plus LPS activated macrophages through nitric oxide (NO·) and TNFα mRNA expression (evaluated by RT-PCR). The VEE were obtained from a 1.7 cc gasoline engine vehicle operated under the FTP-75 method using Mexican commercial gasoline and sampled under EPA/625/R-96/010b specifications. The organic extracts from gas and particle phases were tested at a dose equivalent to a 160 m of urban operation conditions. No cytotoxicity assayed by MTT was observed. Immunodetection after exposure to the particle and gaseous phase showed induction of Bax expression with an inversion on the Bcl-2/Bax ratio in both phases. At the time points evaluated no induction of p53 was observed. On activated-macrophages VEE inhibited NO· production and the TNFα mRNA expression a higher effect was observed in cells exposed to the particle phase extract. These results suggest that the increase in Bax expression with no proportional increase in Bcl-2 is a sensitive biomarker of VEE toxicity and that macrophage activation is an important target of VEE toxicity. Further studies are in course to elucidate on the mechanisms of these toxic effects. (Study supported by FIES-IMP 98-119-VI).

201 *MYCOPLASMA FERMENTANS* INFECTION AUGMENTS IL-6 RELEASE IN HUMAN LUNG CELLS EXPOSED TO RESIDUAL OIL FLY ASH (ROFA) AND TNF-β.

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Mycoplasma (MP) can establish chronic latent infections with little signs of disease. *M. fermentans* is a species of MP associated with arthritis, leukemia, and lung disease, but its presence in normal healthy individuals suggests it to be a co-factor for disease in concert with other stimuli. Particulate matter (PM) is a component of air pollution associated with adverse health effects. We hypothesize that MP modulate the inflammatory effects of chemical stress and alter cellular responses to promote lung disease upon exposure to PM. We assessed IL-6 release in response to PM and TNF-β in human lung epithelial cells (A549) and fibroblasts (HLF) deliberately infected with *M. fermentans* and uninfected controls. Exposure of uninfected HLF to 50 μmg/ml ROFA for 24 hrs increased IL-6 release from 54 ± 11 pg/10^5 cells to 603 ± 145 pg/10^5 cells. MP infection alone produced a similar 10-fold increase in IL-6 release and enhanced the ROFA response greater than that predicted by the sum of each stimulus alone (> 50-fold above uninfected control). A549 cells required 5-fold greater concentrations of ROFA, as well as TNF-β pretreatment to realize the synergistic interactions between ROFA and MP. In both cell types, MP and TNF-β together synergized to augment IL-6. ROFA and other PMs were compared by exposing HFL to 20 μg/ml of urban dust from Dusseldorf, Germany, Mt. St. Helen's ash, or aqueous extract of PM obtained from Provo Valley near Salt Lake City, Utah (SLC). Only ROFA and SLC increased IL-6 release in uninfected cells. None of the other PMs, including SLC augmented IL-6 above that seen with infection alone. Both ROFA and SLC are rich in redox-active metals, but differ in their relative elemental composition (high V in ROFA vs. high Cu in SLC). MP interacts with environmental and endogenous inflammatory stimuli to synergistically activate signaling pathways that control lung cell cytokine production and, thus, can potentially modulate the effects of various environmental chemicals. (Funded in part by EPA STAR Grant R827151)

202 CCSP DEFICIENCY ALTERS BASAL PULMONARY AND SYSTEMIC HUMORAL IMMUNITY.

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Altered levels of Clara Cell Secretory Protein (CCSP), a major component of airway lining fluid, are associated with chronic airway disease in asthmatics, smokers, COPD patients and Silica exposed individuals. CCSP has been proposed to act as an immunoregulatory protein though the mechanism is unknown. We previously demonstrated that CCSP knockout (-/-) mice within a 129/J background possess elevated pulmonary IgA message and protein levels compared to wild type control mice, suggestive of an immunoregulatory defect. Numerous studies have demonstrated that immune responses can vary based on genetic background or strain. Comparison of C57BL/6J wild-type and CCSP -/- mice by northern hybridization revealed that pulmonary IgA message was undetectable in both groups. In contrast, flow cytometry of interstitial pulmonary lymphocytes determined that CCSP deficiency results in a decrease of pulmonary B-2 cells. A two fold increase of IgG1 immunoglobulin in the plasma was also detected, suggesting that the effects of CCSP at steady state are strain dependent and that CCSP deficiency may play a role in B cell population and locality. Evaluation of CCSP -/- B cell populations in the spleen did not reveal alterations to B cell number. These results confirm that CCSP deficiency is associated with immunoregulatory perturbations and that the nature of these alterations is strain-dependent. Acknowledgements: HL70575, ES08964, ES01247, and ES07026.

203 OVALBUMIN-INDUCED AIRWAY INFLAMMATION AND FIBROSIS IN INOS-DEFICIENT MICE: MECHANISMS AND CONSEQUENCES.

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We hypothesized that C57BL mice, a Th-1 responsive strain, would be relatively resistant to ovalbumin exposure as compared with the BALB/c strain, a Th-2 responder. Our results are consistent with this hypothesis, especially with respect to the accumulation of collagen in the airways of the mice exposed to ovalbumin and increased airway reactivity to challenge with methacholine, as measured by the Penh response. BALB/c mice demonstrate significant increases in accumulation of airway collagen after 4 weeks of exposure to ovalbumin aerosol under conditions identical to those used in the current study. Significant increases in airway collagen content were seen only after 6 weeks of exposure of the C57BL mice, as contrasted with 4 weeks in the iNOS knockout animals. At each time point examined, Penh values for the iNOS knockout mice exceeded those observed with the C57BL strain. Thus, responses to ovalbumin exposure of the Th1 responsive C57BL animals were significantly less (or slower) than those of the Th2 responsive BALB/c mice. We found the iNOS knockout mice to be more susceptible to ovalbumin-induced airway fibrosis than the C57BL strain, giving results intermediate between BALB/c and C57BL in the various assays performed. We also measured the response of C57BL and iNOS knockout mice to direct intra-tracheal challenge with TGF-B1. There was no apparent response of C57BL mice to TGF-B1 at 4 or 11 days after TGF-B1 challenge, as evaluated by bronchoprovocation testing. There were significant increases in Penh values measured in iNOS knockout mice exposed to serially increasing concentrations of methacholine, and the observed Penh values were significantly greater in mice that had also received TGF-B1. These results strongly support the hypothesis that the increased sensitivity of iNOS knockout mice to ovalbumin is at least partially dependent on pathways that come into play subsequent to the release of TGF-B1 by effector cells in the lungs of mice exposed to ovalbumin aerosol.

204 RESPONSES TO *MYCOBACTERIUM CHELONAE* FROM METAL WORKING FLUIDS DIFFER FROM *SACCHAROPOLYSPORA RECTIVIRGULA* IN A MURINE MODEL OF HYPERSENSITIVITY PNEUMONITIS.

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Human cases of hypersensitivity pneumonitis (HP) have been reported among machinists for over ten years. Although *Mycobacteria* have been implicated as causal agents, the mechanisms leading to the development of this disease in machinists re-

main unclear. It is recognized that other constituents of in-use metal working fluids (MWF), such as endotoxins may contribute to lung inflammation and may act as adjuvants for the development of HP. Thus, we investigated the potential for microbial agents in MWF to induce HP in an established rodent model and characterized the physiologic and immunologic responses to these MWF exposures and appropriate positive and negative controls. Female C57Bl/6J mice were exposed by nasal instillation to live *Mycobacterium chelonae* (MC) bacteria (isolated from an in-use MWF sample), live *Saccharopolyspora rectivirgula* (SR) bacteria (positive control), saline (negative control), or endotoxin (inflammation control). Mice were exposed three consecutive days per week for three weeks and were necropsied on the fourth day after last treatment. SR-exposed mice demonstrated significantly more total cells, neutrophils, lymphocytes and monocytes in lung lavage; an increased CD4+/CD8+ ratio; and marked pulmonary lymphocytosis on histological exam when compared to saline-treated controls. The cytokine, interleukin (IL)-12 was significantly elevated but IL-6, IL-10 and interferon-γ (IFNγ) were not. Mice treated with MC had significantly elevated lung weights, total lavage cells, neutrophils, and CD4+/CD8+ ratio as compared to saline controls. However, when MC was administered at twice the dose of SR, cellular and cytokines responses were still lower than those observed in the SR-treated mice. These data suggest that MC produces HP-like changes in the mouse but with lower potency and different response profile than induced by SR. *(Supported by UAW-DaimlerChrysler National Training Center.)*

205 RESPIRATORY RESPONSE TO TOLUENE DIISOCYANATE AFTER DERMAL SENSITIZATION IN MICE.

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Aim: Occupational asthma is the principal cause of work-related respiratory disease in the industrial world. In the absence of satisfactory models for predicting the potential of low molecular weight chemicals to cause asthma, we have attempted to produce immunologically mediated respiratory responses in mice using toluene diisocyanate (TDI), a known respiratory sensitizer. Methods: BALB/C mice received TDI (20 µl per ear, 0.3 or 3% solution) or vehicle (acetone/olive oil) on each ear for three consecutive days. On day 7 the mice received a dermal boost of TDI (20 µl per ear, 0.3 or 3% solution). On day 10, they were intranasally challenged with TDI (0.1%, 10 µl per nostril) or vehicle. Lung function was monitored by whole body plethysmography for 40 min after intranasal challenge, and bronchial reactivity to methacholine (0, 10, 25, 50 and 100 mg/ml) was assessed 24 h later. Pulmonary inflammation was assessed by bronchoalveolar lavage (BAL) and histology. Results: Mice sensitized with TDI, boosted with TDI and challenged with TDI did not show a different breathing pattern after intranasal challenge compared to the control. One day later they did not show an increased reactivity to methacholine. However, mice that been "sensitized" with the vehicle and then "boosted" with TDI (0.3% or 3%), experienced marked bronchoconstriction immediately after the intranasal challenge with 0.1% TDI. 24 h later they had pronounced bronchial hyperreactivity and pulmonary inflammation [20% and 40% neutrophils; 1.5% and 2% eosinophils in BAL, for boosts with 3% and 0.3%]. Dry and wet lung weight were also significantly higher in this group compared to the control. Histologic examination revealed a minor influx of eosinophils round the blood vessels. Conclusion: We have been partially successful in our attempt to develop a model of chemical-induced asthma. The role of dermal sensitization and subsequent dermal boosting is proving to be one of the critical factors, the mechanisms of which remain to be explored. DWTC PS/01/43

206 PULMONARY FUNCTION FOLLOWING EXPOSURE TO CARBON-GRAPHITE/EPOXY COMPOSITE MATERIAL SMOKE.

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Four groups of 28 male F-344 rats were exposed to smoke from pyrolysis of 0, 10, 55, or 100 g of carbon-graphite/epoxy composite material for 1 hour. Two groups of 28 animals were exposed to 100 g smoke for 2 hours. No deaths resulted from exposure to 10, 55 g smoke, 4/28 (14%) died immediately after 100 g smoke exposure for an hour, and 24/56 (43%) died during or shortly after 2 hour 100 g smoke exposure. Pulmonary function was assessed in 6 animals from each exposure group at 2, 7 and 14 days post exposure. Significant elevation of tidal volume and minute ventilation was observed 2 days following 2hr-100 g smoke exposure. Lung resistance was not altered in any of the animals, however both dynamic and quasistatic compliance were significantly lower at all post-exposure times in animals exposed to 100 g smoke regardless of exposure duration. Functional residual capacity (FRC), residual volume (RV), FRC/TLC, and RV/FRC were significantly elevated in 100 g animals regardless of exposure duration and post exposure time. Mid-mean expiratory flow and forced expiratory volume at 100 and 50 milliseconds (all normalize forced vital capacity) were significantly elevated at day 2 post-exposure in 100 g smoke regardless of exposure duration. These parameters were elevated at 7 and 14 days post-exposure in animals exposed to 100 g smoke for 2 hours. Diffusing capacity and diffusing capacity normalized to lung volume was significantly decreased at all post-exposure times in animals exposed to 100 g smoke regardless of exposure duration. The arithmetic mean alveolar septal thickness in these animals was significantly elevated at all post exposure times.

207 ACUTE TOXICITY OF CARBON-GRAPHITE/EPOXY COMPOSITE MATERIAL SMOKE: COMPARISON WITH N-GAS MODEL PREDICTIONS.

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One hour exposure to smoke (average nominal concentration (28.4 ±1.6 g/m3) from pyrolysis of 100 grams of carbon-graphite/epoxy composite material (cgeCM) used in the manufacture of military and civilian aircraft was 32% (34/107) lethal to F-344 rats. No lethality was observed from 1 hour exposures to smoke from pyrolysis of 55, 60, or 75 grams of cgeCM. Time weighted average concentrations of CO, CO2, HCN, NO2, SO2, and O2 in the 100 g smoke were 3282, 8932, 56, 254, 218, and 195000 ppm, respectively; and were used to predict acute cgeCM smoke toxicity using the N-gas model (Levin, 1996). The average N-gas number for five 100 g smoke exposures was 2.25 indicating a much higher than expected mortality. However, carboxyhemoglobin (COHb) level in the surviving animals was approximately 45% which was much lower than the 85 to 90% predicted by the Coburn-Forster-Kane equation. This was attributed, in part, to a reduction of the Haldane coefficient by a decrease in blood pH, with concomitant reduction of COHb formation. Adjusting the N-gas model proportionately resulted in an average N-gas number of 1.49. The N-gas model was further modified to remove CO and CO2 synergism because of the relative concentrations of these gases in the smoke and to reflect the non-linearity of CO2 stimulation of ventilation. The result was an additional small reduction of the N-gas prediction to 1.40 which also predicts greater lethality than observed. This suggested that other constituents in the smoke inhibited uptake of more toxic components, possibly by suppression of breathing from SO2 and particulate irritation of the airways.

208 INDUCTION OF STROMELYSIN BY PULMONARY INSTILLATION OF TOXIC DUST.

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Stromelysin is a collagenase of the matrix metalloprotease family that is involved in collagen remodeling of scar and wound tissues. It is not normally present in the lungs. In this study, we tested the hypothesis that the matrix metalloprotease, stromelysin, is induced by instillation of toxic particles into the lungs. Male, Fischer 344 rats (body weight 255±8 g, mean±SE, N=6) were intratracheally instilled with saline vehicle or 4 mg of either crystalline silica, titanium dioxide or particulates from air sampling of citrus and grape fields. After 4 weeks, rats were sacrificed and the lungs were analyzed for total lung collagenase activity using a fluorometric assay. Matrix metalloprotease 2 (MMP2, the principal form of collagenase normally present in the lungs) and stromelysin were localized using immunohistochemistry. Total lung collagenase activity was 407±130, 464±64, 644±110, 895±126 and 906±129 nmol substrate/min/lung (mean±SE, N=6) for saline, titanium dioxide, citrus, grape and silica treated lung, respectively. Total lung collagenase activities of citrus, grape and silica exposed lungs were significantly greater than saline and titanium dioxide treated lungs. Immunohistochemistry demonstrated significant expression of stromelysin in silica, citrus and grape instilled lungs. Stromelysin was not present in the saline controls or titanium dioxide treated lungs. MMP2 was significantly elevated above saline and/or titanium dioxide in silica, citrus and grape treated lungs. The results suggest that stromelysin may play a role in lung remodeling in response to toxic particles, and that it may be used to differentiate between toxic and non-toxic particles.

209 ASSESSMENTS OF THE BARRIER EFFECTIVENESS OF PROTECTIVE CLOTHING FABRICS TO AEROSOLS OF CHRYSOTILE ASBESTOS FIBERS.

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This study measured the performance of flashspun polyethylene clothing fabrics and the spunbonded polypropylene composite (spc) fabrics as barriers to chrysotile asbestos fibers. The spc fabric samples were taken from clothing samples obtained from several manufacturers. Ten fabric samples and two controls were concurrently exposed to chrysotile asbestos aerosols. Fibers that penetrated the fabric specimens were captured on sub-micron filters. These fibers were counted and sized from SEM micrographs of random sections of those filters. The aerosol challenge concentration was obtained from filters directly exposed to the asbestos aerosol without an intervening fabric. Penetration percentage was based on the number of fibers that penetrated the fabric versus the number of fibers observed on the uncovered filters. Five different exposures were performed. The penetration of chrysotile fibers through flashspun polyethylene fabrics ranged from 0.24% to 1.0%. In contrast, the penetration of chrysotile fibers through the commercial spc fabrics ranged from 13.4% to 26.8% under the conditions at which these evaluations were conducted. The results demonstrated that the method consistently differentiates performance of protective clothing materials to chrysotile fiber penetration and that flashspun polyethylene fabrics provide greater resistance to asbestos fiber penetration when compared to several different spc fabrics available from several manufacturers.

210 SILICA EXPOSURE *IN VITRO* STIMULATES THE ACTIVITY AND EXPRESSION OF MATRIX METALLOPROTEINASES IN MOUSE MACROPHAGES. .

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Exposure of mouse macrophages *in vitro* to crystalline silica elicits numerous inflammatory mediators including the matrix metalloproteinases (MMPs) which participate in extracellular matrix remodeling. MMP activity has been implicated *in vivo* in the development of pulmonary fibrosis in rodents and humans. In a rat model of silicosis, MMP-2, -9, and -13 were found in silicotic granulomas (Perez Ramos et al:Am J Respir Crit Care Med. 160:1274, 1999). Furthermore, gene analysis of lungs of both humans and mice implicate MMP-7, matrilysin, as a mediator of fibrosis (Zuo et al: PNAS 99:6292, 2002). However, few studies have examined the profile and regulation of MMP expression *in vitro*. To investigate the expression and activity of MMP-2, -7, and-9, RAW 264.7 cells were stimulated with LPS (1 ug/ml), TNFα(20 ng/ml), crystalline silica (cristobalite), and amorphous silica for varying times and with different concentrations of particles. The results were analyzed using zymographic and western blot methods. The zymogram indicated constitutive levels of MMP-9 activity in cells stimulated with silica; no activity for MMP-2 was detected. Increased gelatinolytic activity was detected in the silica treated cells (25, 12, and 6 ug/cm2) after 48 hours of stimulation that was not apparent in the LPS, media, or amorphous silica treated groups. The apparent molecular weight of this active moiety was ~28kDa, consistent with that of MMP-7. Western blot analysis and further zymographic analysis will confirm the identity of this moiety. These data suggest that MMP expression and activity are integral to the cell's response to a silica insult. (Funded by R15ES09433 and the University of CT Research Foundation)

211 BIOMATHEMATICAL MODELS OF EXPOSURE-DOSE-RESPONSE TO RESPIRABLE QUARTZ IN FISCHER 344 RATS, CYNOMOLGUS MONKEYS, AND HUMANS.

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A biomathematical model was developed to describe clearance and retention kinetics of respirable quartz and pulmonary responses, including the recruitment of alveolar macrophages (AMs) and polymorphonuclear leukocytes (PMNs) and the production of superoxide dismutase (SOD) in bronchoalveolar lavage (BAL) fluid, as well as hydroxyproline (HP) in lung tissue. The model was first calibrated in Fischer-344 rats exposed to respirable crystalline silica (15 mg/m^3 for up to 6 months) and validated using data from two additional rat studies (respirable quartz exposures 0.74 mg/m^3 for 2 years, and 10 mg/m^3 for 75 days). The model was then extrapolated to cynomolgus monkeys and humans by adjusting model parameters for species differences such as breathing rates, lung mass, and surface area. To evaluate model fit, BAL data and limited quartz lung burden data were available in cynomolgus (10 mg/m^3 quartz for up to 26 months) and in coal miners (<0.1 mg/m^3 mean quartz in respirable coal mine dust for 17 years). The model predicted well the end of study lung burden in monkeys and the lavageable quartz mass in two humans; however, it overpredicted the pulmonary cell responses by several fold. This was consistent with the observed dose-response, in which the rat PMN and AM counts were several times greater than those in monkeys at higher doses (cell counts relative to controls and normalized to lung surface area). Human predicted dose-response data were in the low dose region, where responses were similar across species, except for two individuals, whose PMN counts exceeded those expected from the rat data. The relative increases in SOD and HP were comparable across species. Despite some differences in magnitude, similar patterns of nonlinear dose-response were observed in all three species. These biomathematical models may be useful in risk assessment by providing a mechanistic basis for extrapolating rat responses to various inhaled particulates in humans.

212 EVALUATION OF THE CHEMICAL AND PHYSICAL PROPERTIES OF CELLULOSE INSULATION AEROSOLS AND THE POTENTIAL ACUTE PULMONARY TOXICITY.

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Cellulose insulation (CI) is a type of thermal insulation produced primarily from recycled newspapers. The newspapers are shredded, milled and treated with fire retardant chemicals. CI is installed by a blowing process that generates significant quantities of airborne material. These studies were conducted to characterize the chemical and physical properties of the CI aerosol and to evaluate the potential acute pulmonary toxicity of the respirable particles. An aerosol generation system was designed to simulate the process used during installation of CI at work sites and to separate CI particles based upon aerodynamic size. Aerosols were generated using CI from four different manufacturers. The mean equivalent diameter of aerosol particulates ranged from 3.5 to 11.4 microns in the cyclone collection bag and 0.6 to 0.7 microns in the sampling chamber. For all four CI samples, less than 0.1% was collected as the small respirable particle fraction. X-Ray diffraction analysis of the respirable particle fraction did not indicate the presence of any cellulose material. The fraction consisted mainly of fire retardants and smaller quantities of clays. Concentrations of the toxic elements lead, chromium, arsenic, and selenium were very low in all samples tested. The respirable fraction was administered by intratracheal instillation to male F344 rats at doses of 0, 0.125, 0.25, 0.5, and 1 mg/rat, and the bronchoalveolar lavage fluid (BALF) evaluated 3 days later. BALF was evaluated for cellularity and cell differentials. The total number of cells in the BALF was significantly increased only in the high dose group. The %PMNs was increased and the %AMs was decreased in a dose-related manner. These results indicate that few respirable particles or fibers are generated during the application of CI, and that intratracheal instillation of respirable particles in rats resulted in only a minimal inflammatory response.

213 PULMONARY TOXICITY OF CARBON NANOTUBES IN MICE 7 AND 90 DAYS AFTER INTRATRACHEAL INSTILLATION.

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Single-walled carbon nanotubes (SWNT; individual tube: ~1 nm x 1, 000 nm) are a novel material possessing many desirable properties and potentially have widespread applications in the electronics, computer, and aerospace industries. NT are light and could become airborne. Little is known about the toxicity of this fiber/rope-like material. The pulmonary toxicity of three NT products, obtained from Rice University or Carbolex Inc. was investigated in mice by intratracheal instillation. These products were made by different methods, and contained different types or amounts of residual catalytic metals. Metal analysis showed that the Rice HiPco-prepared NT contained 27% (w/w) iron in the raw form, and 2% iron after purification; Carbolex electric-arc product contained 26% nickel and 5% yttrium. Male mice (B6C3F1, ca. 30 g, 4 to 5 per group) were each intratracheally instilled once with 0, 0.1 or 0.5 mg of NT suspended and ultrasonicated in 50 µl of mouse serum. Carbon black and quartz, two standard reference dusts, were prepared similarly for instillation. Carbon black elicited minimal effects and high-dose quartz produced moderate inflammation in the lung. All the NT products, regardless of the type or amount of metal, induced a dose-dependent formation of epithelioid granulomas in the centrilobular alveolar septa and, in some cases, interstitial inflammation in the animals of the 7-d groups. These lesions persisted and, in most cases, became worse in the 90-d groups. The granulomas in NT-treated mice consisted of aggregates of macrophages laden with black NT particles. Our results show that, for the test conditions described here, if SWNT reach the lung, they can be more toxic than quartz.

214 INFLAMMATORY EFFECTS OF QUARTZ SAMPLES AFTER INTRATRACHEAL INSTILLATION IN RATS.

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Objectives: This 28-day study compared the inflammatory potential of quartz with geologically ancient surfaces to well-characterized reference quartz DQ 12 (RQ). A differentiation of subspecies of quartz dusts is important in regard to a wide exposure and a potential carcinogenicity. Methods: A quartz sample in a naturally occurring respirable size (quartz isolate-QI) was separated centrifugally from an unprocessed bentonite dispersion, as were clay minerals (clay isolate-CI). QI contained approx. 67% quartz with the remainder being predominantly clay minerals, and CI contained <0.035% quartz. Lung effects of QI and CI were evaluated in rats at 3 and 28 days after administering a total dose of 15.2 mg/kg in 4 equal doses. To rank the effects, a positive control (RQ) and a negative control (TiO2) were included. Results: Bronchoalveolar lavage showed a severe acute inflammatory effect in the CI group at 3 days after last dosing, which recovered mostly after 28 days. QI induced moderate inflammatory effects after 3 days, which did not change significantly by 28 days post treatment. In contrast, RQ showed a high acute inflammatory response on day 3, persisting through day 28. Histopathologically pulmonary inflammation in lungs at day 28 was most pronounced in the RQ group, whereas QI and CI showed significantly lower effects. In contrast to the quartz groups, inflammation in the CI group was not persistent or progressive but was a late recovery phase of a severe acute inflammatory effect. Conclusion: Pulmonary inflammatory effects of various quartz subspecies were significantly different.

215 PULMONARY TOXICITY OF ADVANCED COMPOSITE MATERIAL COMBUSTION ATMOSPHERES IN RATS.

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Advanced composite materials (ACM) provide a lighter alternative to their metal counterparts without compromising strength. A downside to the use of ACM is their ability to burn. To investigate the toxicity of ACM combustion atmospheres F-344 rats were exposed for 1 hour in whole-body chambers to the smoke produced from the pyrolysis of 60 g of carbon-graphite/epoxy material. Control rats were exposed to filtered air. After 1, 3, and 7 days post-exposure, the animals underwent bronchoalveolar lavage. The lavaged cells were quantitated and identified morphologically. The lavage fluid was analyzed for the inflammatory cytokines Interleukin-1 beta (IL-1β), IL-6, and Tumor Necrosis Factor-alpha (TNF-α) by ELISA. The results included significant increases in cell counts, polymorphonuclear leukocytes (PMN)s, IL-1β, IL-6, and TNF-α as compared to controls. In conclusion, exposure to ACM combustion atmospheres produces time-dependent pulmonary toxicity manifested by the recruitment of inflammatory cells, release of inflammatory cytokines and the development of pulmonary inflammation.

216 ACUTE PULMONARY RESPONSE OF INDUCIBLE NITRIC OXIDE SYNTHASE KNOCKOUT *VERSUS* WILD TYPE MICE FOLLOWING ASPIRATION OF LIPOPOLYSACCHARIDE PLUS INTERFERON-γ OR QUARTZ.

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Exposure of mice to lipopolysaccharide (LPS) plus interferon-γ (IFN-γ) or to quartz increases nitric oxide (NO) production, which has been proposed to play a role in the resulting pulmonary damage and inflammation. To determine the role of NO in these acute lung reactions, the responses of inducible nitric oxide synthase knockout (iNOS KO) versus C57BL/6J wild type (WT) mice to aspiration of LPS+IFN-γ or quartz were compared. Male mice (6-8 weeks) were exposed by aspiration to LPS (1.2 mg/kg) + IFN-γ (5000 University), quartz (40 mg/kg), or saline vehicle. At 24 hours post-exposure, lungs were lavaged with 10 aliquots (1 ml each) of Ca+2 and Mg+2 free phosphate-buffered saline. The acellular fluid from the first bronchoalveolar lavage (BAL) was analyzed for total antioxidant capacity, lactate dehydrogenase (LDH) activity, albumin, tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 (MIP-2). The cellular fraction of the total BAL fluid was assayed for alveolar macrophage (AM) and polymorphonuclear leukocyte (PMN) counts, and AM zymosan-stimulated chemiluminescence (AM-CL). Exposure to LPS + IFN-γ decreased total antioxidant capacity, increased BAL AMs and PMNs, LDH, albumin, TNF-α and MIP-2, and enhanced AM-CL to the same extent in both iNOS KO and WT mice. Exposure to quartz decreased AM yield, increased PMNs, LDH, albumin, TNF-α and MIP-2, and enhanced AM-CL. However, iNOS KO mice exhibited less AM activation (activation status was defined as an increased AM-CL and decreased AM yield) than WT mice. These data suggest that NO may play a role in the acute pulmonary response to quartz exposure; however, evidence for a role of NO in the acute reaction to LPS+IFN-γ was not obtained.

217 INTERACTION BETWEEN PRIMARY ALVEOLAR MACROPHAGES(AM) AND PRIMARY ALVEOLAR TYPE II (TII) CELLS UNDER BASAL CONDITIONS AND AFTER LIPOPOLYSACCHARIDE (LPS) OR QUARTZ EXPOSURE.

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This study evaluated the mutual interactions between AM and TII cells under unstimulated or LPS (10 μg/ml) or quartz (100 μg/ml) exposure conditions. AM were obtained by bronchoalveolar lavage of rats, while rat TII cells were isolated by enzymatic digestion and purified by panning (~20 million cells/rat, 95% pure). AM and TII cells were co-cultured either separated by transwell inserts or in a single well to allow physical contact. After an 18 hour culture period in the absence or presence of stimulant, the medium was assayed for tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-2 (MIP-2), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and nitric oxide (NO). Cell viability, which was measured as lactate dehydrogenase (LDH) released from the cells into the medium, was not affected by either transwell or contact co-culture under basal or stimulated conditions. Under basal conditions, co-culture of AM and TII cells in transwells significantly potentiated the release of TNF-α, MIP-2, IL-6 and NO above the sum of the production by these cells cultured separately. Physical contact between AM and TII cells mitigated this potentiation, which was further decreased by exposure to LPS or quartz. Indeed, under stimulated conditions, physical contact actually decreased the production of some of these inflammatory products below the sum of the production by these cells cultured separately. These results indicate that cross-talk between AM and TII cells is complex. It appears to vary with the distance and/or contact between the two cell types, and with exposure to different stimulants.

218 CYTOTOXICITY OF SIZE-SELECTED MANVILLE CODE 100 (JM-100) GLASS FIBERS ON HUMAN ALVEOLAR MACROPHAGES.

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A previous study using rat alveolar macrophages (AMs) demonstrated that glass fibers > 17μm long were larger than these pneumocytes and resulted in frustrated phagocytosis, while fibers < 7μm long were completely engulfed. Frustrated phagocytosis was associated with a substantially greater cytotoxicity of long versus short fibers (Blake et al. J Toxicol Environ Health. Part A, 54:243, 1998). Human AMs are larger than rat AMs, approximately 18 and 13μm in diameter, respectively. Therefore, the objective of this study was to evaluate the cytotoxicity of fibers of different lengths on human AMs. JM-100 glass fiber samples of 8, 10, 16, and 20μm lengths were obtained by classification of airborne fibers by dielectrophoresis. Human AMs were obtained by segmental bronchoalveolar lavage of healthy, non-smoking volunteers. AMs were treated with three different doses (determined by fiber numbers) of the sized fiber samples for 18 hours *in vitro*. Cytotoxicity caused by the fiber samples was then determined by monitoring membrane damage (leak of lactate dehydrogenase [LDH]) and loss of function (decrease in zymosan-stimulated chemiluminescence [CL]). Microscopic analysis indicated that human AMs were large enough to completely engulf fibers which were 20μm long. All fiber length fractions tested exhibited equal cytotoxicity, i.e., increasing LDH and decreasing CL in the same dose-dependent fashion. The data indicate that because human AMs are larger than rat AMs they are able to phagocytize longer fibers and the absence of frustrated phagocytosis results in lower fiber toxicity in human AMs. These differences in the AM response to long fibers between human and rat phagocytes should be considered when designing *in vivo* exposures using the rat model.

219 SILICA-INDUCED TOXICITY: *IN VITRO* AND *IN VIVO* PROTECTIVE EFFECTS OF TAURINE.

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Taurine has been suggested to have cytoprotective actions through different mechanisms including antioxidant effects. Taurine has been proposed to be a membrane stabilizer thereby preventing oxidative damage. The aim of the present studies was

to test whether taurine might act to attenuate silica-induced toxicity *in vitro* and *in vivo*. The presence of taurine resulted in a reduction of cytotoxicity with alveolar macrophages exposed to crystalline silica. To investigate further whether taurine would function effectively in the prevention of *in vivo* toxicity from exposure to silica we exposed rats by intratracheal instillation to a single dose of 5 mg silica coated with 100 mM taurine and compared the pulmonary response to rats exposed to silica or vehicle saline. Rats were sacrificed 1, 3, or 7 days postexposure, and lungs were lavaged to monitor inflammatory cells (alveolar macrophages, neutrophils, lymphocytes, eosinophils), leakage of albumin, protein and enzymes (LDH, NAG), and chemiluminescence as markers of inflammation, cytotoxicity and reactive oxygen species generated, respectively. Surface coating and coexposure of silica with taurine significantly decreased silica-induced lung injury. In conclusion, taurine showed beneficial effects in both *in vitro* and *in vivo* models of silica toxicity. This result confirms previous studies obtained in other models of lung injury.

220 ASBESTOS AND RADIATION AS COMBINED EXPOSURES IN PULMONARY FIBROSIS.

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Worker protection standards are based upon single exposures and do not account for the constellation of exposures frequently encountered in the workplace. Workers exposed to asbestos may encounter radiation as radon or from other sources. Both asbestos and radiation are etiologic agents in pulmonary fibrosis. The aim of this study is to determine whether concomitant radiation exposure in asbestos exposed workers increases the incidence of fibrosis demonstrated by International Labor Organization (ILO) opacity profusion category on chest radiographs, or spirometric evidence of restrictive disease. 1037 asbestos exposed former nuclear weapons workers from a medical surveillance program make up the study cohort. Most are male (85.5%) with an average age of 64.9 years. 663 workers had complete work histories. The demographic and fibrosis endpoints did not differ significantly between groups. Asbestos exposure based upon years in a potentially exposed job was divided into low (less than 13 years) and high (> 13 years) dose groups. 8.0% of the high dose verses 3.6% of the low dose group had ILO scores >0/1 indicating pulmonary fibrosis while 29.3% of the high dose group verses 21.8% of the low dose group have spirometry indicating restrictive disease consistent with pulmonary fibrosis. In a 2 x 4 table analysis (binary fibrosis x binary asbestos and radiation exposure) 38.6% in the high asbestos/high radiation group met the cases definition for fibrosis vs. 25.4 % in the low/low group suggesting an additive effect. This publication was prepared with the support of the US Department of Energy, under Award No. DE-FG26-00NT40938. Opinions, findings, conclusions, or recommendations expressed herein are those of the author and do not necessarily reflect the views of the DOE. Additional support: Center for Ecogenetics and Environmental Health at the University of Washington and Center for Environmental Health Sciences at the University of Montana.

221 EFFECTS OF AVASTIN™, AN ANTI-ANGIOGENIC ANTIBODY TO VASCULAR ENDOTHELIAL GROWTH FACTOR, IN A RABBIT MODEL OF VENOUS THROMBOSIS.

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Avastin™ (bevacizumab) is a recombinant humanized monoclonal antibody directed against vascular endothelial growth factor (VEGF). It blocks the interaction of VEGF with its receptors, thereby inhibiting angiogenesis. Avastin is intended as a treatment for various cancers. Several thrombotic episodes were observed in clinical trials of Avastin. This non-clinical toxicology study was performed to determine the effect of Avastin in a rabbit model of venous thrombosis. Adult male New Zealand White rabbits were given 75 mg/kg Avastin or Avastin vehicle intravenously daily for eight consecutive days. Following the final dose, a thrombus was induced in the jugular vein by application of a flow-reducing stricture proximal to the site of a clamp-induced damage. The presence or absence of occlusion was noted, as well as time to occlusion and weight of the excised clot. Cuticle bleeding time was measured. Coagulation and fibrinolysis assays were performed *ex vivo*: prothrombin time, activated partial thromboplastin time, whole blood recalcification time, activated clotting time, platelet aggregation to adenosine diphosphate, and d-dimer concentrations. Complete blood cell counts were measured. Immunohistochemical assay for von Willebrand Factor/Factor VIII complex was performed on sections of the jugular vein adjacent to the site of clot formation and on sections from the contralateral undamaged vessel. No differences were found in any of the parameters between Avastin- and vehicle-treated animals. These data suggest that rabbits subacutely treated with Avastin do not develop a prothrombotic or hyper-coaguable state in this venous thrombosis model nor is such a state demonstrable by the biomarkers measured.

222 TH9507: SAFETY STUDIES OF A GROWTH-HORMONE RELEASING FACTOR (GRF) ANALOGUE.

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TH9507 is a GRF analogue which has been stabilized by anchoring a hydrophobic moiety to the peptide, while preserving its amino acid sequence. TH9507 is in Phase II trials (doses range from 1.7 to 33.3 µg/kg) for multiple indications (muscle wasting in COPD, recovery after hip fracture surgery, sleep maintenance insomnia and immune dysfunctions). The mode of action involves induction of growth hormone (GH) release, followed by GH-induced release of Insulin-like Growth Factor–1 (IGF-1). Acute to 4-month toxicology and safety pharmacology studies have permited continuation of ongoing clinical trials, and are presented below. Acute IV studies in rats & mice at doses of 100 & 200 mg/kg produced 80% mortality at 200 mg/kg and severe but transient clinical signs at 100 mg/kg. Range-finding 2-week IV studies in rats & dogs revealed no toxicity up to the highest dose (100 µg/kg/day). IND-enabling 4-week IV studies in rats & dogs employing doses of 100 to 600 µg/kg/day revealed no adverse effects; increased body weight, adrenals weight, food intake, bilirubin, triglycerides and/or cholesterol, were attributed to the pharmacological action of TH9507. Three- and 4-month SC studies (with 1-month recoveries) in rats & dogs, respectively, employing doses of 100 to 600 µg/kg/day, revealed increased body weight, food intake, reversible hepatocellular vacuolization and/or reversible increases in phosphorus, triglycerides, cholesterol and proteins. Dose-related injection site irritancy, common to peptides, was also observed. Toxicokinetic evaluations showed a dose-related increase in TH9507 plasma levels. Bioactivity was confirmed by increased rGH and cIGF-1, in rats & dogs, respectively. In conclusion, although no dose level was free of effects, with the exception of local irritancy, all effects were judged to be due to the pharmacological activity. Furthermore, single-dose SC respiratory and CNS safety pharmacology studies in rats at doses of 0.6 to 50 mg/kg, revealed no adverse effects.

223 TWENTY-EIGHT DAY TOXICITY STUDY OF THE CANCER CHEMOPREVENTIVE AGENT 4-BROMOFLAVONE IN RATS.

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4-Bromoflavone (4BF) is being developed as a cancer chemopreventive agent. We have examined the toxicity of 4BF after 28 days of daily gavage administration to 4 groups of 20 CD® rats/sex at 0, 100, 300 and 1000mg/kg/day. No animals died. Clinical signs included dehydration and rough coat in a high dose group male (1000 mg/kg/day) and 5 males in mid dose group (300mg/kg/day) on days 1-3. Reduced total body weight gain with a corresponding reduction in food consumption was seen in all 4BF-treated groups except the low dose (100mg/kg/day) male group. Plasma levels of 4BF increased as a function of dose level, with male groups showing 2.1 & 1.5 times higher plasma drug levels than female groups at high and mid doses. Treatment-related ophthalmic lesions were absent. Non-hemolytic anemia was noted at high, mid and low dose groups due to reductions in one or more hematologic parameters (hematocrit, RBC count, MCV, hemoglobin, MCH) and increased no. of reticulocytes (high dose group only). The primary target tissues for 4BF were liver and thymus in both sexes, pituitary gland in males, and mammary gland in females. Dose-dependent hyperproteinemia, hypercholesterolemia and hypotriglceridemia were noted. These, accompanied by hepatomegaly and increased hepatocyte glycogen depletion in all 4BF-treated groups were suggestive of liver as a major target organ for the metabolic effects of 4BF rather than hepatotoxicity. Thymic lymphoid depletion in all 4BF-treated groups and hyperplasia/hypertrophy of clear cells of pituitary gland in all 4BF-treated male dose groups were also noted. Increased secretory activity of mammary glands (all doses of 4BF) and reduced weight of ovaries/fallopian tubes (at mid and high doses) were seen in female dose groups. The histopathologic changes were interpreted as related to the metabolic effects of 4BF on liver, pituitary and mammary glands (Support: NCI Contract N01-CN-85173).

224 STABILIZATION AND ANALYSIS OF PYROGALLOL (PG) IN RAT BLOOD AND IN RECEPTOR FLUID MEDIA.

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Pyrogallol, (1, 2, 3-trihydroxybenzene, PG), a metabolite of plant hydrolysable tannin gallic acid, is used in hair dyes, as a mordant in wool dying, in the manufacture of pharmaceuticals and pesticides and in topical formulations. Because of its wide

spread use and lack of carcinogenicity data, PG has been selected for toxicological evaluation by the NIEHS. In this work a reverse phase HPLC/UV method was validated for both rat blood and receptor fluid media at pyrogallol concentrations of 4 to 200 μg/mL. Sample concentrations were determined using the peak area ratio, PAR, with resorcinol, 2 μg/mL, as the internal standard. In addition since resorcinol is a pyrogallol metabolite found in urine, sample concentrations may also be determined by peak area alone. During the development of the analysis method, it was observed that PG spiked into rat blood or into receptor fluid media, was rapidly lost. For pyrogallol in rat blood at 0.5 mg/mL, 10% of the initial concentration was lost within 0.4 hr. If spiked into pH 7 receptor fluid media at 0.1 and 0.5 mg/mL, 10% PG was lost within ~ 10 and ~ 30 hours, respectively. This indicated that, for a collected sample, a significant loss of PG in solution will occur prior to analysis if not stabilized. Here we report on the stabilization of PG in rat blood and receptor fluid media samples by adjusting the sample to pH 3 with phosphoric acid and by adding at least 1.5% ascorbic acid, final concentration, as an antioxidant. Once stabilized, spiked rat blood and receptor fluid media samples may be stored for at least 3 weeks under frozen conditions without significant PG loss, allowing for a reasonable time for analysis. Using direct injection mass spectrometry, identification of the PG degradation products was evaluated. Although no degradation components were observed using HPLC/UV, the MS data suggested the presence of a diol metabolite.

225 PHARMACOKINETICS OF GT56-252, A NOVEL ORALLY AVAILABLE IRON CHELATOR IN RAT, DOG AND CYNOMOLGUS MONKEY.

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GT56-252 is a novel orally-available iron chelator, under development for the primary treatment of iron overload. Pharmacokinetic studies were conducted as part of a preclinical programme to support Phase 1 clinical studies, and were complemented by toxicokinetic data. Single dose pharmacokinetic studies were performed with ^{14}C labeled and unlabeled compound in the rat and dog, and with ^{14}C-labeled compound in the monkey. Excretion balance, biliary excretion and tissue distribution were measured in the rat. GT56-252 was orally bioavailable in all three species, and measurements in the dog revealed a significant food effect. Elimination of GT56-252 from plasma was moderately slow in all 3 species, with terminal half-lives ranging from 3 hours in the rat to 7 or 8 hours in the dog. Overall exposure for unchanged drug in both species was lower than that for radioactivity. Tissue distribution in the rat was similar following oral gavage or IV administration. The organs with the highest concentrations were the adrenals and intestines, while drug levels were also measured in the kidneys and liver. Urinary excretion was the principal route of elimination in both the rat and dog, and more than 80% of the administered dose was eliminated in the urine within 24 hours. Faecal administration accounted for 10 to 20 % of the excreted material, whether given by the oral or IV route, suggesting the involvement of biliary excretion. This was confirmed by measurement of biliary excretion in the rat where 7-8% of an intravenously administered dose was found to be eliminated in the bile in the first 3 hours after dosing. Toxicokinetic data from 28 day oral toxicity studies were broadly consistent with the pharmacokinetic data. In both normal and iron loaded animals, the toxicokinetics data showed good proportionality between administered dose and systemic exposure. There was no evidence that systemic exposure was substantially modified as a result of repeated administration for 28 days.

226 THE PROTEASOME INHIBITOR PS-341 INDUCES COX-2 IN MURINE AND HUMAN ENDOTHELIAL CELLS.

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PS-341, a novel compound selectively inhibits the 26S proteasome that is integral in the proteolytic degradation of intracellular proteins. During preclinical safety studies, hypotension was observed in cynomolgus monkeys within hours after administration of an acutely toxic dose that caused >90% proteasome inhibition. Thus, we investigated the ability of PS-341 and lactacystin, a structurally unrelated inhibitor, to induce synthesis of vasoactive mediators in murine RAW 264.7 macrophages, human aortic endothelial cells (HUAEC), and murine b.END3 endothelial cells. Nitric oxide was not detected in endothelial cells, but was produced in LPS and IFN-γstimulated RAW 264.7 cells, which was completely abrogated by treatment with 10 μM PS-341 or 10 μM lactacystin. In HUAEC and b.END3 cells, both proteasome inhibitors induced a robust increase in levels of PGI2 and PGE2, and a mild increase in Thromboxane B2, but not Leukotriene B4. Lactacystin and TNF-α, had a similar but less robust effect. Northern analysis of treated HUAECs showed that PS-341and lactacystin induced Cox-2, but not Cox-1. TNF-α induced VCAM-1 expression was inhibited 83% and 47% by PS-341and lactacystin, respectively. In b.END3 cells Cox-2 mRNA appears by one-hour and peaks at 8 hours post-treatment with PS-341. By Western analysis there was an 11-fold induction of Cox-2 protein that was first detected at 4 hours and reaches a plateau by 24 hours post-treatment. Also, Hsp70 was induced ~12-fold by PS-341, which is a well-documented effect of proteasome inhibitors. To verify that PS-341-mediated Cox-2 upregulation was independent from Hsp70 induction, cells were heat shock treated, which induced Hsp70, but left Cox-2 expression unaffected. Cox-2 gene induction was confirmed *in vivo* by demonstrating strong induction of Cox-2 mRNA expression in peripheral blood mononuclear cells from cynomolgus monkeys administered PS-341. These data demonstrating that PS-341 induced Cox-2 and prostanoids suggest that a peripheral vasodilatory mechanism may mediate hypotension.

227 INVESTIGATIVE CARDIOVASCULAR STUDY OF THE PROTEASOME INHIBITOR PS-341 IN THE MOUSE.

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During preclinical safety pharmacology studies in macaques with PS-341, acutely toxic IV doses (0.3 mg/kg) caused initial tachycardia, followed by progressive bradycardia, hypotension and death. To investigate mechanisms underlying these observations, PS-341 was given to Balb/c mice and ECG tracings using a free ranging remote ECG recording platform (AnonyMOUSE, Mouse Specifics, Inc., Boston MA). Following a single IV dose of 0.1, 0.3, 1.0, 3.0 or 10 mg/kg, at 10 mg/kg showed there was initial tachycardia, followed by a precipitous decrease in heart rate and moribundity. ECG tracings during the period of bradycardia revealed prolonged PR intervals and spiked T waves. No significant ECG changes were seen at ≦3.0 mg/kg. Increased parasympathetic vagal tone was not a considered a contributing factor to the bradycardia as atropine treatment had no effect on heart rate in PS-341 treated mice. Body temperature monitoring showed a decrease in body temperature coincident with decreased heart rates. To confirm a direct association, PS-341 treated animals had body temperature maintained by ambient temperature control and during this period, bradycardia was not seen. Using an *ex vivo* perfused heart preparation, hearts from PS-341 treated mice showed no difference in heart rate and force of contraction compared with untreated controls. In separate but parallel experiments, we investigated the possible role of PS-341 induced soluble vasoactive mediators in the hypotension observed in macaques (Csizmadia, SOT 2003). These experiments demonstrated that PS-341 promoted robust expression of the vasoactive mediators PGE2 and PGI2 by human aortic endothelial cells, which was associated with induction of Cox-2, but not Cox-1 mRNA and protein. Collectively, these data indicate acutely toxic doses of PS-341 had no direct effect on heart rate or contractility and that bradycardia was mediated indirectly through altered thermoregulation, which can be abrogated by maintenance of body temperature.

228 ENDOTHELIAL CELL-DERIVED NITRIC OXIDE MEDIATES SMOOTH MUSCLE CELL FAS EXPRESSION INDUCED BY PHOSPHODIESTERASE INHIBITION.

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Using an established model of phosphodiesterase inhibitor-induced vasculopathy, we demonstrated increased levels of smooth muscle cell apoptosis in mesenteric vessels of female Wistar rats given 3 daily 750-mg/kg oral doses of the phosphodiesterase (PDE) subtype IV inhibitor CI-1018. Inducible nitric oxide synthase was observed by immunohistochemistry in vascular endothelium and inflammatory cell infiltrates in the vessel media. Because nitric oxide is a documented intercellular signaling molecule between vascular endothelial and smooth muscle cells as well as an initiator of apoptotic signaling pathways, we hypothesized that nitric oxide elaborated by endothelial cells may play a role in vascular smooth muscle cell apoptosis induced by CI-1018. We hypothesized further that nitric oxide release from endothelial cells upon treatment with CI-1018 induces vascular smooth muscle cell apoptosis *via* Fas-Fas ligand pathway. In the present study, human pulmonary artery smooth muscle cells (HPASMC) were cultured with or without overlying monolayers of human lung microvascular endothelial cells (HMVEC-L). Cultures were treated with vehicle or CI-1018 at 125 μM for 72 hours. RT-PCR analysis of smooth muscle cell lysates demonstrated that CI-1018 induced Fas expression in HPASMC only and only when co-cultured with HMVEC-L. Induction of Fas expression was accompanied by an increase in the levels of the nitric oxide metabolites nitrate and nitrite in the shared medium. Pretreatment of HPASMC/HMVEC-L co-cultures with the nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester at 0.1 mμM followed by treatment with CI-1018 diminished Fas mRNA levels in HPASMC in co-culture with HMVEC-L. Fas expression in HMVEC-L was not changed by treatment with CI-1018. These data suggest an important paracrine role for endothelial nitric oxide in PDE IV inhibitor-induced Fas expression in PASMC with important implications in the etiology of vascular smooth muscle cell apoptosis induced by PDE IV inhibition *in vivo*.

229 PYROLYTIC PRODUCTS OF METHAMPHETAMINE HYDROCHLORIDE AS POTENTIAL MARKERS FOR SMOKED METHAMPHETAMINE.

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Sponsor: M. Davis.

Methamphetamine hydrochloride and other smoked drugs of abuse pyrolyze during volatilization to produce breakdown products with potential lung toxicity. Although pyrolysis products of cocaine and phencyclidine have been identified in urine and used as markers for the detection of smoked drugs of abuse, there are currently no markers identified for methamphetamine abuse by smoking. Smoking of methamphetamine HCl laced tobacco yields several volatile products. In order to identify pyrolytic products of methamphetamine in the absence of potentially reactive substances found in tobacco, differential scanning calorimetry (DSC) was used as a pyrolysis chamber to generate thermal breakdown products of methamphetamine hydrochloride. Sample sizes of 0.6-1.0 mg of methamphetamine hydrochloride were pyrolyzed using a Perkin Elmer Differential Scanning Calorimeter 7. Samples were crimped in air-tight and then the sample was placed in the sample holder with a reference pan. Heating rates of 20 and 40 °C/min were evaluated in the temperature range of 275 to 450 °C. Samples were eluted with ethanol and 15 microL portions of the eluant were injected onto a C-18 reverse phase HPLC column with 70:30 MeOH:H_2O mobile phase at a flow rate of 1 mL/min. Samples were also analyzed by gas chromatography-mass spectrometry on a DB5 capillary column with oven temperature 50 °C held for 1 min then programmed at 15 °C/min to 280 °C and by direct injection electrospray ionization ion trap mass spectrometry. Methamphetamine hydrochloride degraded when heated in the DSC instrument above its melting point. At least five decomposition products were observed by HPLC and mass spectral analysis. Beta-methylstyrene (1-propenylbenzene) was one of the major degradation products identified and is a good candidate for use as an indicator of methamphetamine hydrochloride abuse by smoking.

230 CJC-1131, A LONG-ACTING GLP-1 ANALOGUE, EXHIBITS SAFETY AND TOLERABILITY IN DOGS.

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CJC-1131 is an anti-diabetic compound being developed for the control of Type 2 diabetes. By applying the Drug Affinity Complex (DAC) technology to glucagon-like peptide-1 (GLP-1), the hormone selectively and covalently binds to plasma proteins, primarily albumin, after parenteral administration. Pharmacokinetic data demonstrated a long half-life of ~75 hrs following subcutaneous (SC) administration of CJC-1131 in dogs. The acute toxicity of CJC-1131 was evaluated in Beagle dogs following single SC doses of 2, 4 and 8 mg/kg. No treatment-related effects on survival, physical examinations, blood pressure, organ weights, clinical pathology and gross necropsy were observed. Clinical signs included decreased fecal output at doses of ≥4 mg/kg, and emesis at 8 mg/kg. Transient decreased food consumption was noted at all doses in both sexes, as was decreased water consumption in the males at all doses. Both effects were related to the pharmacological action of GLP-1. A sub-chronic study in dogs was also conducted with SC administration every other day (q2d) at doses of 0.25, 1 and 4 mg/kg for 14 days. All dogs survived to termination and no treatment-related effects were noted during physical, ophthalmic and ECG examinations, or on hematology, urinalysis and gross necropsy. Slight dose-dependent weight loss (<6%) was noted at all doses, which correlated with the transient decreases in food (↓6-66%) and water (↓3-41%) consumptions when compared to control animals. Decreases in food and water consumptions were more pronounced on dosing days and were of a lesser magnitude during week 2. Cholesterol values were lower (↓18-31%) in dogs treated at 4 mg/kg. Mild reversible inflammation, noted microscopically in SC tissue at the injection site in all dose groups, including controls, was attributed to administered dose volume and repeated injections in the same site. In conclusion, CJC-1131 up to 4 mg/kg was generally well tolerated in Beagle dogs after SC administration q2d for 14 days.

231 CALCIFICATION OF MUCOSA IN GLANDULAR STOMACH OBSERVED DURING WITHDRAWAL PERIOD AFTER SEVELAMER HYDROCHLORIDE TREATMENT IN RAT.

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Sponsor: S. Tsuda.

Calcification of glandular part of gastric mucosa was observed after 4-week withdrawal in 1-month toxicity study in rats with RenaGel®(Sevelamer Hydrochloride), which is used for hyperphosphatamia of hemodialysis patients. This study is to investigate the time course change of calcification in glandular stomach during withdrawal period. Normal diet or 10 g/kg/day of RenaGel was administered by diet mixture for 4 weeks to 25 male rats (Slc:SD, 6-week-old), respectively. Then, at the end of administration (day 0 of withdrawal), and on days 3, 7, 14 and 28 of withdrawal, histopathological examination and clinical pathology were carried out. 5 animals/group were assigned for each time point. On day 0 of withdrawal, serum inorganic phosphorus (IP) and calcium (Ca) x IP product (Ca x IP product) decreased, and chloride (Cl) increased, although histopathology revealed no lesions in glandular stomach. On days 3 and 7 of withdrawal, serum IP and Ca x IP product increased, and then those resumed to control level by day 14 of withdrawal. Serum Cl decreased on days 3 and 7 of withdrawal, and then recovered to control level by day 14 of withdrawal. Serum Calcium was maintained control level during withdrawal. In histopathological examination, mucosal calcification in glandular stomach had been most remarkable on days 3 and 7 of withdrawal, afterward, its frequency and severity declined. Furthermore, hypertrophy of parietal cell was observed on day 3 of withdrawal, suggesting hypersecretion of gastric acid after early period of the withdrawal. Susceptibility of mucosal calcification in glandular stomach during withdrawal period after RenaGel treatment was increased by: (i) immediate increase of blood Ca x IP product after the start of the withdrawal, and (ii) elevated pH in the interstium of glandular stomach by hypersecretion of gastric acid immediately after withdrawal (which may be compensating the disappearance of RenaGel derived Cl from the stomach cavity).

232 FLOW CYTOMETRY METHOD TO EVALUATE BONE MARROW TOXICITY IN THE RAT.

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The use of flow cytometry has led to significant advances in the understanding of toxicity of some drugs in the bone marrow. We have previously reported several methods to assess toxicity by flow cytometry in the bone marrow such as using cell sorting on Forward and Side Scatter properties; using CD45R and CD71 staining; using Rh123 and nonyl acridine for mitochondria membrane potential evaluation, and a special combination of paraformaldehyde and digitonin fixation with propidium iodide (PI) staining for cell cycle analysis. The present study describes new flow cytometry methods for our investigations on the bone marrow toxicity. CD71 and CD45 have proved to be particularly useful in evaluating the effects of drugs on bone marrow. Two-color analysis using CD45 and CD71 staining provides a means where erythroid precursors, myeloid precursors, lymphocytes and granulocytes can be visualized on one histogram. Other specific stains, such as CD45R or CD11b can be used to evaluate specific cell types on an as-needed basis. Combination of CD45 or CD71 with MitoFluor Green and LDS-751 staining allowed assessment of the mitochondria function for specific cell lineages. Apoptosis has been evaluated in human bone marrow cells using Annexin V in conjunction with PI. However in rats, Annexin V cross-reacts with immature B-cells in the marrow making identification of apoptotic bone marrow cells difficult. Therefore, staining with an activated fluorescent marker of apoptosis, Caspase 3, was evaluated. Combining Caspase 3 with 7AAD as the nuclear stain has allowed the addition of a third marker, such as CD45R or CD71. Thus using 3-color analysis is possible to evaluate cell death and apoptosis in a specific cell lineage. Cells can also be specifically stained for the presence of a drug receptor to determine whether toxicity is due to pharmacology. Flow cytometry methods in cell sorting, apoptosis, cell cycle and immunophenotyping techniques not only provide a rapid bone marrow cells evaluation, but also offer a reliable and fully understanding for the mechanism of toxicity.

233 MEDIATORS OF INFLAMMATION IN PHOSPHODIESTERASE INHIBITOR-INDUCED MESENTERIC VASCULOPATHY IN RATS.

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Mesenteric vasculitis induced in rats by the phosphodiesterase (PDE) subtype IV inhibitor CI-1018 is characterized by infiltration of mixed inflammatory cells and is associated with increases in peripheral blood neutrophils. We sought to detail the time course of appearance of inflammatory cytokines and chemokines as potential contributors to inflammatory cell activation, differentiation, and recruitment to affected tissues. Eight groups of four female Wistar rats each were given daily oral doses of vehicle or CI-1018 at 750 mg/kg for 3 days. Animals were euthanized 2, 4, 8, 16, 24, 48, and 72 hours after the first dose. Vascular lesions were evident at 48 hours with exacerbation at 72 hours. Serum levels of interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and granulocyte macrophage colony stimulating factor (GM-CSF) were determined by enzyme immunoassay. TNF-α and IL-10 were transiently elevated 2 hours after administration of CI-1018. IL-6 was increased at

48 hours and sustained through 72 hours. No changes in serum IL-1β, GM-CSF, or IFN-γ were noted. In concordance with serum data, semiquantitative RT-PCR analysis of mesenteric vessels revealed elevated mRNA levels of IL-6 and TNF-α. Similarly, mRNA levels encoding for chemoattractant factor interferon-inducible protein-10 (IP-10) were increased. The rapid increases in serum TNF-α, IL-10, IL-6 and tissue IP-10 mRNA suggest their involvement in the early pathogenic events leading to frank vasculitis. In particular, the qualitative correlation between the incidence of vasculitis and the magnitude of changes in serum IL-6 support its consideration as a principle component in any proposed battery of biomarkers for vasculitis.

234 CJC-1131, A LONG-ACTING GLP-1 ANALOGUE, IS WELL TOLERATED IN RATS UP TO 14 DAYS.

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Sponsor: S. Wilson.

CJC-1131 is a novel agent being developed as an anti-diabetic compound for the control of Type 2 diabetes. It is a synthetic modification of the hormone glucagon like peptide-1 (GLP-1) that allows the compound to selectively and covalently bind to albumin, following parenteral administration. In this way, CJC-1131 retains the activity of the GLP-1 pharmacophore while adopting the pharmacokinetic profile of albumin. Pharmacokinetic data in rats has shown that CJC-1131 exhibits a long plasma elimination half-life of approximately 21-28 hours, following a single subcutaneous (SC) administration. The acute toxicity of CJC-1131 was evaluated in CD rats following single SC doses of 0.75, 1.5, 3 and 6 mg/kg. CJC-1131 was generally well tolerated with decreased fecal output, and decreased food and water consumption at all dose levels. Transient decreased activity was noted at doses ≥1.5 mg/kg with recovery noted within 1-2 hours postdose. In a sub-chronic study, the toxicity of CJC-1131 was investigated in CD rats, following every other day SC injections for 14 days at 0.12, 0.6 and 3 mg/kg. Similar clinical observations were noted, including decreased fecal output, reduced food intake (↓7-87%), reduced water consumption (↓16-66%) at 3 mg/kg only, and dose-dependent decreases in the body weight gain (↓19-70%) when compared to the control animals. Food and water consumption effects were less pronounced on non-dosing days. There was no effect on survival, ophthalmology, hematology, urinalysis, clinical chemistry, organ weights, macroscopic and microscopic pathology that was clearly attributed to treatment. Microscopically, minimal depletion of vacuoles in the cytoplasm of hepatocytes was found in female rats at 0.6 and 3 mg/kg and considered related to the decreased body weight gain rather than evidence of hepatotoxicity. In conclusion, CJC-1131 was well tolerated in rats, following single and repeat SC administration at dose levels up to 3 mg/kg.

235 REPEATED DOSE SUBCUTANEOUS TOXICITY AND TOXICOKINETICS STUDY OF NAHBED IN BEAGLE DOGS.

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The objective of this work was to evaluate potential toxic effects and determine plasma drug concentrations in male and female beagle dogs following repeated daily subcutaneous administration of the monosodium salt of N, N-di(2-hydroxybenzyl)ethylenediamine-N, N-diacetic acid (NaHBED). NaHBED is an iron-specific chelating agent that has been shown to be effective in producing net iron excretion in the iron-loaded Cebus monkey model and has been selected as a candidate therapeutic agent for treating patients with transfusional iron overload. In this study dogs were administered 14 consecutive daily doses of NaHBED in aqueous solution at 32.13 (15% or 20% w/v), 96.39 (20% w/v), or 192.78 mg/kg/day (20% w/v) by slow bolus injection. Administration of 192.78 mg/kg/day caused mortality. Some minimal injection site irritation was observed in all groups with the incidence and severity being lower in controls than in treated animals but comparable among all the NaHBED-exposed groups. High dose animals also exhibited increased emesis, hypoactivity, hunched posture, body weight loss (~19-24%), elevated ALT levels, hepatic biliary leukocyte infiltration, pigmented Kupffer cells, and renal tubular vacuolation. The liver lesions were also present in dogs administered 20% formulations of NaHBED at 32.13 and 96.39 mg/kg/day. Serum iron was notably elevated in all dose groups that received NaHBED relative to controls. Iron-to-total iron-binding capacity was also elevated. NaHBED was readily absorbed as shown by a Tmax of approximately 0.5 hr on Day 1. The Cmax and AUC values increased with dose in both sexes. Based on mortality and moribundity seen the maximum tolerated dose is considered to be at least 96.39 mg/kg/day but less than 192.78 mg/kg/day in dogs administered NaHBED in this dosing regimen. The liver and kidney were identified as target organs. Based on the biliary effects, the no-observed-adverse-effect-level is considered to be 32.13 mg NaHBED/kg/day when administered as a 15% (w/v) formulation.
This work was supported by NIDDK Contract No. N01-DK-3-2201.

236 RAT SPECIFIC MECHANISM OF SRA88O-INDUCED RHABDOMYOLYSIS.

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The ergoline and somatostatin receptor antagonist (sst1) SRA880 was found to decrease serum creatine kinase (CK) activity (> 90 %) and to induce myonecrosis (rhabdomyolysis) after histopathological examination in a 2 week rat study. The underlying mechanism and the relevance of this finding to man was investigated *in vitro* in rat skeletal muscle cells (rSkMC) with and without metabolic activation in a rSkMC-hepatocyte coculture. The results indicate that the SRA880 parent compound was neither toxic in rSkMC, nor did it cause CK inhibition when given to rat or human serum. However, after preincubation with rat hepatocytes SRA880 was toxic to rSkMCs and caused CK inhibition in rSkMCs and rat serum. SRA880 did not cause any effects when it was preincubated with human hepatocytes. While the O-demethylated metabolite 227-450, which is the main SRA880 metabolite in human, was not toxic in rSkMCs, 227-809, the main metabolite in the rat was toxic. Indicators of toxicity were decreased CK activity and ATP content as well as an increased intracellular lactate/pyruvate ratio. The present result suggests that SRA880 can cause rhabdomyolysis in rats but not in man by the formation of metabolite 227-809. By inhibition of the cellular energy supply 227-809 is causing increased anaerobic glycolysis with acidification and damage of the skeletal muscle fibres.

237 ABSENCE OF TOXICITY IN A 13-WEEK INTRAVENOUS INFUSION STUDY WITH INTERCEPT PLASMA IN SPRAGUE-DAWLEY RATS.

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The INTERCEPT Blood System for Plasma has been shown to inactivate a broad range of viruses, bacteria, parasites and leukocytes using the psoralen derivative, amotosalen HCl (formerly S-59), in an *ex vivo* treatment process. After addition to plasma, amotosalen reversibly intercalates into nucleic acids. Upon exposure to UVA light, amotosalen reacts to form covalent DNA and RNA cross-links. After the treatment process, the levels of free photoproducts and residual amotosalen are reduced by exposure to a Compound Adsorption Device (CAD). The study was designed to determine any potential adverse effects of treated rat plasma with and without CAD exposure when administered *via* 2-hour daily intravenous infusion at 40 mL/kg to three groups of 15 rats/sex for 13 weeks (91 days), followed by a four-week observation period for five rats/sex/group. The vehicle control was rat plasma. The mean doses of amotosalen given to the rats were ~14.6 and ~993 µg/kg/day for rats receiving treated plasma, with and without CAD exposure, respectively. The high dose was ~250 fold the anticipated human dose, based on a 1L plasma transfusion. Rats were observed for evidence of mortality, moribundity and clinical signs. Body weights and feed consumption were measured. Ophthalmological examinations were performed twice. Hematology, clinical chemistry, and urinalysis evaluations were performed. All rats were evaluated macroscopically on necropsy, organ weights recorded, and tissues processed for histopathologic evaluation. On the first day and during the last week of dose administration, blood samples were taken for evaluation of toxicokinetic parameters. On Day 1 of dosing, the amotosalen C_{max} values were 9.73 and 37.4 ng/mL in rats administered ~14.6 and ~993 µg/kg, respectively. The AUC_{last} values after dosing were 47.5 and 109 ng·hr/mL and the $t_{1/2}$ values were 6.7 and 6.0 hours. There was no observed accumulation of amotosalen in plasma after 91 days of dosage. There was no amotosalen related toxicity in this study.

238 TOXIC EFFECTS OF HMG-COA REDUCTASE INHIBITIORS IN THE HUMAN SKELETAL MUSCLE PRIMARY CELL CULTURE.

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Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors are well established as pharmacologic agents to improve the lipid profile of dyslipidemic patients as well as decrease cardiovascular-related morbidity and mortality. Rhabdomyolysis is a rare but clinically relevant side effect of statin mono and combination therapy in man, with a higher prevalence for simva- and lovastatin as compared to other statins. The mechanisms leading to HMG-CoA reductase inhibitor mediated myonecrosis are not yet understood. In the present study we compared simva- lova-, atorva-, prava- and fluvastatin in human skeletal muscle cell primary culture (hSkMC) concerning their effects on energy status, caspase-3 activation and cytoplasmic membrane damage (LDH-release). After 2 hours of incubation 50 µM simavastatin and 100 µM lovastatin induced almost similarly, sig-

nificant increases in caspase-3 activity and in parallel increased ATP levels. After 20 hours of incubation with simva- and lovastatin the ATP pools were significantly decreased in comparison to controls and cells started to become leaky for LDH. At the same concentrations, none of the other investigated statins, fluva-, atorva- and pravastatin showed effects comparable to the findings seen with simva- and lovastatin. Effects were seen only at concentrations of 400 µM. These results suggest that induction of capase-3 activity might be an important early event in the development of the statin-induced rhabdomyolysis. Since the caspase-3 activity is depending upon the intracellular ATP levels, the results further suggest that the intracellular energy state might serve as a checkpoint, which could drive cells into necrosis.

239 SAFETY AND ABSORPTION OF PULMONARY DELIVERED HUMAN INTERFERON BETA-1A IN THE NON-HUMAN PRIMATE.

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Safety and absorption of pulmonary-delivered human interferon beta-1a (IFN beta-1a) were evaluated in the rhesus macaque to assess an alternate delivery route. IFN beta-1a was administered *via* inhalation with a dose range of 0-4500 micrograms per week. Serum interferon beta and neopterin, an interferon-induced biological response marker, were measured throughout the study. Safety assessments were performed following one, three, and six months of treatment, the latter followed by a 4-week recovery period prior to sacrifice. Inhaled IFN beta-1a was not associated with any significant changes in safety parameters. In the lung, histomorphologic changes consisted of minimal to mild subchronic alveolitis, which increased slightly with dose but did not progress over six months. Resolution of pulmonary lesions was seen in all recovery animals. Approximately 70% of the blisterpack content was available to the monkeys for inhalation over the exposure period. There was a dose-dependent increase in IFN beta serum concentration levels in treated compared to control animals at doses up to 900 micrograms per week and in the biological response marker, neopterin. Neutralizing titers of anti-IFN beta antibodies (NAbs) were evident in the repeat dose groups beginning at the third week of dosing, which correlated with decreased serum IFN beta and neopterin levels in those animals testing positive for NAbs. However, the lungs were exposed to active IFN beta throughout the entire six-month dosing period as evidenced by analysis of bronchioalveolar lavage fluid. In conclusion, these studies show that chronic pulmonary administration of human IFN beta-1a is generally safe and that sufficient systemic absorption occurs to induce biologic responses in the macaques. These studies suggest that the pulmonary route of administration is a viable alternate route for the systemic delivery of IFN beta-1a.

240 EVALUATION OF SOMATROPIN INHALATION POWDER IN A SINGLE DOSE AND 4-WEEK MONKEY INHALATION TOXICOLOGY STUDY.

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The toxicity of Somatropin Inhalation Powder (SIP), a dry powder formulation consisting of large porous particles of recombinant human growth hormone and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine, was investigated in single dose and 4-week inhalation toxicology studies in young rhesus monkeys. Standard toxicology study parameters plus respiratory function (respiratory rate, tidal volume, minute volume), bronchial alveolar lavage parameters and serum levels of immunoreactive growth hormone (GH) and GH-specific antibodies were monitored in each study. Body weights, urinalysis parameters and cardiovascular function were also monitored during the 4-week study. Necropsies were conducted 1 day after the 4-week exposure and after a re-challenge dose following an approximate 2-week recovery period. Organ weights were analyzed and gross and histopathological examinations, including lung tissue stained with proliferating cell nuclear antigen (PCNA) to evaluate a potential mitogenic response, were conducted for all animals. In the single dose study, systemic bioavailability of human growth hormone (hGH) was approximately 10% relative to subcutaneous (sc) injection. Serum pharmacokinetic profiles were similar between pulmonary and sc administration. A moderate immunogenic response was observed in monkeys exposed for 4 weeks. There was no evidence of neutralizing antibodies and there was no anaphylactic response upon single-dose re-challenge after the 4-week exposure. There were no histopathological or other toxicological effects attributable to SIP in any of the parameters evaluated following single doses, during the 4-week exposure period, or following re-challenge. In conclusion, no adverse effects were noted in young rhesus monkeys exposed to single inhaled doses up to 4.0 mg hGH/kg bw or exposed daily to inhaled doses up to 5.5 mg hGH/kg bw for 4 weeks. In sc-administered equivalents, these data in monkeys provide margins of safety well above the recommended clinical doses of hGH.

241 LACK OF GENOTOXICITY WITH THE NOVEL ANTI-INFECTIVE PRODRUG DB289 AND ITS ACTIVE METABOLITE DB75, A 2, 4-DIPHENYL FURAN DIAMIDINE WITH DNA MINOR GROOVE BINDING ACTIVITIES.

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The antimicrobial activities of DNA minor groove binding agents have been well-established. DB289 (2, 5-Bis-[4-(N-methoxyamidino)phenyl]furan) is the dimethoxime prodrug of DB75 (2, 5-Bis[4-amidinophenyl]furan), a potent anti-infective that binds to the minor groove of DNA. To support initiation of clinical trials with DB289, the genotoxicity of DB289 and its active metabolite DB75 were evaluated. The potentials for DB289 and DB75 to induce reverse mutations in an Ames assay were assessed at concentrations up to 5000 (DB289) or 333 (DB75) mg/plate using various Salmonella typhimurium strains and 5000 mg/plate using E. coli strain WP2uvrA, with and without metabolic activation. The potentials for DB289 and DB75 to induce structural and numeric chromosomal aberrations in CHO cells at concentrations up to 120 (DB289) and 75 mg/ml (DB75) were assessed, with and without metabolic activation. Finally, potential *in vivo* clastogenicity of DB289 was assessed in a rat micronucleus assay. In duplicate trials of the Ames assay, DB289 and DB75 did not significantly increase the mean number of revertants beyond vehicle control levels in all strains tested. In duplicate trials of the CHO cell chromosomal aberration assay, DB289 and DB75 did not significantly increase either the number or type of chromosomal aberrations beyond vehicle control levels. In the rat micronucleus assay, no significant increase in micronucleated polychromatic erythrocytes was detected in rats treated with DB289 up to 1000 mg/kg. Similarly, DB75 was not clastogenic in this assay since it was present as a major metabolite. Thus, DB289 and DB75 are not mutagenic or clastogenic, and binding of DB75 to the minor groove of DNA is not associated with genotoxicity in these assays.

242 SIMULTANEOUS DETERMINATION OF ACETYLSALICYLIC ACID NON-ENZYME MEDIATED OXIDATIVE PRODUCTS.

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Aspirin (ASP) is a commonly used analgesic and anti-inflammatory agent. After ingestion, it is hydrolyzed to salicylic acid (SA) by esterase in the gastrointestinal tract and in liver. We found that ASP can be oxidized to SA without enzyme. On the other hand, SA can react with hydroxyl radical to form its derivatives, 2, 3-dihydroxybenzoate and 2, 5-dihydroxybenzoate. The reaction system consisted of hydrogen peroxide (H2O2), ferrous iron (Fe2+) and ASP or SA. The hydroxyl radical production in the Fenton reaction was confirmed by electron spin resonance (ESR) in combination with spin trapping technique. The assay of ASP oxidative products was performed on high-performance liquid chromatography (HPLC) equipped with UV-visible diode array detector and an XTerra Phenyl column (3.5 um, 2.1 mm x 150 mm). The mobile phase consisted of 0.3% formic acid and acetonitrile at the gradient ratios from 85:15 for 3 min to 80:20 for 6 min and kept for 1 min, then back to 85:15 for 1 min at a constant flow rate of 0.3 mL/min. The compounds were monitored at 230 nm and a reference wavelength 360 nm with 100 nm widths. Benzoic acid was used as the internal standard for quantification. A detection limit of less than 5 ng per injection for these products could be achieved. The reproducibility was found to be better than 10%. The method also could be used to evaluate the *in vivo* and *in vitro* oxidative stress induced by toxicant dermal exposure.

243 OTOTOXICITY OF NORVANCOMYCIN: AN EXPERIMENTAL STUDY IN GUINEA PIGS.

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Norvancomycin (NV), a structural analogue of vancomycin, is used to treat methicillin-resistant staphylococcus infections. The aim of this study was to investigate the ototoxic effects of norvancomycin on the inner ear. Guinea pigs with positive Preyer's reflex were given fourteen consecutive daily doses intravenously of norvancomycin at either 56, 108 or 216 mg/kg. Control animals received equivalent volumes of saline. For a comparison, other animals received daily vancomysin (V) at either 56, 108 or 216 mg/kg for 14 days. The duration of post-rotatory nystagmus and threshold of auditory brainstem response stimulated by click and pop tone were recorded before and after the treatment. The cochleas were dissected for hiar cell counting by the surface preparation, and examined by transmission and scan-

ning electron microscopy. The results showed that there was no changes in duration of post-rotatory nystagmus after treatment in all groups.There was no significant difference in hearing loss between the saline controls and the animals treated with 56 and 108 mg/kg NV . The threshold shifts after administration with NV 216 mg/kg were about 5dB. There was no significant difference in threshold shifts between the groups treated with NV and V at all doses. No inner hair cells were missing and about 2% loss of the outer hair cells of the basal turn were observed in the animals treated with NV and V of 216 mg/kg. Scanning electron microscopy showed a normal appearance of stereocilia and cuticular plateof hair cells in all groups. There is also a normal intracellular ultrastructure of hair cells in the coahleas with NV and V at all doses. Conclusion: There is no clear evidence for the existence of ototoxicity of norvancomycin.

244 PRE-CLINICAL EVALUATION OF ITRACONAZOLE NANOSUSPENSION FOR INTRAVENOUS INJECTION.

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The commercially available Itraconazole, a broad-spectrum antifungal agent, contains Cyclodextrin, a potentially toxic excipient. Therefore, a new Itraconazole nanosuspension was prepared without Cyclodextrin, but using a proprietary microprecipitation and homogenization process. This preparation was found to be stable both at room temperature and at 5°C for more than 1 year without any changes in potency, particle size or agglomeration. Regarding acute toxicity, intravenous injection of nanosupension to Sprague-Dawley rats resulted in no lethality with up to 8 times the dose of the commercially available solution. The NOEL for the nanosuspension was 80 mg/kg in contrast to 20 mg/kg for the commercial solution. The nanosuspension appeared more bioavailable as indicated by the pharmacokinetic parameters. For example, the plasma half-life of the nanosuspension was 14 h, in contrast to 5 h for the commercial Itraconazole solution at an equivalent dose (20 mg/kg). Similarly, the mean resident time for the nanosuspension was 13 h in contrast to 5h for the commercial Itraconazole suspension. Higher PK values were also observed for the metabolite, hydroxy-itraconazole, using the nanosuspension. In efficacy studies, the nanosuspension appeared more efficacious than the commercial solution in eliminating *Candida albicans* from the body, in that alternate day dosing of nanosupension was more effective than every day dosing of the commercial solution. Furthermore, a dose of 80 mg/kg of nanosuspension completely eliminated the Candida colonies from the kidney of rats, whereas dosing with the commercial solution was limited by toxicity. After a high dose (320 mg/kg) intravenous injection of nanosuspension, histopathological studies revealed the appearance of nanoparticles in rat splenic macrophages. In summary, Itraconazole nanosuspension is less toxic and appears to be a more effective antifungal agent than the commercially available solution we evaluated.

245 POLYISOTYPIC ANTIBODY AND MIXED CYTOKINE RESPONSES TO THE MAJOR PEANUT ALLERGENS IN AN ORAL MOUSE FOOD ALLERGY MODEL.

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Sponsor: M. van den Berg.

Peanut is responsible for the majority of fatal food-induced allergic reactions and peanut-allergy is known for its persistence for life. The mechanism of sensitization is largely unknown. To investigate this, we used a mouse food allergy model. The animals were orally or intra-peritoneally exposed to a peanut protein-extract (PE) together with cholera toxin in order to investigate cytokine production and both Th2 and Th1 antibody responses to the extract and purified peanut allergens Ara h1, Ara h2 and Ara h3. B-cell responses to PE, Ara h1, Ara h2 and Ara h3 were characterized by serum Elisa's and Elispot. After one week, PE-specific IgG1 antibodies could be detected in 2 out of 8 animals orally exposed to PE. Only T-cells of IgG1 positive animals showed IL-4 and IL-5 production upon restimulation with PE. Remarkably, IFN-γ levels were also clearly elevated in cultures of these animals compared to IgG1-negative and control animals. After 3 weeks, peanut specific antibody levels IgE, IgG1 as well as IgG2a were increased in the serum of orally and i.p. peanut-exposed mice. A few animals from the orally dosed groups did not respond and showed no antibody production at all. PE-specific IgA levels were significantly higher in orally dosed animals compared to i.p. treated mice. Strong mixed antibody responses (both IgG1 and IgG2a) were also observed to the purified allergens Ara h1 and Ara h3, whereas the response to Ara h2 was more skewed to Th2. In the animals with serum antibody responses to PE, the PE -induced antibody secreting cells were located in the bone marrow. These results indicate that cytokine production in an early stage may serve as a mechanistic tool to study the onset of sensitization and also as a predictive factor for the development of food allergy. Furthermore, data imply that immune responses to the strong allergen peanut, are not restricted to Th2 induced phenomena but involve Th1/Th2 cytokine production and polyisotypic antibody responses.

246 EVALUATION OF RESPIRATORY SENSITIZATION POTENTIAL OF PHTHALATE ESTERS.

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Bronchial asthma is mediated, in part, by the immunoregulatory cytokines IL-4 and IL-13. These cytokines stimulate IgE synthesis which is associated with airway hyperresponsiveness, a hallmark of asthma. Compounds that stimulate IgE synthesis and elicit bronchial reactivity are generally considered to be respiratory sensitizers. A recent publication (Tox Sciences Late-Breaking Absts Suppl, Vol 66, No 1-S, p. 22, 2002) showed that DEHP did not stimulate IgE in mice, and, therefore is unlikely to produce respiratory sensitization. To extend these observations, the potential for three other commercial phthalate ester plasticizers, DINP, DIHP and BBP, to induce respiratory sensitization was assessed. Testing followed the method of Dearman et al. (1992): topical application (and challenge) of the phthalate esters to mice followed by measurement of serum IgE. In addition, auricular lymph nodes were harvested for measurement of IL-4 and IL-13 proteins as well as IL-4 and IL-13 mRNAs. Because skin absorption of higher molecular weight phthalate esters is limited, liver weight increase, a measure of peroxisome proliferation, was monitored for DEHP and DINP. Liver weights were significantly increased indicating that these phthalates achieved systemic distribution. ELISA and RNAse protection assays showed that levels of IgE, IL-4 protein, IL-13 protein, IL-4 mRNA and IL-13 mRNA in phthalate ester treated animals were similar to control values. The positive control, a respiratory sensitizer, trimellitic anhydride, produced large and statistically-significant increases in all parameters, demonstrating responsiveness of the assay. As expected, dinitrochlorobenzene, a contact sensitizer, produced small but significant increases in IgE and in mRNA for IL-4 and IL-13 but not in the levels of these cytokines. In summary, DEHP, DINP, DIHP and BBP were without activity in the mouse IgE test suggesting that they would not induce respiratory sensitization.

247 INDUCTION OF IgE ANTIBODY FOLLOWING SYSTEMIC EXPOSURE OF MICE TO RESPIRATORY SENSITIZING PROTEINS.

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With the advent of genetically modified crop plants there is an increased interest in the development of methods for the identification of novel proteins as potential allergens. We have demonstrated previously that food proteins display a differential capacity to stimulate IgE antibody following systemic (intraperitoneal; ip) exposure of BALB/c mice which is consistent with their apparent differing allergenic potential (in man). An additional concern is that inhalation exposure to the novel allergens *via* plant pollens may lead to sensitization. We have therefore investigated the induction of IgE antibody following systemic (ip) exposure of mice to the detergent enzymes Lipolase® and Termamyl®, proteins for which the primary route of sensitization is *via* inhalation. BALB/c strain mice received an ip injection of protein (1%) on days 0 and 7. Control animals were exposed to ovalbumin (OVA), a relatively common food allergen which can also cause occupational asthma. Seven days later, serum samples were analyzed for specific IgE antibody by homologous passive cutaneous anaphylaxis assay and for IgG antibody by enzyme-linked immunosorbant assay. Administration of OVA and Lipolase each induced high titer IgG antibody responses (ranging from 1in 1600 to 1 in 3200 and 1in 512 to 1 in 1024, respectively). Both proteins provoked vigorous IgE antibody production, with all animals testing positive for IgE and titers of 1 in 16 and 1 in 256 recorded for OVA and Lipolase, respectively. Despite the fact that the IgG antibody response was considerably less vigorous (titers ranging from 1 in 32 to 1 in 64), all Termamyl-treated mice were IgE responders and an IgE titer of 1 in 2 was recorded. These data show that those proteins which have respiratory sensitizing capacity may also be characterized by their ability to stimulate IgE responses following systemic treatment of BALB/c strain mice.

248 THE PESTICIDE METARHIZIUM ANISOPLIAE HAS AN ADJUVANT EFFECT ON THE ALLERGIC RESPONSE TO OVALBUMIN IN MICE.

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Metarhizium anisopliae is a parasitic fungus employed as a biological control agent against vermin and used in the US for indoor control of cockroaches. Sensitization to cockroach allergens is associated with development of asthma. This pesticide is

non-pathogenic for humans and is therefore an alternative to more toxic chemicals. However, in previous studies M. anisopliae has been shown to cause allergic and asthma-like responses in mice. We examined the adjuvant activity of M. anisopliae crude antigen (MACA), consisting of different parts of the fungus including mycelium, and mycelium alone, on the response to the model allergen ovalbumin (OVA) in the poplietal lymph node assay (PLNA). Groups of 8 mice were injected with MACA + OVA, mycelium + OVA, MACA, mycelium or OVA alone in the right hind footpad. On day 21 the animals were boosted with OVA and then exsanguinated and the PLN removed on day 26. The levels of OVA-specific serum IgE and IgG1 (Th2 immune response), and IgG2a (Th1 immune response) were determined by ELISAs. There was a statistically significant increase in the levels of anti-OVA IgE and IgG1, but not anti-OVA IgG2a in the group given mycelium + OVA compared with OVA alone. In the group given MACA (1/5 of concentration for mycelium) + OVA the levels of anti-OVA IgG1 increased in one out of two experiments. Moreover, both MACA and mycelium + OVA compared with OVA alone induced increased weight and cell number of the excised PLN. Our study shows that exposure to M. anisopliae together with OVA results in an increased local inflammatory response and Th2-dependent immune response to a model allergen in mice. This indicates that M. anisopliae has the ability to increase an allergic response to allergens in the environment. In particular, use of this pesticide for cockroach control in homes, may result in an enhanced allergic reactions in individuals who are sensitized to cockroach allergens. (This abstract does not reflect EPA policy.)

249 PYROGALLOL INDUCES WEAK CONTACT SENSITIZATION BUT STRONG IRRITATION IN FEMALE BALB/C MICE.

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The reducing agent pyrogallol (PYR) was used as topical therapy for chronic plaque psoriasis, and has found extensive use as an antioxidant in industrial and commercial processes. The objective of this study was to determine the potential for PYR to elicit an allergic response when applied dermally to female BALB/c mice. Measurement of the contact hypersensitivity response was initially accomplished using the local lymph node assay (LLNA). Because PYR was soluble at a concentration of 75% (w/v) in the AOO (Acetone:olive oil at 4:1) vehicle, the exposure level selected was 2.5%, 5%, 10%, 25% and 50% in the first LLNA. Since PYR at all the concentrations produced a significant increase in the proliferation of lymph node cells, two additional LLNAs were performed. The concentrations of PYR used in the second study were at 0.5%, 1% and 2.5%, and the third at 0.25%, 0.5%, 1.0%, 5% and 10%. Significant increases in the proliferation of lymph node cells were observed at the concentrations of 0.5% and higher. Based on the results of the LLNA, a Primary Irritancy Assay (PIA) study was conducted using exposure levels at 0.125%, 0.25%, 0.5%, 1%, 5% and 10%. The results suggest that PYR is an irritant at concentration as low as 0.125%. To further determine if PYR is primarily an irritant, two Mouse Ear Swelling Tests were performed. In the first MEST, mice were sensitized with PYR at concentrations of 0.25%, 1%, and 5%, and challenged with 0.25% of PYR. There was no significant difference when the naïve, vehicle, and three dose groups were compared to background control at either 24- or 48-hr time point. In the second MEST, mice were sensitized with PYR at concentrations of 1% and 5%, and challenged with 1% of PYR. Significant increase in mouse ear thickness was observed at 66 hr after challenge in mice that were sensitized with 5% PYR. Taken together, these results demonstrate that PYR is a weak sensitizer but a strong irritant in female BALB/c mice. (Supported by the NIEHS Contract ES 05454).

250 GENE EXPRESSION CHANGES IN AN ANTIGEN-SPECIFIC SECONDARY RESPONSE USING A DENDRITIC CELL / T CELL CO-CULTURE.

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The defining event of skin sensitization is the stimulation of T cell activation and proliferation by antigen presenting dendritic cells (DC). Therefore a reasonable approach for the development of an *in vitro* test is to examine at the cellular and molecular levels the interactions between these cells, utilizing a culture system comprising both DC as antigen presenting cells and responding T cells. Therefore, to increase our understanding of the changes in gene expression that are induced by antigen-specific T cell/DC interactions in humans we examined changes in gene expression that occur in a secondary antigen-specific response using cells from a sensitized donor. T cells isolated from a dinitrochlorobenze (DNCB) sensitized subject were cultured with untreated autologous DC or with DC treated with dinitrobenzene sulfonic acid (DNBS), the water soluble analog of DNCB. Total RNA was obtained after 6hrs incubation and changes in gene expression were analyzed using Affymetrix U95Av2 Genechips®. Comparison of mean signal values from triplicate cultures found 29 genes that were significantly different ($p \leq 0.001$) between T cells cultured with untreated versus hapten-treated DC. 25 genes were up-regulated with fold increases of +1.5 to +10.6. Some of the up-regulated genes of interest include interferon regulatory factor 1, interferon-γ, interleukin-2, STAT1 and guanylate binding protein 2. Four genes were down-regulated with fold decreases of -1.1 to -3.7 and only one of the four could be associated with T cells. It is our aim to utilize these data to develop endpoint measures which might form the basis of an *in vitro* approach for the identification of skin sensitizing chemicals.

251 COMPARISON OF UREA-TYPE REACTION PRODUCTS OF METHYLENE DIPHENYL DIISOCYANATE (MDI) AND THE BIS-THIOCARBAMATE METHYLENE DIPHENYL DIISOCYANATE CYSTEINE METHYL ESTER (MDI-CME).

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Diisocyanates (dNCOs) are a class of chemicals used worldwide in polyurethane products such as foams, spray paints and wood products. Diisocyanates have been associated with respiratory irritation, asthma, hypersensitivity pneumonitis, bronchitis, irritant and allergic dermatitis, and conjunctival irritation. They are the most commonly reported cause of chemically induced occupational asthma, but the antigenic component is unknown. Reaction of dNCO with free thiol gives rise to bis-thiocarbamate formation. It has been established that the bis-thiocarbamates can form rapidly, and although reversible, are more stable in biological systems than the parent dNCO. The urea-type reaction products obtained from the reactions of both methylamine and HSA with MDI and MDI-CME, were compared. Reactions were carried out under aqueous conditions and analyzed using HPLC, measurement of primary amine content, and 1 and 2-dimensional (D) gel electrophoresis. It was found that at least some of the reaction products of MDI and its bis-thiocarbamate were different. MDI was able to cross link HSA causing loss of primary amine and altered migration in 1D gels. Two methylamine reaction products were evident with the less polar product predominant. The MDI-CME caused no apparent loss of primary amine or 1D gel shift. HSA conjugation was noted by both 2D gel shifts and HPLC-UV analysis. The more polar methylamine MDI-CME product was predominant. This data, along with our previously reported MDI-CME (consecutive) hydrolysis kinetics, suggest potentially different antigenic products may be formed from reaction of MDI vs MDI-thiocarbamates to endogenous proteins.

252 CHARACTERIZATION OF LAMOTRIGINE-SPECIFIC T-CELLS FROM HYPERSENSITIVE PATIENTS.

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Lamotrigine causes severe hypersensitivity reactions. It has been suggested that these reactions have an immune aetiology, and T-cell involvement has been postulated. However, there is little evidence for this. The aim of this study was to characterize the role of T-cells in lamotrigine hypersensitivity. Lymphocytes from hypersensitive patients proliferated when exposed to lamotrigine. T-cell clones were generated by serial dilution and characterized in terms of their phenotype, functionality and mechanisms of antigen presentation (including structure-activity relationships and processing pathways) and cytotoxicity. Forty-four drug-specific T-cell clones were generated; most were CD4+ with occasional CD8+ cells. All clones expressed the αβ T-cell receptor with use of a single Vβ chain, and the skin-homing receptor CLA. Although the Vβ repertoire was heterogeneous, several Vβ 5.1(+) or 9(+) T-cell clones were generated. Lamotrigine-stimulated T-cells were cytotoxic and secreted perforin, IFN-γ, IL-5 and MIP-1α, MIP-1β and I-309. RANTES was expressed constitutively. Lamotrigine was presented on MHC class II (both DR and DQ) by HLA matched antigen presenting cells, in the absence of overt drug metabolism and antigen processing. The T-cell receptor of certain clones could accommodate structural analogues of lamotrigine but no cross reactivity was seen with other anticonvulsants. The data show that Th1 secreting T-cells mediate lamotrigine hypersensitivity. Demonstration of IL-5 secretion, expression of the skin homing receptors CLA and perforin-mediated killing are consistent with clinical features of hypersensitivity including eosinophilia and the observed tissue damage in skin.

253 PATHOLOGIC AND IMMUNOLOGIC RESPONSES IN THE RESPIRATORY TRACT OF A/J MICE AFTER INTRANASAL SENSITIZATION AND CHALLENGE WITH TRIMELLITIC ANHYDRIDE.

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Sensitization of the respiratory tract to low molecular weight chemicals (LMWC) including trimellitic anhydride (TMA) is a leading cause of occupational asthma and allergic rhinitis in industrial settings. Mucus hypersecretion and airway inflammation consisting of lymphocytes and eosinophils are pathologic features of such allergic airway diseases (AAD). Many experimental models have linked LMWC-induced AAD to Th2 cytokine expression. Most murine models, however, use systemic administration (e.g., topical) to sensitize mice. The present study tests the hypothesis that intranasal sensitization and challenge with TMA will induce the immunologic and pathologic responses characteristic of LMWC-induced AAD in the nasal and pulmonary airways. A/J mice were intranasally sensitized and then intranasally challenged twice with TMA or 1:4 ethyl acetate/olive oil vehicle. Nasal and pulmonary airways were processed for light microscopic examination. Right lung lobes were analyzed for Th2 cytokine mRNA expression using real-time PCR and total serum IgE was measured using an ELISA. Only mice that were intranasally sensitized and challenged with TMA had a marked allergic rhinitis characterized by an influx of eosinophils, lymphocytes and plasma cells, 24 h after the final challenge. By 96 h, the nasal airway epithelium exhibited increases in stored mucus and a regenerative hyperplasia. Only TMA-sensitized and challenged mice exhibited an increase in lung-derived IL-5 mRNA and elevated total serum IgE that was 4-fold the control level by 24 h after exposure and persisted at that level by 96 h. However, no pulmonary lesions were found in any group. This study is the first to demonstrate that intranasal administration of an LMWC is an effective method of sensitization resulting in the hallmark features of allergic rhinitis and the up-regulation of critical mediators of AAD, IL-5, within the lung after challenge, and total serum IgE (Supported in part by American Chemistry Council Grant #0051).

254 AN *IN VITRO* SENSITIZATION TEST USING HUMAN CELL LINE, THP-1, FOR THE EVALUATION OF WATER INSOLUBLE CHEMICALS.

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In our previous study, we found that the expression of CD54 and/or CD86 on human monocytic leukemia cell line, THP-1, were enhanced after a 24-hour exposure of allergens but not non-allergens. These results suggested that measuring CD54 and CD86 expression on THP-1 cells could be used as an *in vitro* sensitization method. However, water insoluble chemicals add a degree of complexity to a cell culture system (i.e., lack of homogeneity). The aim of this study was to identify suitable conditions to evaluate the insoluble chemicals in our *in vitro* system. Under normal conditions, the insoluble chemicals are first solubilized in vehicle (DMSO) at a high concentration and added to the culture medium to make the final concentration of DMSO 0.1%. However, when the insoluble sensitizers, α-hexylcinnamaldehyde (HCA) and benzocaine, were evaluated, the expression of CD54 and CD86 was not enhanced. We first studied a way to evaluate the insoluble chemicals using DNCB as a positive control. When the vehicle concentration was increased to 2% DMSO and exposure time was shortened to 4 hours, the CD54 and CD86 expression was not enhanced. Interestingly, when the cells were cultured in the medium for an additional 20 hours following a 4-hour exposure, the expression of CD54 and CD86 was augmented. We then evaluated HCA and benzocaine using this procedure; namely, the cells were exposed to HCA and benzocaine in 1% DMSO vehicle for 4 or 8 hours and then cultured in the medium to make the total culture time 24 hours. Under these conditions, the insoluble sensitizers enhanced CD54 and/or CD86 expression. It was postulated that the antigens could stimulate the cells during the 4-hr time period and that the surface markers would be expressed on the cell surface later. The results suggest that insoluble chemicals can be evaluated in an *in vitro* culture system by modifying the test conditions. With these modifications, our skin sensitization assay using THP-1 cells can evaluate a larger variety of chemicals.

255 CYTOKINE EXPRESSION IN A ASTHMATIC MURINE MODEL INDUCED BY TOLUENE DIISOCYANATE.

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This study was aimed at investigated the cytokine status and their gene expressions in airways of asthmatic mouse caused by toluene diisocyanate (TDI). After the mice were exposed to TDI vapor for 5 consecutive days, bronchoaveloar lavage (BAL) was carried out and the cells in the BAL fluid were counted. Lung histological examine was coducted to analyse the inflammatory status in the airways. Total serum IgE and IL-4 and IL-5 concentrations in the BAL fluid were determined with ELISA kits. RT-PCR was applied to investigate IL-4 and IL-5 mRNA from BAL cells. The airway cell count and histological analysis showed that TDI exposure resulted in airway inflammatory responses characterized by marked infiltration of eosinophils in the central and peripheral airways. Total serum IgE was significantly increased in the TDI-exposed mice. IL-4 and IL-5 productions in the BAL fluid were significantly enhanced in the mice exposed to TDI, but in BAL cells IL-4 and IL-5 mRNA were slighlty enhanced without any significant difference compared to the control mice. These results further support the hypothesis that Th2 cytokines are predominantly produced in TDI-induced airway hypersensitivity. However, our data was not able to indicate the exact source of these cytokines and we could not determine whether the change of cytokines in the airways was controlled by gene transcription level.

256 OXIDATIVE STRESS AND PROSTAGLANDIN E2 RELEASE FROM AIR PARTICULATE PM1648 TREATED MACROPHAGES.

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Epidemiological studies reveal associations between air pollution and the occurrence of respiratory symptoms characteristic of asthma, but the mechanisms of this effect are not yet clear. The prevalence of asthma has been increasing since the 1970's. Asthmatics are particularly sensitive to attacks brought on by air pollution. Air pollution particulate matter is cleared from the lung by the alveolar macrophage, and the effects of the particulates seem to be mediated by macrophage derived cytokines and lipid mediators. PM1648, a characterized urban particulate, and air particulates in general cause an increased asthmatic, Th2-driven response. Because eicosanoids may play a role in the asthmatic response, this study was based on the hypothesis that PM1648 would affect eicosanoid release from macrophages, possibly through an oxidative stress response. RAW 264.7 cells were treated with PM1648, and oxidative stress was measured as increased dichlorofluorescein oxidation and subsequent increases in intracellular glutathione observed at 6 and 12 hours following treatment with PM1648. Prostaglandin E2 (PGE2) measured by EIA in culture supernatants was also significantly elevated 6 hours after treatment. This increase in PGE2 could be blocked with addition of cell permeable glutathione monoethylester. We have shown that instillation of PM1648 *in vivo* exacerbates bronchial hyperresponsiveness in a mouse model of asthma. These results together suggest that PM1648-induced oxidative stress may lead to changes in eicosanoid metabolism that may affect hyperresponsiveness in the lung. This work was supported by the NIH Grant ES11120 and the Stella Duncan Memorial Research Foundation.

257 TRIMELLITIC ANHYDRIDE (TMA)-INDUCED CELL INFILTRATION IN A MOUSE MODEL OF OCCUPATIONAL ASTHMA IS NOT COMPLEMENT DEPENDENT.

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Trimellitic anhydride (TMA) is a small molecular weight chemical known to cause occupational asthma. Recent studies in ovalbumin-induced asthma in guinea pig and murine models suggest that the complement system plays a role in mediating symptoms of asthma, particularly airway hyperresponsiveness. Also, in a guinea pig model of TMA-induced asthma, complement depletion reduced eosinophil infiltration into the airspace. In the present study, to determine if an intact complement system is essential in TMA-induced asthma in the mouse, animals were depleted of complement with cobra venom factor (CVF) and the effect on TMA-induced cell infiltration into the lung determined. BALB/c mice were sensitized as follows: days 1 and 3 intradermally with TMA in corn oil vehicle, and day 12 intratracheally with TMA conjugated to mouse serum albumin (TMA-MSA). To elicit the allergic response, mice were challenged intratracheally on day 19 with 400 ug TMA-MSA or control MSA. To deplete complement, animals were treated with CVF or its vehicle (Veh) for 4 days, beginning 24 hours before challenge. Eosinophil and neutrophil infiltration and concentrations of complement component 3 (C3) in the bronchoalveolar lavage (BAL) were assessed 72 hr after challenge in 4 to 8 animals per group. Challenge of sensitized animals with 400 ug of TMA-MSA resulted in significant eosinophil infiltration into the lung compared to MSA challenge. Veh treatment itself reduced TMA-induced eosinophilia in BAL. However, CVF treatment did not significantly reduce TMA-induced eosinophil infiltration into the BAL or lung when compared to Veh treatment. Measurements of C3 in the BAL confirmed that CVF treatment significantly depleted complement compared to controls. These data demonstrate that complement depletion with CVF does not

inhibit TMA-induced eosinophilia in the mouse and that the presence of an intact complement system is not essential for TMA-induced occupational asthma in this model. (Supported by Graduate School and Academic Health Center of University of Minnesota).

258 PARTIAL CHARACTERIZATION OF ALLERGENS IN EXTRACTS OF *STACHYBOTRYS CHARTARUM*.

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Exposure to *Stachybotrys chartarum* has been associated with the development of serious health problems in humans, including asthma. Previous studies in this laboratory demonstrated respiratory exposure to an extract of a pool of 5 *S. chartarum* isolates caused inflammatory and allergic biochemical, immunological, and physiological responses similar to those observed in human allergic asthma. The goal of the present study was to characterize the major allergens responsible for the observed immune responses. Five isolates of *S. chartarum* obtained from wallboards in water-damaged houses were grown and combined in approximately equal weight amounts, extracted using Hanks' Balanced Salt Solution (HBSS) + Tween-80, and filter sterilized to form a crude antigen preparation (SCE-1). A hyperimmune serum was generated by injecting female BALB/C mice with a 25 µg dose of SCE-1 in alhydrogel adjuvant, followed by additional 15 µg doses of SCE-1 in HBSS at 21 and 35 days. Five days after the 3rd dose, the mice were exsanguinated by cardiac puncture. The resulting serum was used to probe western blots of individual *S. chartarum* isolates, loaded at equal total protein amounts and using an anti-IgE secondary antibody to visualize the major IgE-positive antigens. IgE-positive protein bands were observed in all the extracts; however, the positive bands varied in intensity, suggesting that the isolates differ in their overall antigen profile. (Supported by NCSU/EPA Cooperative Training Agreement CT826512010.) (This abstract does not reflect EPA policy.)

259 STRUCTURE ACTIVITY RELATIONSHIPS OF EPOTHILONE B AND ANALOGUES IN THE MURINE LLNA.

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Epothilone B (EPO906) is a non-taxane microtubule-stabilizing agent intended for the therapy of cancer. EPO906 and related structures (CGP085720 and CGP085217) were tested in the murine local lymph node assay (LLNA) to identify a putative skin sensitising potential. Test items were applied to female, 7 to 9 weeks old BALB/c mice on the dorsum of both ears on 3 consecutive days. 24 hours after the last application mice were sacrificed and ear biopsies as well as ear-draining lymph nodes (LN) were taken in order to assess skin irritation and LN hyperplasia. A concentration range of EPO906 from 20% to 0.0001% (w/v) was applied in order to identify the concentration, which would lead to a 1.05-fold increase in ear weight (circular biopsy of 0.5qcm), and a 1.3-fold in LN cell count, when compared with the vehicle-treated control group. These increases had been shown by analysis of historical data to serve as a threshold at which usually statistically significant ear skin irritation and LN hyperplasia had been observed. Testing of EPO906 in the LLNA resulted in a bell-shaped concentration response curve with a maximum increase at 0.1% for ear weight, LN weight as well as LN cell count. Minima were identified at 20% and 0.0001% for all parameters. Since body weight was significantly reduced at concentrations above 0.1%, and were not changed at the lower concentrations, it is assumed that general toxicity at higher concentrations is responsible for the sub-maximal ear skin and LN parameters. The threshold concentrations for the ear skin and the LN cell count were calculated as 0.0152% and 0.0032%, respectively. CGP085720 is a structural analogue of EPO906 lacking the macrocycle epoxy-group potentially responsible for the postive effects of EPO906. With CGP085720 similar threshold concentrations as with EPO906 were obtained in the LLNA. CGP085217, an EPO906 analogue containing the respective epoxy group but lacking the macrocycle lactone moiety, produced threshold concentrations above 0.1%, indicating that the lactone group may be involved in the positive LLNA reaction.

260 DETECTION OF ANTI- R-SP-C ANTIBODIES IN SERA OF DOGS AND MONKEYS USING A SPECIFIC ELISA SYSTEM.

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Rationale: Recombinant surfactant protein C (r-SP-C) represents an important option for the reconstitution of the alveolar surfactant system in patients undergoing intensive medical care. In studies investigating the biological activities of the recombinant protein, the induction of r-SP-C-specific antibodies in r-SP-C-surfactant treated monkeys and dogs has to be considered. The appareance of those antibodies during the treatment phase indicates the development of a specific immune reaction of the animals against the protein. Therefore an ELISA was established, detecting the r-SP-C-specifc IgG, IgA and IgM-antibodies. Methods: The sera of r-SP-C-surfactant treated monkeys or dogs were prediluted 1:100 and then titrated 1:2 over 8 wells in plates coated with the antigen. After a washing step the bound antibodies of the sera were detected using polyclonal antibodies against IgG, IgM or IgA of dogs or monkeys. The coating of the antigen was investigated using an antibody directed against r-SP-C to ensure a positive signal. To evaluate the data of the treated animals, a negative group of at least 71 untreated animals were investigated to determine the range of the response of a normal group. Results: Three concentrations of r-SP-C-surfactant were investigated in the studies. Only in single animals slightly increased titers of r-SP-C-specific antibodies could be detected. The appearance of the antibodies did not correlate with the treatment or the dose given to the animals. Conclusion: The treatment with r-SP-C-surfactant used in the approach did not indicate a specific immunogeneic risk of the protein in monkeys and dogs. The ELISA assay developed for this specific surfactant protein represented an useful tool for the investigation of the induction of antigen-specific antibodies of several subclasses in these species.

261 THE RELATIONSHIP BETWEEN CELLULAR DISPOSITION AND CYTOKINE POLARIZATION FOR SIMPLE HAPTENS.

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2, 4-Dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA) are haptens that induce a polarized cellular immune responses. Cutaneous exposure of BALB/c strain mice to DNCB results in vigorous production of type 1 cytokines such as IFN-g and IL-12 by lymph node cells (LNC) draining at the site of exposure. The opposite is true for TMA; with the production of low levels of type 1 cytokines and higher levels of the type 2 cytokines IL-4, IL-5 and IL-10 by activated LNC. In an attempt to study whether such qualitatively divergent immune responses are associated with differential disposition we have applied a Western Blot method. Briefly, DNCB and TMA (50-500mM in 1% dimethylsulfoxide) were incubated with the human macrophage-like cell line U937 in the presence or absence of 10% fetal calf serum. At one hour cells and serum were separated and assayed. The proteins were separated by SDS-PAGE, and hapten binding was analyzed using anti-DNCB and anti-TMA antibodies. DNCB was found to bind selectively to cells when the chemical, serum and cells were incubated together. In contrast, TMA bound selectively to serum. However, both chemicals were found to bind covalently to cells and to serum when incubated with either cells or serum alone. In conclusion, we show that TMA and DNCB interact differentially with cellular and extracellular proteins, and propose that this may be an important factor in the development of polarized cellular immune responses to chemical allergens.

262 RELATIONSHIP OF CD86 SURFACE MARKER EXPRESSION AND CYTOTOXICITY ON DENDRITIC CELLS EXPOSED TO CHEMICAL ALLERGEN.

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Human peripheral blood derived dendritic cells (PBMC-DC) respond to a variety of chemical allergens by upregulating expression of the co-stimulatory molecule CD86. We recently reported that PBMC-DC, treated with the highest non-cytotoxic concentrations of chemical allergen, marginally upregulated CD86 expression and concluded this measure did not provide the dynamic range for assessing a chemical's sensitization potential. For the work presented here, DC were derived from either human monocytes in GM-CSF and IL-4 or from CD1c+ leukocytes cultured in the presence of GM-CSF, IL-4 and TGFb. DC were treated with various concentrations of the chemical allergens dinitrochlorobenzene (DNCB) or dinitrochlorobenzene sulfonic acid (DNBS) for 48 hrs in the presence of GM-CSF and IL-4. Mean fluorescence intensity of CD86 expression, measured by flow cytometry, was upregulated in 5/5 preparations (150%-340% control) on PBMC-DC treated with concentrations of DNBS which induced 10-15% cytotoxicity. In one case, CD86 was upregulated with a non-cytotoxic concentration of DNBS (182% control). CD86 expression was upregulated in 2/2 experiments (130% and 420% control) with CD1c-derived DC treated with DNCB at a concentration which induced slight cytotoxicity. The irritant sodium dodecyl sulfate did not induce upregulation of CD86 expression when tested at concentrations which induced similar cytotoxicity. Our results illustrate that concentrations of chemical al-

lergen which induce slight to moderate cytotoxicity are capable of inducing upregulation of CD86 expression. We did not observe differences in the ability of DC to respond to chemical allergens based on the way in which the DC were isolated and cultured. An association between CD86 expression and cytotoxicity appears evident, but the mechanisms underlying this phenomenon are unknown and should be understood if assessment of changes in CD86 expression is to be used as a predictive tool for skin sensitization.

263 THE CCR7 RECEPTOR IS UP-REGULATED BY HAPTENS ON HUMAN DENDRITIC CELLS.

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The frequency of allergic diseases in industrial countries is still increasing. Chemical haptens are responsible for a large part of those immune pathogenesis. These chemicals penetrate into the organism and are taken up by immature dendritic cells (DC) and then presented to naive T-lymphocytes thus eliciting hypersensitivity reactions. Activation of DC by haptens in cooperation with cytokines produced by DC themselves or by keratinocytes induces the maturation of DC. During this process, DC leaves peripheral tissues and migrates to lymph nodes to activate naive T-lymphocytes. Migration of DC involves many factors including the expression of the chemokine receptor CCR7. In this study, we tested the effects of two well-known chemical haptens, dinitrochlorobenzene (DNCB) and nickel sulfate (NiSO4), on the expression of CCR7 on immature DC. These cells were differenciated from cord blood CD34+ hematopoietic progenitor cells in the presence of GM-CSF, TNF-alpha and Flt-3 ligand for 7 days. Treatment of immature DC by DNCB (25 microM) or NiSO4 (500 microM) during 24 hrs induced the maturation of these cells as assessed by the expression of CD83 and CD86. CCR7 mRNA was not detected by RT-PCR on unstimulated DC. However, DC incubated for 4 hrs with DNCB or NiSO4 express the CCR7 mRNA and this expression increases with time. As measured by cytofluorometry DC stimulated 24 hrs by DNCB or NiSO4 express the protein CCR7 on cell membrane respectively. Using microchambers Transwell we showed that DC stimulated by these two haptens were able to migrate in response to CCL19, a ligand of CCR7. Up-regulation of CCR7 was independant of IL-1beta as shown using an antagonist of IL-1 Receptor (IL-1Ra). However, inhibition of TNF-alpha could partially reverse the effect of haptens on CCR7 expression. Taking together, these results suggest that DNCB and NiSO4 are able to provoke the migration of human DC through the expression of CCR7 independently of IL-1beta secretion.

264 PRION PATHOGENESIS: A JOURNEY THROUGH GUT, SPLEEN, & NERVES.

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Prion neuroinvasion consists of an ordered sequence of events resulting in infection of the central nervous system. Successful oral challenge requires transepithelial migration of prions, which may be accomplished by M-cells. Depletion of lymphocytes from the intestinal mucosa by ablation of $\alpha_4\beta_7$ integrins does not prevent pathogenesis, yet mice exhibiting reduced number of Peyer's patches are virtually uninfectible orally. After gaining access to the body from peripheral sites, prions colonize lymphoid organs of mice, humans, and sheep: the failure of peripherally administered prions to elicit disease in immune deficient mice indicates that this is crucial for pathogenesis. B-lymphocytes are required for neuroinvasion upon intraperitoneal administration, probably (but not necessarily only) because they provide lymphotoxins to secondary lymphoid organs, thereby maintaining follicular dendritic cells: genetic or pharmacological interference with lymphotoxin signaling effectively impairs patho-genesis. We have recently generated mice expressing lymphotoxins in lymphoid organs despite the absence of B lymphocytes: this should help determining the precise contribution of B lymphocytes to pathogenesis. The sympathetic nervous system appears to be involved in prion transfer to brain, since sympathectomy delays or prevents pathogenesis, whereas sympathetic hyperinnervation accelerates it. Topographic relationships between follicular dendritic cells and sympathetic endings in lymphoid organs control efficiency of pathogenesis: CXCR5 deficient mice, whose follicular dendritic cells are juxtaposed to sympathetic endings, effect neuroinvasion more efficiently than wild-type mice. Various components of the complement system are modifiers of neuroinvasion efficiency, and their pharmacological or genetic ablation interferes with neuroinvasion. Although isogenic prions are immunologically inert, expression of anti-PrP^C antibodies in transgenic mice has uncovered that (1) autoreactivity to PrP^C does not necessarily tolerize B-cells, and (2) sustained anti-PrP^C IgM titers can prevent peripheral prion pathogenesis.

265 FREE RADICALS IN THE TOXICOLOGY OF ALCOHOLS.

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Evidence exists supporting the postulate that alpha-hydroxylethyl free radical is involved in the toxicity of alcohols. This free radical has been detected in animal and human models by ESR and immunological techniques. Nonetheless, the metabolic origin of the oxygen free radical species responsible for this free radical formation remains obscure with evidence suppporting the role of both hepatocytes and Kupffer cells. The purpose of this symposium is to bring together leading experts representing different viewpoints in this important area. Several investigators have combined detection of free radicals with ESR with knockout mice to provide new insights. Another investigator is transfecting cell lines with specific radical-generating enzymes. Another investigator is the world leader in antibodies to the alpha-hydroxyl ethyl radical in humans and rodent models. This syposium will address controversial issues in the area of free radicals and alcohol-induced hepatotoxicity.

266 PRONOUNCED HEPATIC FREE RADICAL FORMATION PRECEDES PATHOLOGICAL LIVER INJURY IN ETHANOL-FED RATS.

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Ethanol is metabolized to a free radical intermediate, the 1-hydroxyethyl radical, after acute and chronic administration to both rats and mice. Furthermore, large acute doses of alcohol, as well as chronic alcohol administration in liquid diets, stimulate radical formation from endogenous compounds that are assumed to be lipids. In order to explore the role of radicals in development of liver injury, we used spin trapping and EPR spectroscopy with rats that had received intragastric alcohol feeding in diets that contained either saturated fat or fish oil. When alcohol is fed in combination with saturated fats, livers develop steatosis, but no further evidence of injury. In this dietary model, biliary concentrations of lipid radical adducts were similar in alcohol-fed and control rats after two weeks of feeding. In contrast, when identical experiments were conducted with diets containing fish oil, alcohol-fed rats formed lipid radical adducts at rates that were 5 to 6 times those of controls. Furthermore, enhanced rates of lipid radical formation continued even after blood alcohol concentrations had returned to zero. Rats given alcohol and fish oil develop hepatic inflammation, fibrosis and necrosis after about four weeks of feeding. Thus, liver pathology develops subsequent to pronounced radical formation in rats fed fish oil and alcohol. In contrast, feeding alcohol with saturated fats does not enhance radical formation or produce liver injury.

267 CYP2E1-DEPENDENT TOXICITY AND OXIDATIVE STRESS IN HEPG2 CELLS.

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CYP2E1 metabolizes toxicologically important substrates including ethanol. Oxidative stress is a mechanism by which ethanol is hepatotoxic. Induction of CYP2E1 is one pathway by which ethanol generates oxidative stress. We established HepG2 cell lines, which express CYP2E1 by retroviral infection (E9 cells) or by plasmid transfection methods (E47 cells). Ethanol, iron or polyunsaturated fatty acids was more toxic to E9 cells than control cells. Toxicity was associated with elevated lipid peroxidation and prevented by antioxidants. Mitochondrial membrane potential was lowered in E47 cells. Mitochondria appear to be an important target for CYP2E1-dependent toxicity. Toxicity of ethanol, iron, or arachidonic acid was enhanced when GSH was depleted. The E47 cells had an increased GSH content due to transcriptional activation of the gGCS gene. Levels of catalase, alpha-, and microsomal-glutathione transferases were also increased in the E47 cells. Up-regulation of these antioxidant genes may reflect an adaptive mechanism to remove CYP2E1-derived oxidants. Hepatic stellate cells (HSC) are central to the fibrotic response to liver injury and ROS can activate HSC. A possible interaction between CYP2E1, and CYP2E1-derived diffusable mediators, with HSC was evaluated developing a co-culture model. There was an increase in collagen levels in HSC co-incubated with E47 cells compared to C34 cells. Catalase and vitamin E completely blocked the increase. This suggests that the E47 cells are releasing ROS such as H2O2 and lipid peroxidation-derived products that contribute to the elevation in collagen protein. HepG2 cells expressing CYP2E1 appear to be a valuable model to characterize biochemical and toxicological properties of CYP2E1. While several mechanisms contribute to alcohol-induced liver injury, the linkage between

CYP2E1-dependent oxidative stress, mitochondrial injury and GSH homeostasis and elevation of collagen levels of HSC by CYP2E1-derived ROS may contribute to the toxic action of ethanol on the liver.

268 HYDROXYETHYL FREE RADICALS AS A TRIGGER FOR ALCOHOL-INDUCED IMMUNO-TOXIC REACTIONS.

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Hydroxyethyl free radicals (HER) originate as side products of ethanol oxidation by cytochrome P450 2E1 (CYP2E1). HER are reactive intermediates capable of interacting with a variety of molecules including nucleic acids and proteins. HER-protein adducts are stable enough to be recognised by the immune system and lead to the production of antibodies against HER-derived epitopes (anti-HER ab). These antibodies are detectable in alcohol-fed rats as well as in 50-60% of alcoholic patients. The formation of anti-HER antibodies in both experimental animals and humans strictly depends on CYP2E1 activity and alcoholics with low-inducible CYP2E1 phenotype do not show appreciable immunization by HER-derived epitopes. Although, HER alkylate several hepatic proteins, the binding to CYP2E1 is particularly interesting from the toxicological point of view. We have observed that HER-alkylated CYP2E1 is present on the outer surface of the plasma membranes of isolated hepatocytes exposed to ethanol where it is recognized by human anti-HER antibodies. Furthermore, the reaction of anti-HER IgG with plasma membrane epitopes triggers antibody-dependent cell mediated cytotoxicity when ethanol-treated rat hepatocytes are incubated with normal human periferal blood mono-nucleated cells. Furthermore, the analysis of patients with advanced alcoholic liver disease has revealed that subjects immunized against HER-derived antigens have a 4 fold higher risk of developing auto-immune reactions against CYP2E1 than those non-immunized. Such a risk is further increased up to 22 fold in patients caring a functional mutation in immune regulatory molecule CTLA-4 that reduce inhibitory signals regulating lymphocyte proliferation. This indicates that HER might participate to the development of alcohol liver damage by triggering immuno-toxic reactions as well as by favouring the breaking of immune tolerance towards CYP2E1 that can in turn further contribute to cause immune-mediated hepatic injury.

269 AN *IN VIVO* ESR SPIN-TRAPPING STUDY: FREE RADICAL GENERATION IN RATS FROM METHANOL AND FORMATE INTOXICATION— ROLE OF THE FENTON REACTION.

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Electron spin resonance spectroscopy (ESR) has been used to study free radical generation in rats with acute sodium formate poisoning. The *in vivo* spin-trapping technique was utilized with alpha-(4-pyridyl-1-oxide)-*N*-t-butylnitrone (POBN), which reacts with free radical metabolites to form radical adducts. The radical adducts were detected in the bile and urine samples from Fischer rats. The use of [13C]-sodium formate and computer simulations of the spectra identified the twelve-line spectrum as arising from the POBN/carbon dioxide anion radical adduct. The identification of POBN-carbon dioxide anion radical adduct provides for the first time direct ESR evidence for the formation of carbon dioxide anion radicals during acute intoxication by sodium formate, suggesting a new metabolic pathway. To study the mechanism of free radical generation by formate, we tested several known inhibitors. Both allopurinol, an inhibitor of xanthine oxidase, and amino-benzo-triazole, a cytochrome P450 inhibitor, decreased free radical formation from formate, which may imply a dependence on hydrogen peroxide. In accord with this hypothesis, the catalase inhibitor 3-aminotriazole caused a significant increase in free radical formation. The iron chelator Desferal decreased the formation of free radicals up to twofold. Presumably, iron plays a role in the mechanism of free radical generation by formate *via* the Fenton reaction. The detection of formate free radical metabolites generated *in vivo* and the key role of the Fenton reaction in this process may be important for understanding the pathogenesis of both formate and methanol intoxication. The mechanism of oxidation of methanol to the alpha-hydroxymethyl radical is also being investigated.

270 INFLAMMATORY CELL-DERIVED OXIDANTS IN ALCOHOLIC LIVER DISEASE: STUDIES *IN VIVO.*

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The evidence that alcohol causes oxidative stress is extensive. Both *in vitro* and *in vivo* data support the hypothesis that alpha-hydroxyethyl free radical is critical adduct formed during alcohol exposure. Furthermore, we have shown that delivery of the antioxidant Cu/Zn-superoxide dismutase (SOD) *via* adenovirus (Ad) also prevented alcohol-induced liver injury and alpha-hydroxyethyl radical formation. While gene delivery of SOD with Ad clearly demonstrates a role for superoxide ($O_2^{\cdot-}$) in alcohol-induced liver injury, there are many superoxide-producing enzymes *in vivo*. Potential sources of $O_2^{\cdot-}$ *in vivo* include xanthine oxidase, NADPH oxidase on macrophages or neutrophils, or CYP2E1 and mitochondria, which are located predominantly in hepatocytes. In addition, $O_2^{\cdot-}$ is not itself a potent oxidant; for example, it cannot attack ethanol to form the alpha-hydroxyethyl radical. These data suggest that $O_2^{\cdot-}$ reacts with other biological molecules to create a more reactive oxidizing species. There are multiple pathways involving $O_2^{\cdot-}$ that can lead to the formation of potent oxidants. Applying knockout technology to the mouse enteral model of alcohol-induced liver injury now makes it possible to determine the specific role of oxidants during alcohol exposure. For example, injury and free radical production is completely blocked in mice deficient in the superoxide-producing enzyme NADPH oxidase (p^{47phox} knockout), suggesting a key role of $O_2^{\cdot-}$ production from this enzyme in alcohol-induced liver injury. Further, we recently showed that mice deficient in inducible nitric oxide synthase (iNOS knockout mice) were completely protected against alcohol-induced liver injury and radical formation, analogous to studies with NADPH oxidase deficient mice. These results support the hypothesis that the potent oxidant formed during alcohol-induced liver injury is dependent on both $O_2^{\cdot-}$ and $NO^{\cdot}$, such as $ONOO^{-}$, an oxidant that is indeed potent enough to form the alpha-hydroxyethyl radical.

271 GENE-ENVIRONMENT INTERACTIONS *IN UTERO*: THE FETAL BASIS OF ADULT DISEASE.

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The purpose of this symposium is to address an important and emerging area of developmental toxicology: the effect of *in utero* exposures that cause functional changes that are not overtly teratogenic but that result in increased susceptibility to disease/dysfunction later in life. It is becoming apparent that there is an environmental component to every disease. In some cases the environmental trigger is exposure to the adult. However, it is now clear that in many cases the fetus is more sensitive to the same environmental insults and that the effect of exposures during development may have a more detrimental effect on the etiopathology of the disease. Indeed, there is overwhelming epidemiological and clinical evidence that intrauterine conditions alter development of tissues and organs that lead to increased susceptibility to diseases. During development fetuses respond to adverse conditions by favoring the metabolic demands of the growing brain and heart at the expense of other tissues . The long-term consequences of this response are that the individual is protected from death but is more prone to diseases later in life. While epidemiology studies have identified the phenomenon of metabolic programming, little is known about mechanisms by which fetal insults lead to altered programming and to disease later in life. In addition, emphasis thus far has been on alterations in nutrition during development with virtually no focus on the role that exposures to environmental agents either alone or in combination with alterations in nutrition might have on this phenomenon. This symposium will focus not only on the role of altered nutrition in fetal programming but will also show evidence that some environmental agents, especially those with endocrine agonist or antagonist activity, may alter developmental programming *via* alteration in gene expression or gene imprinting and that these changes will also result in increased susceptibility to disease later in life.

272 PRENATAL PROGRAMMING OF ADULT CARDIOVASCULAR DISEASE.

K. Thornburg, M. Morton, G. Girard, A. Barbera, J. Maylie, S. Jonker, N. Sundgren, P. Ahmed, J. Shultz and L. Davis. *Heart Research Center, Oregon Health & Science University, Portland, OR.* Sponsor: J. Heindel.

Known risk factors for coronary disease are based upon statistical associations with high plasma cholesterol, smoking, hypertension and physical inactivity. However, all of the known risk factors combined are believed to account for only about 1/2 of the known cases of coronary disease within the USA. The theories regarding the etiology of coronary artery disease include oxidized lipid damage to endothelium, infectious agents, low production of endothelial dilators and direct oxidative damage. Since the report by Barker et al. (1989), there have been numerous studies showing an inverse relationship between adult onset ischemic heart disease/diabetes and term birth weight in men and women. At present there are no proven underlying mechanisms relating coronary disease to fetal undergrowth. Several hypotheses have been proposed: 1) Increased fetal plasma glucocorticoids. 2) Intrauterine hypox-

emia. 3) High placental vascular resistance. 4) Maternal under-nutrition. We studied the fetal sheep model to test the hypotheses that intrauterine pressure load and anemia re-program the developing myocardium for life. We occluded the main pulmonary artery (PA) of the near term fetal sheep for 10 days to increase PA pressure by a mean of 10 mmHg. Loading dramatically reduced the pool of cardiac cells able to divide. In fetuses made anemic for 6 days, coronary conductance doubled. When these same sheep were studied as full-grown adults with normal hemaotcrits since birth, their coronary conductance was still 2X that of their undisturbed twin. We conclude that prenatal loading and anemic stress alter heart development in ways that predict pathological changes later in life.

273 MATERNAL-FETAL PROGRAMMING *IN UTERO*: ADULT METABOLIC DISEASE AND DIABETES.

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Epidemiological studies in humans have shown that poor growth *in utero* predisposes to later development of type-2 non-insulin-dependent diabetes and hypertension in adulthood. Animal studies have similarly found that pregnant rats fed low protein diets have alterations in glucose metabolism and blood pressure in their offspring. Dietary protein deficiency during pregnancy has long-term effects on the structure and function of the developing endocrine pancreas. In pregnant rats fed isocaloric low protein diets, the fetal pancreas has been shown to have reduced b-cell proliferation, islet size and vascularization, as well as well as impaired insulin secretion *in vitro*. Exposure to a low protein diet *in utero* and through lactation has been associated in young animals with increased pancreatic β-cell apoptosis, decreased proliferation and altered growth factor expression. The livers of low protein offspring also show morphological changes in zonation and alterations in enzymes involved in glucose metabolism and insulin sensitivity. Study of fetal hepatic function, however, is complicated because the fetal liver is a major hematopoietic organ that is primarily comprised of nonhepatic hematopoietic stem cells until the time of birth. Our studies have found that the low fetal liver content of hepatocytes significantly impacts on the evaluation of the effect of environmental exposures on hepatic function. This developmental shift in the cellular composition of the fetal liver is an important factor that must be considered in studies designed to compare fetal hepatic function and gene expression with patterns found in postnatal and adult liver. In summary, multiple mechanisms influence the nutrient and endocrine milieu which can alter the expression of the fetal genome and the adult functioning of the pancreas and liver.

274 ENVIRONMENTAL EFFECTS ON PERINATAL RESPIRATORY/NEURAL DEVELOPMENT: LASTING CONSEQUENCES OF EARLY EXPOSURE.

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The respiratory and nervous systems are complex organs composed of multiple cell types involved in a variety of functions. Exposure to a wide range of environmental toxicants during perinatal life has the potential to significantly affect the growth, maturation, and function of both respiratory and neural processes. we examined the effects of perinatal exposure to environmental tobacco smoke (ETS) on lung and neural development in rats and monkeys. Exposure to ambient levels of ETS (1 mg/m3 of total suspended particles) was for 6 h/day, 5 d/week from early gestation to late postnatal age (pre-puberty). We assumed that such exposures could likely affect critical molecular signals expressed during distinct stages of development. We also factored in the tenet that respiratory/ neural development is a multi-step process. Therefore, some studies involved only *in utero* exposure, others only postnatal exposure and others both *in utero* and postnatal exposure. We found differential effects in both the lungs and the brain following ETS exposure. Some respiratory effects (airway hyper-reactivity and neuroendocrine cell hypertrophy) were not evident with only *in utero* or only postnatal exposure, but demonstrated significant effects with combined exposure; these effects persisted weeks after exposure to ETS had ended. Neural consequences displayed a wider window of vulnerability. In both rats and monkeys, we measured significant effects of either prenatal or postnatal ETS that could lead to lasting adverse consequences of asthma, cardiovascular compromise and neurological deficits. Many of these changes were consonant with abnormalities associated with sudden infant death syndrome (SIDS), immune imbalance and lingering respiratory hyper-reactivity. We conclude that critical windows of exposure to ETS during perinatal life can alter the growth and development of neural and respiratory systems. Perinatal exposure to common environmental pollutants may thus have adverse consequences that emerge later in life.

275 EFFECTS OF ENVIRONMENTAL ANTIANDROGENS DURING FETAL REPRODUCTIVE DEVELOPMENT: CONSEQUENCES FOR THE ADULT.

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There has been significant concern that environmental agents are contributing to increased human male reproductive disorders (decreases in sperm counts, increases in reproductive tract malformations, cryptorchidism and testicular cancer). Experimental evidence in rats indicates that a number of environmental antiandrogens can produce a range of reproductive effects identical to those for which there is human concern. Two agents, di-n-butyl phthalate (DBP) and linuron (L), produce major malformations, predominantly of the epididymis, in the absence of toxicity to the dam when administered during critical windows of *in utero* development (GD 12-21). The epididymal malformations are observable postnatally, with resulting testicular effects occurring at adulthood. However, DBP and L operate *via* different mechanisms that affect fetal androgen signaling. An analysis of gene expression changes in fetal and neonatal testes and epididymides by cDNA microarrays with confirmation by RT-PCR has indicated significant down regulation of genes in a number of critical pathways. DBP in fetal testes (GD 19 and 21) produced a down regulation of several genes in the steroidogenic pathway producing testosterone (T), consistent with lowered fetal testicular T levels, while changes in c-kit expression are consistent with aberrant gonocyte development. Interestingly, while mRNA levels for the androgen receptor (AR) were not changed in the fetal epididymis, the distribution of AR protein between epithelial and mesenchymal cells was altered. In the case of L, fetal (GD 21) and neonatal (PND 7) epididymides had decreases in gene expression in the Bone Morphogenic Protein, EGF and IGF signaling pathways, all consistent with a failure of normal Wolffian duct development and altered interaction between epithelial and mesenchymal cells. The range of gene changes noted following *in utero* exposure to DBP and L could be directly related to morphological disturbances in perinatal animals and ensuing injury noted in later life.

276 DIETHYLSTILBESTROL(DES) EXPOSURE DURING DEVELOPMENT ALTERS UTERINE GENE EXPRESSION: INFLUENCE ON CANCER LATER IN LIFE.

R. Newbold. *Environmental Toxicology Program, NIEHS, Research Triangle Park, NC.* Sponsor: J. Heindel.

Estrogenic substances like DES alter development of reproductive tract tissues leading to structural, functional, and long term abnormalities including neoplasia later in life. Since the developing organism is uniquely sensitive to perturbation by chemicals with hormone-like activity, the present study addresses whether exposure to these chemicals during critical stages of differentiation will permanently alter normal gene expression during uterine development, so that the uterus responds atypically to further estrogen stimuli later in life. Outbred CD-1 mice were treated by subcutaneous injections with DES (0.0001 - 1, 000 mg/kg) dissolved in corn oil, or corn oil alone (control), on days 1-5 of neonatal life. Mice were weaned at 17 days prior to puberty and challenged with 3 daily doses of 17b-estradiol (500 mg/kg) or DES (10 mg/kg). On the 4th day, uterine wt./body wt. ratios were determined and uterine tissues evaluated for both cellular and molecular changes. Neonatal DES exposure resulted in altered uterine gene expression and response to estrogen at puberty. The response varied depending on the dose of neonatal exposure; neonatal exposure to the low dose of DES (0.01 mg/kg) caused an enhanced response to estrogen at puberty as compared to controls, whereas higher neonatal doses of DES caused reduced uterine response. To determine if these altered uterine responses were permanent, an additional group of mice was neonatally treated with DES (0.001-10 mg/kg) and housed until 4-5 months of age. Following ovariectomy, these adult mice challenged with estrogen showed similar altered gene expression and uterine response as the immature mice. DES-associated transgenerational events involving an increased susceptibility for tumors were also studied to determine if altered gene expression was playing a role in uterine neoplasia observed in second generation DES exposed mice.

277 HEALTH RISK ASSESSMENT OF HEXAVALENT CHROMIUM IN DRINKING WATER: CARCINOGENICITY, RESEARCH, AND REGULATION.

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It has been well established that hexavalent chromium [Cr(VI)] can cause lung and nasal cancer among workers exposed to high airborne concentrations in certain industries. However, the potential oral carcinogenicity of Cr(VI) has been a matter of

controversy and conjecture in recent years. It is known that Cr(VI) is detoxified by reduction to Cr(III) in the acidic and reducing environment of the stomach. USEPA cited the gastric reductive capacity in support of their decision to raise the chromium maximum contaminant level (MCL) from 50 ppb to 100 pbb. USEPA and other agencies have also determined that there is insufficient evidence at this juncture to indicate that Cr(VI) poses an oral cancer risk. However, some uncertainties remain with respect to the quality of the available data, and as a result, the National Toxicology Program recently initiated a subchronic and chronic drinking water toxicity research project. The purpose of this study is to supplement the available animal pharmacokinetic and toxicity information. The potential hazards posed by Cr(VI) in drinking water, and the practical implications for assessing risk and setting health-based standards, depends on the weight of evidence from many scientific disciplines, including mechanistic and whole-animal toxicology, epidemiology, and kinetics. Significant new research in these fields has been conducted and will be discussed in this symposium. The use of new data for assessing the health risk from drinking water exposures, and the basis for setting health-based standards, will be presented with a case study of a recent risk assessment for Cr(VI) drinking water exposure in San Fernando Valley, California.

278 MECHANISM OF HEXAVALENT CHROMIUM [(CR(VI)] TOXICITY AND CARCINOGENICITY.

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Long term occupational inhalation exposure to chromium(VI)-containing dusts has been associated with an increased risk of respiratory cancers whereas other routes of exposure (dermal, ingestion) have not been associated with significant adverse health effects. Conversely, chromium(III) is an essential trace element in humans and animals and is essentially non-toxic. The basis for the differences in the biological effects of these two chromium valence states, and for the selective toxic effects of chromium(VI) which are restricted by chemical and physical form, route of exposure, and target tissue toxicity, are explained by the unique biology and chemistry of chromium and have been summarized in the cellular uptake-reduction model of Wetterhahn and the *in vivo* reduction model of De Flora. Chromium(III) is taken up very poorly by the gut and also does not easily cross cell membranes. An organic complex containing chromium(III) interacts with cell surface receptors to elicit effects on cell signaling that underlie its essential trace element role in glucose homeostasis. Chromium(VI) has the potential to cross cellular membranes through the anionic transport system but does not normally reach internal cellular targets due to the high reductive capacity of the body, especially the GI system and the skin, except under very high dose conditions which can overwhelm this capacity. However, under historical occupational conditions in which workers were exposed to very high concentrations of chromium(VI) dusts daily over several decades, the reductive capacity of the respiratory system was likely overwhelmed leading to increased risk of cancer, perforating ulcers and fibrosis. The available data, in conjunction with these models, is consistent with the prediction that there is no significant health risk associated with most environmental exposures to chromium(VI) including exposure *via* drinking water.

279 CHROME EXPOSURE AND LUNG CANCER, STOMACH CANCER AND OTHER CAUSES OF DEATH: AN EPIDEMIOLOGIC META-ANALYSIS.

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We reviewed the entire epidemiologic literature on chromium exposure and cancer published since 1950. This included more than 100 papers, 72 of which contained useful information on cancer risks. Eight epidemiologic design features of each study were evaluated and scored independently by the authors. The studies were then divided into two groups of higher and lower scoring studies. Findings were then compiled for All Causes of Death Combined, All Cancer Combined, Cancer of the Lung, Cancer of the Stomach and for each of six additional forms of cancer. This was done separately for the higher and lower-scoring studies. Nearly all of the studies related to occupational, not to drinking-water, exposures. However, 20% to 40% of inspired chromium particulates eventually reach the stomach. Among persons exposed to Cr(VI), there was a slight but significant deficit of All Causes of Death. There was a minimal, but significant, excess of deaths from All Cancer; this was due almost entirely to an excess of lung cancer. For lung cancer, the overall meta-standardized mortality ratio (mSMR) was 148 (95% CI=140-155) but among studies that controlled for cigarette smoking the mSMR was 120(113-128). There was a slight (mSMR=106) and non-significant excess of stomach cancer. However, among studies that controlled for the economic status of their subjects, the mSMR was actually depressed to 79 (67-93). Discrepancies between the higher and lower-scoring studies will be discussed. This comprehensive evaluation indicates that there probably is a causal relationship between exposure to Cr(VI) and lung cancer. However, this relationship previously has been over-estimated because early studies did not adjust for the heavy smoking of their subjects. The findings also suggest that there is no association, much less a causal relationship, between exposure to Cr(VI) and stomach cancer. The implications of the findings for other forms of cancer will be discussed.

280 REFINING THE PBPK MODEL FOR CHROMIUM(VI) IN HUMANS.

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A physiologically based pharmacokinetic (PBPK) model for trivalent and hexavalent chromium [Cr(VI)] in humans was recently published (OFlaherty et al., 2000). A critical parameter within the model is the rate at which Cr(VI) is reduced to Cr(III) in the gastrointestinal tract (GI). Two limitations of the current model are: 1) that it relies on limited data regarding the gastric reduction capacity of humans, and 2) the GI tract is described as a single combined stomach and small intestine (SI) compartment. This latter limitation requires that absorption and reduction processes be described as simultaneous competing reactions, whereas physiologically, reduction of Cr(VI) occurs primarily in the stomach followed by absorption in the SI. This work describes refinements to the current PBPK model. Specifically, we used new *in vitro* reduction rate data, simulating a variety of conditions (e.g., fasted, fed, antacids, diluted stomach fluid), to better quantify the rate of Cr(VI) reduction in the stomach, focusing on the rate within the first few minutes before transfer of stomach contents to the SI occurs while fasting. Based on these new data, first-order reduction rate constants and gastric half-lives were calculated to range from 10 to 0.7 minutes, with an average of 2.7 minutes. These rates are up to 14 times faster than that used in the current PBPK model. We also modified the current model, separating the stomach compartment (where reduction occurs) from the SI compartment (where reduction continues, but at a slower rate, and absorption occurs). The revised model provides vastly different estimates of the amount of Cr(VI) that might be absorbed systemically by humans following ingestion of Cr(VI), as compared to the first model. These results are valuable for understanding the potential risks associated with Cr(VI) in drinking water and the potential threshold for absorbing Cr(VI) following ingestion.

281 NATIONAL TOXICOLOGY PROGRAM STUDIES OF HEXAVALENT CHROMIUM.

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Hexavalent chromium (CrVI) is a known human carcinogen through occupational exposures, but there is inadequate information to characterize its carcinogenic hazard when consumed in drinking water. Consequently, private individuals, the State of California and members of the California Congressional delegation nominated CrVI to the NTP. In preliminary studies, CrVI (as sodium dichromate dihydrate in all studies) was administered in drinking water (1 to 300 mg Cr/L) for 3 weeks to rats, mice and guinea pigs. Significant differences were not apparent in the shapes of the dose response curves for total Cr accumulation (measured once following a two-day wash out) in blood and kidney in the three species, although absolute levels differed. In subsequent studies, rats and mice received 62.5 to 1000 mg sodium dichromate dihydrate/L (24 to 384 mg CrVI/L) in drinking water for 13 weeks. Significant toxic effects were limited to metaplasic (mice) and inflammatory and/or erosive lesions (rats) of the glandular stomach at 1000 mg/L, a microcytic, hypochromic, responsive anemia in all dosed groups of rats, and effects secondary to decreased water consumption primarily in the 1000 and 500 mg/L dose groups. Based on these results, a 2-year drinking water study in F344 rats and B6C3F1 mice is under design. Additional 13-week studies are examining the comparative toxicity of CrVI in BALB/c and C57BL/6 mice, and *in vivo* mutagenicity in the am3-C57BL/6 mouse. Immunotoxicity studies will be performed in female B6C3FI mice. Protocols can be accessed at http://ntp-server.niehs.nih.gov/htdocs/Studies/HexChromium/hexchromiumpg.html

282 RISK ASSESSMENT FOR HEXAVALENT CHROMIUM IN DRINKING WATER.

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Hexavalent chromium has been detected in the drinking water of a number of communities in California. An evaluation of risk associated with chemicals in the drinking water supply should consider all relevant routes of exposure, such as inges-

tion and the inhalation of water generated during showering. The assessment of exposure to hexavalent chromium in drinking water is complicated by the classification of hexavalent chromium as carcinogenic by inhalation, while there are no high quality cancer studies in animals and humans by the oral route. Mechanistic and genotoxic studies suggest a reason for concern about increased levels of hexavalent chromium (and perhaps trivalent chromium) within cells. Differences in the pharmacokinetics of orally administered hexavalent chromium and trivalent chromium compounds have been observed in both humans and animals. Compared to trivalent chromium, a higher fraction of orally administered hexavalent chromium is absorbed, higher levels of chromium are detected in the blood and tissues, and more chromium is recovered in the urine. A prolonged urinary half-life of chromium following the administration of hexavalent chromium in humans also suggests orally administered hexavalent chromium is taken up into tissues and then slowly released. Limited carcinogenicity data and uncertainties regarding interpretation of the pharmacokinetics, mechanism of action, and genotoxicity prompted OEHHA to nominate hexavalent chromium for NTP cancer bioassays. A risk assessment for hexavalent chromium based on the presently available data considers non-cancer effects by the oral route, and the potential for inhalation of respirable droplets in showering, to arrive at predicted health-protective levels in drinking water.

283 WORLD TRADE CENTER AFTERMATH: LOOKING BACK TOWARDS THE FUTURE.

H. Zenick and S. H. Gavett. *USEPA NHEERL, Research Triangle Park, NC.*

The World Trade Center (WTC) disaster of September 11, 2001 galvanized the public health community to respond rapidly with environmental pollutant monitoring, exposure assessments, toxicological research, and health risk assessments. This symposium draws upon the WTC experience to better understand the major challenges that were presented, and the key response, research, and data needs that emerged. An effective public health response to environmental health emergencies requires a unified infrastructure of institutions with a coordinated communications strategy, preparedness response plans, and capacities in medical care, environmental monitoring, and surveillance. Exposure assessments of the general population and workers involved in recovery and cleanup utilize newly developed analysis and computational tools, monitoring databases, and personal exposure monitoring. The destruction of the WTC and associated fires caused the release of pollutants which are possibly unique in composition and toxicity. Toxicological assessment of particulate matter (PM) from the WTC relative to previously characterized emission and ambient air PM samples contributed to the health risk assessment of WTC-derived airborne pollutants. The human health risk assessment approach integrates information from compositional analysis, toxicological assessments, ambient air monitoring, modeling of the plume movement, and comparisons against historical pollutant levels and benchmark health values. As the WTC recovery effort moves into the indoor residential environment where cleaning efforts are ongoing, the long-term health risks to local residents are being addressed by establishing clearance criteria of contaminants of potential concern. This symposium provides an integrated view of the role of research in helping the Nation to better anticipate, prepare, detect and respond to environmental health threats in the future. [This abstract does not necessarily reflect USEPA policy.]

284 ON-THE-GROUND PUBLIC HEALTH RESPONSE TO THE WTC COLLAPSE: LESSONS LEARNED.

P. Meehan. *National Center for Environmental Health, CDC, Atlanta, GA.* Sponsor: H. Zenick.

Public health is a critical part of the response system for disasters, terrorism and other domestic and international emergencies. The role of federal health agencies, as defined in the various federal response plans, is described in this presentation. The requirements of a prepared public health system include an incident command and support structure, preparedness response plans, effective communications, epidemiology and surveillance capacity, chemical and biological laboratory capabilities, medical care surge capacity, and capacity in occupational and environmental health. The events in New York City since Sept. 11, 2001, vividly demonstrate the importance of these capacities and provide lessons that are useful in improving the public health response system. On Sept. 11, staff from the Centers for Disease Control and Prevention (CDC) were on the ground to assist the New York City Department of Health (NYCDOH) in its response to this disaster. Immediate issues were the need to institute effective emergency response coordination, the need for surveillance for possible biological or chemical terrorism, and response to a request to provide materiel from the National Pharmaceutical Stockpile. Over the next several weeks, CDC assisted the NYCDOH in response to worker health and safety issues and environmental contamination and toxicology. As a major part of the federal health response system, the Centers for Disease Control and Prevention has developed a comprehensive approach to terrorism and emergency public health that includes the development of internal capacities, support for the development of state and local capacity building and the development of a national stockpile of medical supplies and pharmaceuticals.

285 PLUME RECONSTRUCTION AND MICROENVIRONMENTAL MODELING FOR ASSESSING EXPOSURES TO CONTAMINANTS ASSOCIATED WITH THE WTC FIRE AND COLLAPSE.

P. G. Georgopoulos. *EOHSI (Environmental and Occupational Health Sciences Institute), UMDNJ - R. W. Johnson Medical School and Rutgers University, Piscataway, NJ.* Sponsor: H. Zenick.

This project has been focusing on the development and implementation of an integrated computer-based Exposure Information System (EXIS) intended to support assessments of population exposures and doses for contaminants released from and following the World Trade Center (WTC) fire and collapse on 9/11/2001. This system, WTC-EXIS, includes both observational databases and prognostic/diagnostic computational modules, jointly used to characterize the spatial and temporal attributes of the contaminant plume and deposition over multiple scales, and the air quality of affected local microenvironments (street canyons, residences, offices, stores, etc.). Distributions of microenvironmental parameters and of human activities are being developed at resolutions that depend on distance from the WTC site (the highest being for the census blocks of the 84 census tracts within 2.5 km distance from the WTC site). Components of WTC-EXIS include: (a) multiscale atmospheric transport and fate analysis tools, utilizing University. Colorado's prognostic mesoscale Regional Atmospheric Modeling System (RAMS), coupled with EOHSI's Computational Chemodynamic Laboratory's (CCL) dynamic fate modules and with local computational fluid dynamics (CFD) modules; (b) computational tools of the MENTOR/SHEDS (Modeling Environment for Total Risk with Stochastic Human Exposure and Dose Simulation) system, developed jointly by CCL and USEPA's National Exposure Research Laboratory (NERL); (c) relational databases and geodatabases, of contaminant-related observations (from both local monitoring efforts and remote sensing stations), meteorological, land-use, microenvironmental, and demographic information; and (d) a Geographical Information System (GIS) interface (implemented in ArcInfo/ArcGis), to facilitate usage of information by researches involved in related health impact studies.

286 EXPOSURE ASSESSMENT OF WORKERS INVOLVED IN CLEANUP OPERATIONS AT THE WORLD TRADE CENTER DISASTER SITE.

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The destruction of the World Trade Center (WTC)on September 11, 2001 created a large amount of airborne contamination including crushed concrete, metals, asbestos, plastics, foams, solvents, insulations, glass, and asphalt. Fires, which burned for several weeks, generated potentially high concentrations of toxic volatile organic compounds (VOCs). The purpose of this study was to assess the personal exposures of cleanup workers to airborne contaminants at the WTC disaster site. The study was conducted in October 2001 with a follow up assessment in April 2002. Truck drivers hauling rubble away from the debris pile were recruited to wear personal monitors during their 12-hr work shifts. Personal monitoring was conducted every day of the week in both day and night shifts. Area monitoring was conducted at street intersections in the immediate vicinity of the disaster site, and also directly in the debris pile in October and in the pit in April. Thirty-two percent of drivers reported being at the site during the first week after the disaster. During any work shift 52% reported spending at least half the shift outside their truck walking around the site. In October personal exposures to Total Suspended Particles ranged from 65.8 to 765.8 $\mu g/m^3$. Personal and area concentrations of VOCs were low; the highest area concentration was measured for benzene (17.8 ppb), with the highest personal exposure found for m, p-xylene (13.7 ppb). Overall area concentrations and personal exposures to asbestos were low. Area monitoring and personal exposure assessment to airborne contaminants at the disaster site was necessary to understand exposure in the context of the potentially wide range of resulting possible health outcomes. Preparation for future environmental disasters must include a comprehensive and coordinated air monitoring and exposure assessment plan.

287 RESPIRATORY TOXICOLOGICAL EFFECTS OF WORLD TRADE CENTER FINE PARTICULATE MATTER IN MICE.

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The destruction of the World Trade Center (WTC) caused the release of high levels of airborne pollutants which were reported to cause adverse respiratory responses in rescue workers and nearby residents. We examined whether WTC-derived fine particulate matter ($PM_{2.5}$) has detrimental respiratory effects in mice. Samples of

WTC $PM_{2.5}$ were derived from settled dust collected at several locations around Ground Zero on September 12 and 13. Chemical analysis showed high levels of calcium sulfate and calcium carbonate. Aspirated samples of WTC $PM_{2.5}$ induced mild to moderate degrees of pulmonary inflammation one day after exposure, but only at a relatively high dose (100 micrograms). This response was not as great as that caused by 100 μg $PM_{2.5}$ derived from residual oil fly ash (ROFA) or Washington DC ambient air PM (NIST 1649a). However, this same dose of WTC $PM_{2.5}$ caused airway hyperresponsiveness to methacholine aerosol comparable to NIST 1649a and to a greater degree than ROFA. Mice exposed to lower doses by aspiration or inhalation exposure did not develop significant inflammation or hyperresponsiveness. These results show that a high dose of WTC $PM_{2.5}$ can promote mechanisms of airflow obstruction in mice. Airborne concentrations of WTC $PM_{2.5}$ which would cause comparable doses in people are high but conceivable in the immediate aftermath of the collapse of the towers. We conclude that a high-level exposure to WTC $PM_{2.5}$ could cause pulmonary inflammation and airway hyperresponsiveness in people. The effects of chronic exposures to lower levels of WTC $PM_{2.5}$, the persistence of any respiratory effects, and the effects of coarser WTC PM are unknown and were not components of these studies. Degree of exposure and respiratory protection, individual differences in sensitivity to WTC $PM_{2.5}$, and species differences in responses are important elements in the health risk assessment of WTC $PM_{2.5}$. [This abstract does not necessarily reflect USEPA policy.]

288 APPROACHES TO EVALUATION OF POTENTIAL HUMAN EXPOSURES AND HEALTH IMPACTS ASSOCIATED WITH AIRBORNE CONTAMINANTS FROM WORLD TRADE CENTER COLLAPSE/FIRES.

L. D. Grant[1], J. P. Pinto[1] and A. Galizia[2]. *[1]NCEA/Research Triangle Park, USEPA, Research Triangle Park, NC and [2]Region 2, USEPA, New York, NY.* Sponsor: H. Zenick.

The 9/11/01 World Trade Center (WTC) attack resulted in dispersal of numerous potentially toxic materials in the dust/smoke cloud that enveloped lower Manhattan and extended over other New York City (NYC) areas. The US Environmental Protection Agency (U.S. EPA) worked quickly with numerous other Federal, State, and local government agencies to mobilize emergency response efforts. This included cooperative actions to expand rapidly monitoring capabilities by which to measure environmental contamination derived from the 9/11 attack, the collapse of WTC buildings, the ensuing WTC Ground Zero fires, and WTC rescue/recovery operations. Environmental sampling was also carried out by others, e.g., academic investigators sponsored by various government agencies. This paper will mainly illustrate approaches employed, under challenging circumstances and time constraints, to evaluate potential human exposures to WTC-derived airborne pollutants and possible associated human health impacts, based on integrating information derived from: (a) analyses of composition of dust deposited from collapse of WTC buildings and associated toxicity testing of such dust; (b) data from ambient air monitoring at WTC Ground Zero, its immediate perimeter, and at sites in lower Manhattan and elsewhere in NYC metropolitan area; (c) preliminary results of meteorologically-based modeling of WTC plume movement/dispersal; (d) comparison of monitored or estimated concentrations against historical pollutant levels in NYC or other US urban areas; and (e), for those substances discernibly elevated above typical background levels, comparison against health benchmark values judged to be indicative of low risk for adverse health effects due to acute and/or prolonged exposures. [This abstract does not necessarily reflect USEPA policy.]

289 INDOOR AIR ASSESSMENT FOR THE WORLD TRADE CENTER SITE: SELECTING CONTAMINANTS OF POTENTIAL CONCERN AND SETTING HEALTH-BASED BENCHMARKS.

M. A. Maddaloni. *USEPA, Region II, New York, NY.*

As the clean-up and ambient air monitoring of the World Trade Center site comes to an end, health and environmental agencies are directing resources to evaluate the indoor environment for the presence of pollutants that might pose long-term health risks to local residents. Based on historical knowledge of building collapses/fires and review of both outdoor and limited indoor sampling data, six contaminants of potential concern (COPCs) were identified: lead, dioxin, PAHs, asbestos, fibrous glass and crystalline silica. Health-based benchmarks for indoor air and settled dust for each of these COPCs were developed to serve as clearance criteria. Existing environmental standards were utilized where appropriate. The Housing and Urban Development (HUD) standard for lead in settled dust (40 $\mu g/ft^2$) and the National Ambient Air Quality Standard (1.5 $\mu g/m^3$) were employed in this manner. In the absence of existing standards, risk-based clearance levels were developed employing IRIS verified toxicity factors and a residential exposure scenario (30 year duration). Health-based benchmarks for asbestos, dioxin and PAHs were developed in this way. Finally, for substances lacking a widely recognized environmental toxicity assessment, occupational standards with added safety factors were employed. Clearance criteria for fibrous glass and crystalline silica were derived by adding a safety factor of 100X to existing occupational standards.

290 DERMAL EXPOSURE LEADING TO RESPIRATORY TRACT SENSITIZATION AND DISEASE: A TRIVIAL OR CRITICAL LINK?

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Exposure to allergens resulting in respiratory tract sensitization has classically been considered to occur by inhalation. Increasing evidence from epidemiological and clinical studies and data from animal models support the hypothesis that dermal exposure may lead to respiratory sensitization and resultant alterations in pulmonary function. Permeation studies have demonstrated the potential for proteins as well as low molecular weight chemicals to penetrate the skin and mechanistic studies have demonstrated the skin to be a permissive site for the induction of Th2 responses. Animal models have been used to demonstrate specific and non-specific increases in airway hyper-reactivity following dermal exposure to allergens. Using latex allergy, chronic beryllium disease and allergy to low molecular weight chemicals as examples, these presentations will lay the ground-work for a discussion of the relevant clinical and experimental data and the mechanistic basis for the role of skin contact in the development of respiratory sensitization. Only by understanding the mechanisms of sensitization can effective intervention strategies be implemented to protect against respiratory allergens.

291 INFLUENCE OF DERMAL EXPOSURE ON THE DEVELOPMENT OF SENSITIZATION OF THE RESPIRATORY TRACT TO CHEMICAL ALLERGENS.

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Many chemicals are able to cause skin sensitization and allergic contact dermatitis. Fewer in number have been shown to cause allergic sensitization of the respiratory tract; among these being certain diisocyanates, acid anhydrides, reactive dyes and platinum salts. It is often assumed that sensitization of the respiratory tract to chemical allergens is induced exclusively following inhalation exposure. However, an argument can be made on the basis of theoretical considerations, experimental data and anecdotal clinical evidence that this is not necessarily the case. Allergy, by definition, requires the stimulation of a specific immune response and there is reason to suppose that the responses which result in allergic sensitization will normally be systemic in nature. In theory, therefore, dermal exposure to a chemical respiratory allergen may provoke the vigor and quality of immune responses necessary for effective sensitization of the respiratory tract. The results of experimental studies support this argument since it has been shown for instance that topical or intradermal exposure of guinea pigs to a known chemical respiratory allergen is able to sensitize animals such that subsequent inhalation challenge with an aerosol of the same chemical will provoke a respiratory hypersensitivity reaction. Taken together, the available evidence suggests that in certain circumstances respiratory sensitization to chemical allergens may be achieved by dermal exposure.

292 DERMAL EXPOSURE TO TRIMELLITIC ANHYDRIDE (TMA) POWDER INDUCES AIRWAY SENSITIZATION IN AN ANIMAL MODEL.

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TMA is a low-molecular-weight chemical used as a dry, fine powder by industry. Specific IgE and subsequent occupational asthma have been reported in exposed workers. The respiratory tract is considered to be a major route of TMA exposure, but dermal exposure is also possible. The role of exposure route in the development of TMA asthma is not known. The present study investigated the potential role of dermal exposure to dry TMA powder in both immunological sensitization and subsequent pulmonary responses to TMA inhalation challenge using the Brown Norway rat. Various doses of TMA were applied to the back (hair clipped carefully with scissors) on day 0, 7, 14 and 21, occluded with surgical tape and washed away after overnight, or after 5 hours of non-occluded exposure. Sera were collected on day 0, 7, 14, 21, 28 and 35 for specific IgE test. Exposed skin was also taken for morphologic study. TMA aerosol challenge was performed on day 35 and respiratory parameters including enhanced pause (Penh) recorded overnight.

Bronchoalveolar lavage was performed and pulmonary eosinophils assessed. The application area appeared normal, without any sign of inflammation examined grossly or microscopically. Immunohistochemical study (with one exposure only) found TMA-adduct staining in the stratum corneum and hair follicle. Specific IgE was noted by day 14 and levels were TMA dose-dependent. Eosinophilic inflammation and both early (EAR) and late airway response (LAR), were observed after airway challenge. EAR subsided within 30 min following challenge. LAR typically began 2 or more hours following challenge and persisted longer than 8 hours. TMA specific IgE and airway responses occurred in both occluded and non-occluded dermally exposed rats. Dermal exposure to dry TMA powder can induce specific-IgE and subsequent asthmatic-like EAR and LAR following TMA aerosol challenge. This model may be useful for mechanism study of dermal exposure and asthma from low-molecular-weight chemicals.

293 THE ROLE OF DERMAL EXPOSURE IN THE DEVELOPMENT OF LATEX ALLERGY.

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Latex allergy remains a serious risk for health care workers with past prevalence rates reported to range from 2.7-12%. Exposure may occur through inhalation as latex proteins bind to glove powders and are aerosolized during donning and removing gloves or through dermal exposure. Although hand dermatitis has been accepted as a risk factor, little is known of the relative contribution of the two routes of exposure to the development of sensitization. Animal models (BALB/c mice and hairless guinea pigs) have been used to evaluate the role of dermal exposure in the development of latex sensitization and to compare the potential for sensitization by the two routes. *In vitro* penetration studies using human surgical specimens and hairless guinea pig skin have demonstrated the importance of skin condition in the penetration of latex proteins with less than 1% of the applied dose of non-ammoniated latex (NAL) penetrating intact skin while as much as 30% penetrated through abraded skin. Exposure of BALB/c mice to 25µg NAL every 5th day by the dermal or intratracheal (i.t.) routes resulted in similar levels of latex specific IgE as measured by ELISA. A robust latex specific IgA response was seen in animals exposed by the i.t. route as compared to the dermal route. Upon respiratory challenge with methacholine, non-specific airway hyper-reactivity was dose-responsively increased and positively correlated with total IgE levels in animals exposed to latex proteins by either the i.t. or dermal routes. Immunoblot analysis revealed a different spectrum of sero-recognition of individual latex protein when exposure was *via* the i.t. versus the dermal route. Intervention strategies have been aimed at reducing the powder and latex protein content of gloves. Although these efforts have been observed to reduce the prevalence of symptoms in sensitized individuals, these data raise concerns regarding dermal exposure resulting from the continued use of low protein latex gloves. This work was supported in part by NIEHS interagency agreement #Y1-ES-0049-03.

294 INCLUSION OF SKIN EXPOSURE REDUCTION IN A TOTAL HYGIENE PROGRAM TO REDUCE EXPOSURE TO BERYLLIUM: BACKGROUND AND RESULTS.

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Recent epidemiological studies show no or very weak association between the mass concentration of beryllium in air, the traditional metric of exposure, and the prevalence of sensitization to beryllium or chronic beryllium disease. In general in the USA beryllium workplaces have permitted unlimited skin contact with beryllium solutions or particulate. In 1998 the possibility that sensitization to beryllium might occur *via* the skin was raised. Since then measures have been introduced to reduce skin exposure to beryllium solutions and particulate. Also taken were measures to reduce lung exposure to beryllium as well as dispersion of beryllium by various pathways within the workplace. Early data suggests these measures together reduce sensitization to beryllium.

295 GENOMIC MARKERS OF NEPHROTOXICITY IN FEMALE CYNOMOLGUS MONKEYS.

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The emergence of genomic technologies has led to a focused effort to identify early predictive markers of target organ toxicity. We define gene expression changes that correlate with the onset of histopathlogical lesions as genomic indicators of toxicity. For a genomic target to be considered an early predictive marker its expression pattern must quantitatively change before the development of the lesion, and the magnitude of change should intensify with the detection of histopathology. We have studied quantitative gene expression changes in the kidneys of female cynomolgus monkeys dosed with gentamicin (10 mg/kg) and/or everninomicin (30 or 60 mg/kg), an experimental oligosaccharide antibiotic, for seven days. Both drugs have been demonstrated to cause renal tubular necrosis and degeneration in rodents and non-human primates. Monkeys receiving both drugs showed renal lesions as early as day 1, consistent with a potentiation mechanism of gentamicin on everninomicin toxicity. By day 7 monkeys dosed with 60 mg/kg everninomicin alone also developed renal lesions, while the group exposed to both compounds had more extensive renal damage. The modulation of several genes previously identified to be associated with nephrotoxicity in rodent models was confirmed using quantitative real-time PCR. Among these, c-myc, c-jun, and MMP-9 exhibited changes consistent with the definition of a genomic marker of toxicity. Logistic regression modeling demonstrated a high degree of correlation between changes in gene expression and the development of histopathology. In addition, we identified a candidate early marker of toxicity. The expression of clusterin, a protein previously associated with renal necrosis, was significantly different from control levels (≥2-fold change) on day 1, and its level of expression was greater on day 7. These results provide the first data confirming gene expression changes associated with rodent nephrotoxicity in a non-human primate model, and are the first step in the validation of clusterin as an early marker for nephrotoxicity.

296 EXPRESSION OF GENES ASSOCIATED WITH DRUG-INDUCED BILIARY HYPERPLASIA AND CELL PROLIFERATION IN CYNOMOLGUS MONKEYS.

C. M. Bral, F. M. Goodsaid, F. Poulet, L. Li, W. Choy, G. Mandakas, H. Zairov and I. Y. Rosenblum. *Genetic and Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

Bile duct hyperplasia (BDH) can develop following administration of certain drugs. It is an important health concern because there are no predictive biomarkers for BDH that would allow adequate patient monitoring in the early stages. BDH arises as a consequence of cell proliferation within the hepatobiliary tree. We have investigated gene expression changes associated with BDH and cell proliferation in Cynomolgus monkeys treated with an experimental S-P compound. Histopathological evaluation of the livers and determination of a BrdU labeling index (BrdU-LI) for the biliary epithelium were used to separate the treated monkeys into good responders (BDH and high BrdU-LI), poor responders (BDH and low BrdU-LI) and non-responders (no BDH and no change in BrdU-LI). RNA isolated from the livers of these monkeys was hybridized on the Affymetrix U133 human genome chip. Analysis of this data with the GeneLogic Gene Express software revealed gene expression changes associated exclusively with monkeys in each of the good-, poor- and non-responder groups. There was limited overlap between the histopathological results for individual monkeys in each of these groups and between gene sets in these groups. Some of the genes that overlap include cyclins and other genes associated with cell proliferation. We identified 13 gene expression changes that discriminate between good- and non-responders, 63 for discrimination between good- and poor responders, and 23 for discrimination between poor- and non-responders. We also identified 5 gene expression changes that would discriminate between groups of monkeys with hyperplasia and those with no hyperplasia, 7 for high BrdU and low BrdU, and 2 for both of these effects. The predictive value of these changes will be evaluated further with quantitative PCR.

297 GENE EXPRESSION PROFILING OF NORMAL MAMMARY TISSUES FROM RAT STRAINS SENSITIVE AND RESISTANT TO MAMMARY CARCINOGENESIS.

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While human genetic linkage analyses have identified tumor suppressors that are responsible for familial breast cancer, many susceptibility genes are likely to be partially penetrant, due to the modifying effects of diverse genetic backgrounds and gene-environmental interactions. The advantage of using animal models to study inherited cancer susceptibility genes is the ability to use carefully controlled carcinogen exposure in highly inbred strains of animals. Consequently, breast cancer suppressor mutations that would have low penetrance in the human population may present as highly penetrant phenotypes in experimental tumor models. To gain insight into gene-environmental interactions in mammary carcinogenesis, we compared gene expression profiles in normal mammary glands from the sensitive Fisher 344 and resistant Copenhagen rat strains. Gene expression profiles were compared

at the onset of puberty (50 to 55 days of age), when rats are most sensitive to mammary carcinogenesis. Expression levels of ~8, 000 known genes and ESTs were compared among five individual animals from each strain using Affymetrix Rat Genome GeneChips. We identified ~1, 000 genes whose expression levels are significantly different ($P < 0.001$) between the two rat strains. The altered patterns of gene expression implicated several biochemical pathways in the differential sensitivity to mammary carcinogenesis, including a diminished anti-oxidant response. Studies are in progress to compare expression profiles in additional strains before and after exposure to carcinogenic doses of nitrosomethyl urea and dimethylbenz[a]anthracene. Comparisons of expression profiles among differentially sensitive rat strains are expected to identify biochemical mechanisms affected by loss of the rat mammary tumor suppressor(s). This study was supported by PHS Grants U19-ES-011387 & P30 ES-07033 from the NIEHS.

298 USE OF AN EMPIRICAL BAYES SCREENING APPROACH AND GENE ONTOLOGY ANNOTATIONS TO FILTER AND INTERPRET MICROARRAY DATA FROM THE UTERI OF ESTROGEN-TREATED MICE.

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While microarrays can useful in screening compounds *in vivo* for (anti)estrogenic activity, important basic information is still lacking in several key areas. In particular, transcriptional responses to estrogen itself must be better understood, and methods for distinguishing significant responses from baseline or irreproducible signals must be refined. Mu11KSubA GeneChip data (6523 probe sets) was examined from uterine tissue of duplicate immature, ovariectomized C57BL/6 mice treated orally with 0.1 mg/kg ethynyl estradiol or vehicle for 2, 8, 12, 24 hr, or 3x24 hr. A nonparametric empirical Bayes approach identified responses with a treatment and/or time effect (881 probe sets), and a subsequent ANOVA model distinguished treatment effects and treatment by time interactions (392 probe sets) from time-only effects. These 392 responses were clustered into 8 generalized temporal patterns by k-means, and annotated with Gene Ontology (GO) descriptions where available (193 probe sets). GO terms appearing at high frequency in specific k-means clusters, and therefore associated with specific temporal expression patterns, were found to be valuable in interpreting the cellular and tissue responses occurring over time in response to estrogen, both confirming published findings and suggesting novel estrogen-induced responses. Knowledge of the specific processes that are activated by estrogen in the uterus, and the corresponding temporal distribution of these responses, will be very helpful in the design and interpretation of assays aimed at identifying and characterizing xenoestrogens. Furthermore, the filtering, clustering, and annotation strategy described here addresses common microarray study issues of analysis and interpretation. Supported by ES 011271.

299 IDENTIFICATION OF NRF2-DEPENDENT ARE-DRIVEN GENES CONFERRING PROTECTION AGAINST OXIDATIVE STRESS IN PRIMARY CORTICAL ASTROCYTES AND NEURONS: CELL TYPE SPECIFIC NRF2-DEPENDENT ARE-DRIVEN GENE EXPRESSION.

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The antioxidant responsive element (ARE) mediates transcriptional regulation of many phase II enzymes and antioxidant proteins such as NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferases, and glutamate-cysteine ligase. Several lines of evidence demonstrated that Nrf2 binds to the ARE sequence leading to transcriptional activation of ARE-driven genes. Also, many studies demonstrated that Nrf2 plays an important role in protecting cells from oxidative stress induced cellular damage. In this study, we report that b-Zip transcription factor Nrf2 is critical for both NQO1 gene expression and cellular defense in primary mouse cortical astrocytes and neurons. We identified astrocyte- and neuron-specific Nrf2-dependent ARE-driven genes by oligonucleotide microarray analysis. First, primary cortical astrocytes and neurons derived from Nrf2-/- mice have decreased levels of basal NQO1 activity than those derived from Nrf2+/+ cells. Furthermore, tert-butylhydroquinone increased NQO1 activity only in Nrf2+/+ astrocytes and neurons. Second, Nrf2-/- astrocytes were more sensitive to H2O2-induced cytotoxicity compared to Nrf2+/+ astrocytes. Similarly, Nrf2-/- neurons were more vulnerable to rotenone-induced apoptosis suggesting Nrf2 is important for cellular defense against oxidative stress in both astrocytes and neurons. Finally, in support of these observations, microarray analysis revealed that Nrf2-/- astrocytes and neurons have decreased levels of antioxidant and detoxification gene expression compared to Nrf2+/+ astrocytes and neurons, respectively. Taken together, these findings imply that Nrf2 plays a central role in ARE-driven antioxidant/detoxification gene expression contributing to cellular defense against oxidative damage in primary cortical astrocytes and neurons. (Funded by ES08089, ES10042, ES09090).

300 TRANSCRIPTIONAL PROGRAM OF NRF2 IN LUNG CONFERS PROTECTION AGAINST ENVIRONMENTAL TOXICANTS.

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Nrf2 is a bzip transcription factor which is activated in response to various toxicants. Nrf2 plays a pivotal role in the transcriptional induction of various phase 2 enzymes and antioxidant proteins by binding to antioxidant response element (ARE) in their promoters. In order to investigate the role of Nrf2 in lungs, Nrf2 +/+ and Nrf2 -/-mice were exposed to a complex mixture of toxicants, the environmental tobacco smoke (ETS). Nrf2 -/- mice was more sensitive to ETS and tobacco carcinogen, benzo[a]pyrene.In response to ETS, there was an increased infiltration of macrophages and intense staining for 8 Oxo-dG in the lungs of Nrf2 -/- mice. DNA postlabeling method revealed higher amounts of DNA adducts in the lungs of B[a]P treated Nrf2 -/- mice than Nrf2 +/+ mice. EMSA showed the increased binding of nuclear Nrf2 (Nrf2 +/+ mice) to ARE sequence, after exposure to ETS.To elucidate the protective role of Nrf2, the transcriptional profile of lungs from Nrf2 +/+ and Nrf2 -/- mice were compared with and without exposure to ETS (using Affymetrix Murine Genome U74A genechip).Oligonucleotide microarray analysis revealed the induction of sixty genes in the lungs of Nrf2 +/+ mice compared to Nrf2-/- mice. The target genes include various antioxidant and xenobiotic metabolizing enzymes that can counteract a wide spectrum of exogenous as well as endogenous free radicals and toxicants.Screening with GCG Find Patterns Software Program revealed the presence of ARE (s) in most of the genes, suggesting the involvement of Nrf2 in the transcriptional regulation of these genes. The microarray data was validated by northern blot analysis and enzyme assay of selected genes. This study concludes that transcriptional program of Nrf2 in lungs plays an important role in protection against environmental toxicants. (This work was supported by NIEHS center grant P30 ES03819).

301 DIFFERENTIAL GENE EXPRESSION INDUCED BY PEROXISOME PROLIFERATOR, CI-924, IN RATS AND MICE.

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The lipid lowering agent 5, 5{[1, 1-biphenyl]-2, 5-diylbis(oxy)}bis[2, 2-dimethylpentanoic acid] (CI-924) induces peroxisome proliferation to a greater extent in rats than in mice. However, an increase in the incidence of hepatic tumors in mice only, not in rats, was observed following treatment with CI-924 for 2 years. CI-924 is, therefore, an interesting tool for exploring the mechanism of tumor induction by peroxisome proliferators. Previous studies looking at differences between rats and mice in peroxisome proliferation, hepatic enzyme induction, serum biochemistry, cell proliferation and rates of apoptosis did not reveal any clear predictors of subsequent tumor incidence. The current study explored the difference in gene expression between rats and mice. CI-924 was administered to Wistar rats and B6C3F1 mice for 1, 7 and 28 days at 25 and 75 mg/kg. Total RNA was isolated and hybridized to Affymetrix Rat Genome U34A and Mouse GenomeU74A GeneChips respectively. Different expression profiles were observed between rats and mice and the differential regulation was much greater in rats than mice. The expression of genes linked to CYP4A and peroxisomal enzyme genes were induced to the largest extent in the mice. Little impact was observed on genes associated with cell cycle. In the rats, enzymes involved in acyl CoA metabolism, followed by CYP4A, then peroxisomal enzyme genes were induced to the greatest extent. Further biometric analysis to explore the differential tumor response between rats and mice revealed areas of substantial similarity and difference in the gene expression profiles of rats and mice.

302 COMPARISON OF CELL PROLIFERATION ASSOCIATED GENE EXPRESSION BETWEEN REVERSIBLE AND IRREVERSIBLE LIVER PATHOLOGIES IN MALE WISTAR RATS TREATED WITH A SERIES OF HEPATOTOXIC COMPOUNDS AND DRUGS.

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The liver is unique among the major organs in its capacity to regenerate after injury. Proliferation of specific cell populations within the liver is a critical event in the normal repair and regeneration of damaged tissue. Perturbation of this process can

serve as the pathogenesis for irreversible pathologies including fulminate liver failure, cirrhosis, and cancer. There is limited information relating various types of liver histopathology with a comprehensive survey of changes in transcript expression for genes involved in cell proliferation. The GeneCalling® open architecture platform was selected to identify changes in expression of hepatic gene transcripts important in cell proliferation. Male Wistar rats received either a high or low dose of over 100 different hepatotoxic compounds daily over a two-week period. Groups of 5 animals were sacrificed at 1, 3, 7 and 14 days and tissues recovered for gene expression studies and histopathological evaluation. High doses were adjusted to produce one or more liver histopathologies. Low doses were 1/10 of the high dose and in general produced minimal or no histopathological changes. Negative controls included both vehicle treated and untreated animals. Gene expression was altered in a number of biological pathways important to cell proliferation including cell cycle control and cellular communication. Changes in gene expression could be associated with specific cell populations within the liver. Comparisons between gene expression and reversible and irreversible pathological changes were made. Results point to the importance of coupling traditional methods of toxicity assessment (histopathology) with toxicogenomics to provide a more detailed understanding of liver injury and regeneration after toxic insult.

303 TOWARDS ESTABLISHING A TOXIC SIGNATURE FOR VARIOUS ASBESTOS FORMS-MICROARRAY ANALYSIS OF 900 TOXIC RESPONSE GENES.

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In the post-genomics era, the biological questions we ask hopefully take advantage of the new wealth of genomic information available. The generation of a toxic signature for various types of asbestos particles by microarray analysis of gene expression is an example of one such globally directed investigation. Rather than look for changes in gene expression one gene or protein at a time following asbestos exposure in a mouse model, we are assessing expression changes in 900 toxic response genes simultaneously. The set of genes examined represented a conversion of the NIEHS Human ToxChip V1.0 to a mouse version, creating a Mouse ToxChip V1.0. Balb/c mice were exposed *via* intratracheal installation of winchite, tremolite, crocidolite, or chrysotile forms of asbestos, as well as silicate, wollastinite and saline controls. Following a three month long exposure, mice were sacrificed, and RNA was isolated from lungs of mice from each treatment group. Microarray analysis of each exposure set was compared to a mouse reference standard (Stratagene) so that all exposures could be compared. Lungs from each treatment group were preserved for histological observation. Evidence of widespread fibrosis was found in all asbestos or silica exposed mice, but not in wollastinite or saline control mice. Genes found to be reproducibly activated or repressed in each exposure were clustered using Spotfire Decision Suite k-means clustering algorithms, and will be further analyzed at the protein level for their role in the fibrotic process. In this constantly expanding set of toxic-response related genes, many pathways, functional cohorts, and classes of genes are represented, refining our knowledge of cellular response to asbestos. This work was supported by NIH/EPSCoR EPS-0091995.

304 OZONE TOXICITY UPREGULATES EXPRESSION OF THE P44/42 MAP KINASE IN ALVEOLAR MACROPHAGES.

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Inhalation of ozone (O_3) causes lung injury and inflammation in humans and experimental animals. We have previously demonstrated that macrophages recruited to the lung following exposure to this oxidant contribute to the pathogenesis of tissue injury. In the present studies we analyzed mechanisms regulating alveolar macrophage (AM) activation after O_3 inhalation that cause production of inflammatory mediators. We found that exposure of mice to O_3 (0.8 ppm, 3 h) resulted in increased AM expression of inducible nitric oxide synthase (NOSII) and production of nitric oxide, as well as cyclooxygenase-2 (COX-2) and prostaglandin E2. These mediators have been directly implicated in ozone-induced toxicity. The MAP kinases p44/42 (ERK1/2) and p38 kinase are known to be important in regulating expression of NOSII and COX-2. We found that AM constitutively express low levels of ERK1/2 and the p38 MAP kinase. O_3 inhalation caused a marked increase in AM expression of ERK1/2 without altering expression of the p38 kinase. This was evident immediately following exposure of mice to O_3 and was maximal within 6 hr. O_3 inhalation also induced phosphorylation of ERK1/2 in AM. Activation of ERK1/2 is known to downregulate caveolin-1, a negative regulator of the p85 subunit of phosphatidylinositol 3'-kinase (PI3K). We found that caveolin-1 was markedly down-regulated in AM after O_3 inhalation. This was directly associated with activation of PI3K and its downstream target, protein kinase B (PKB), in the cells. We have previously demonstrated that PI3K and PKB regulate NF-κB, a transcription factor important in controlling expression of NOSII and COX-2. Taken together, these data demonstrate that, in AM, O_3 is an effective activator of the MAP kinase ERK1/2, an early upstream component of the signaling that leads to reactive-intermediate producing enzymes. This may be an important pathway by which irritants induce lung injury. Support: NIH ES04738 and ES06897

305 LOSS OF CYCLIN D1 DOES NOT INHIBIT THE PROLIFERATIVE RESPONSE OF MOUSE LIVER TO MITOGENIC STIMULI.

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Cyclin D1 is considered to play a critical role in the progression from G1 to S phase of cell cycle, and its overexpression is seen in many human tumors including the majority of human mammary carcinomas. However, previous studies in cell lines have shown that cyclin D1 is not sufficient to trigger cell replication. To directly test the role of cyclin D1 in the progression of cell cycle, we have examined the proliferative response of hepatocytes to the powerful hepatomitogen 1, 4-bis[2-(3, 5-dichloropyridyloxy)]benzene (TCPOBOP), in mice with homozygous disruption of the cyclin D1 gene. We found that 24 hours after a single treatment with TCPOBOP the number of bromodeoxyuridine-positive hepatocytes was significantly reduced in cyclin D1-/- when compared to cyclin D1+/- mice (LI was 1.9% in knockout mice vs 9.7 % of heterozigous mice); however, no difference in the number of proliferating hepatocytes could be found at 36 or 72 hours after treatment (LI was 16% and 43% in cyclin D1-/- mice vs 20% and 41% of heterozygous mice), thus, indicating that lack of cyclin D1 may transiently delay the entry into S phase, but is not sufficient to inhibit the response of hepatocytes to mitogenic stimuli. Results also showed that while there was no difference in hepatic protein levels of cyclin D2 and D3 between untreated cyclin D1-/- and cyclin D1+/- mice, mRNA and protein levels of cyclin E were much higher in the former. In conclusion, our results showed that cyclin D1 is not essential for liver development and hepatocyte proliferation induced by mitogenic stimuli and suggested that cyclin E overexpression may compensate for the lack of cyclin D1.

306 FUNCTIONAL GENOMIC ANALYSIS OF STRESS-ACTIVATED PROTEIN KINASE SIGNALLING PATHWAYS.

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Mammalian cells respond to a wide variety of external stimuli including growth factors, cytokines, pharmaceutical agents and toxicants *via* protein kinase signalling pathways. We have used transcript profiling to identify novel molecular targets and mechanisms of toxicity associated with stress-activated and mitogen-activated protein (MAP) kinase signalling pathways. Anisomycin, a potent activator of stress-activated and MAP kinase cascades in mammalian cells, induced more than 15 genes in mouse fibroblasts, including several immediate early genes (C-FOS; CYR61; MKP-1) and an EST encoding a novel protein. We have combined transcript profiling with the use of small molecule protein kinase inhibitors to reveal the contribution of specific signalling pathways in mediating the transcriptional response to chemically induced stress. Most of the anisomycin-induced genes we examined were sensitive to the p38 MAP kinase inhibitor SB203580 and thus are regulated *via* p38 MAP kinase activation. In contrast, one anisomycin-induced EST was relatively insensitive to this inhibitor, implying that it may be regulated by a distinct signalling pathway. The mechanisms by which these stress response genes integrate information from complex intracellular signalling cascades are being elucidated using a combination of genomic and biochemical approaches. Data mining of recently completed mammalian genome sequences allows the analysis and identification of target gene regulatory sequences. This genomic information has been used to design chromatin immunoprecipitation assays that measure the recruitment and modification of gene regulatory proteins targeted to stress response genes. Furthermore, we have identified a novel stress response protein kinase activity using a functional biochemical screen. Together, these functional genomic experiments provide insights into the mechanisms governing transcriptional responses to toxic agents.

307 ENHANCED LIVER TISSUE REPAIR IN DIET RESTRICTED RATS UPON TOXIC CHALLENGE: A BATTLE WON BY COOPERATION OF SIGNALING PATHWAYS.

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We have reported that moderate diet restriction (DR, 35% for 21 days) protects from lethal dose of thioacetamide [TA, 70% survival in DR vs 10% in ad libitum (AL)] due to early onset of robust liver tissue repair response. Mechanisms of the augmented tissue repair in DR were investigated over a time course after TA challenge. Our results indicate that the enhanced tissue repair is a result of prompt upregulation of a number of pro-mitogenic signaling pathways. The higher plasma and liver IL-6 in TA challenged DR rats and 2-fold induction of iNOS by DR may explain earlier cell division. MAPK signaling pathway (TGF-α, EGFR, and p38 MAPK) was upregulated in DR rats after TA treatment. Liver levels of TGF-α and EGFR were upregulated along with p38 MAPK in DR rats. The sustained robust DNA synthesis and cell division in DR rats was also consistent with higher levels of HGF and its receptor (c-met) in the liver. Proteomic analysis using 2-DE combined with MALDI-MS revealed additional players in the enhanced liver tissue repair in DR rats. DR rats had higher levels of calmodulin, a Ca^{2+} binding protein known to be involved in cell division. Glucose regulated mediator of cell division, grp78 was induced in TA challenged DR rats. Stimulation of cell division by grp78 is known to be mediated by MAPK, which was upregulated in TA treated DR rats. Apart from higher tissue repair, DR rats also exhibited higher apoptosis after TA administration, a mechanism conducive for efficient tissue repair by ridding damaged and weak cells. These data indicate that the enhanced tissue repair in DR rats is stimulated by a combination of signaling pathways related to stress induced by TA challenge and highlights the augmented ability of diet restricted rats to handle toxicant-induced stress (Supported by ES-9870).

308 SYNERGISTIC ACTIVATION OF ESTROGEN RECEPTOR TARGET GENES BY HORMONAL SIGNALS AND CHEMICAL STRESS.

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Mammalian cells are capable of integrating responses to a wide spectrum of external stimuli including cellular stress, hormonal signals, growth factors and toxicants. In the physiological milieu, such stimulation simultaneously activates a range of signalling mechanisms. Thus, the effects of endocrine-disrupting estrogen receptor ligands can potentially be modulated by other signalling pathways, perhaps amplifying the biological effects of these compounds. We are using genomic and biochemical techniques to investigate the responses to combinations of such signals. We have focused on transcriptional induction of an endogenous classical estrogen-responsive gene, pS2 (TFF1), in a model system, the ERα-expressing MCF-7 breast cancer cell line, in response to estradiol (E2) and 12-O-tetradecanoylphorbol-13-acetate (TPA). TPA is a well-characterised cellular stressor and protein kinase C agonist capable of activating mitogen-activated protein kinase cascades and inducing activator protein 1 (AP1)-mediated transcription. Northern blot analysis has revealed that E2 and TPA (10^{-7}M) are both capable of increasing pS2 mRNA abundance independently, and that a combination of the two stimuli induces transcription in a synergistic manner across a range of E2 doses from 10^{-7} to 10^{-11}M, such that TPA is capable of potentiating E2 responsiveness to a level above that achievable with E2 alone. We are now using large-scale genomic analysis to identify other E2-responsive genes regulated in a similar synergistic fashion. In addition to transcript profiling, we are using biochemical approaches including the chromatin immunoprecipitation (X-ChIP) assay to investigate differences in promoter occupancy by transcription factors and components of the transcriptional machinery in response to E2 and TPA alone and in concert. Comparison of the recruitment profiles observed with E2, TPA and the combined stimulus will further our understanding of the molecular mechanisms underlying the observed synergy between estrogen receptor ligands and signals that induce stress.

309 CHARACTERIZATION OF THE SPECIES-SPECIFICITY OF PEROXISOME PROLIFERATORS IN RAT AND HUMAN CELL LINES.

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Peroxisome proliferation is a well-defined effect that leads to liver tumors in rodents. Since peroxisome proliferation is much less pronounced in humans than in rodents and, so far, no increased liver tumor incidences have been reported in humans who are exposed to peroxisome proliferators like fibrates, this effect is considered to be a species-specific phenomenon. The peroxisome proliferation activated receptor (PPAR) α has been identified as the key regulator in peroxisome proliferation. PPARα mediates effects of peroxisome proliferation by inducing gene expression and subsequently enzyme activity. Our aim was therefore to define the species-specificity and potency of peroxisome proliferators by analyzing enzyme activity, PPARα-mediated transactivation and PPAR regulated target gene expression in human and rodent cell lines as well as in primary hepatocytes. The human HepG2 cell line showed weak induction of peroxisomal enzyme activity by fibrates, whereas in the rat FAO and MH1C1 cells, fibrates strongly induced acyl-CoA oxidase activity and highest enzyme activities were induced in primary rat hepatocytes. A similar ranking of sensitivity towards fibrates could also be shown in transient transactivation assays using rat or human PPARα on a rat consensus PPAR response element (rPPRE). We then analyzed whether a rPPRE is more sensitive for peroxisome proliferators than a consensus human PPRE (hPPRE). Indeed, in transient transfection experiments, clofibrate and Wy 14, 643 were more active on the rPPRE than on the hPPRE, indicating that the higher susceptibility of rodents vs. man may be due to species specific PPREs. In on-going studies we are analyzing expression of PPAR target genes acyl-CoA oxidase, catalase and PCNA in rat and human cells with defined expression of either rat or human PPARα by TaqMan PCR. This comprehensive analysis in human and rat hepatic cell lines should help in further deepen our understanding of the species-specific effects of peroxisome proliferators.

310 THE COORDINATE ACTIVATION OF ERK AND P38 MAPK IS REQUIRED FOR HISTONE H3 PHOSPHORYLATION IN RESPONSE TO ROS-INDUCED DNA DAMAGE.

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2, 3, 5-Tris-(Glutathion-S-yl)hydroquinone (TGHQ) is a reactive metabolite of the nephrotoxicant hydroquinone. TGHQ catalyzes the rapid formation of DNA single strand breaks in renal proximal tubular epithelial cells (LLC-PK_1), in a reactive oxygen species (ROS)-dependent manner, resulting in oncotic/necrotic cell death. TGHQ induced mitogen activated protein kinase (MAPK) activation and histone H3 phosphorylation precede cell death. Both cell death and histone H3 phosphorylation were attenuated by inhibition of extracellular signal-regulated kinase (ERK) (PD98059). Inhibition of p38 MAPK (SB202190) also protected cells from death, whereas inhibition of c-Jun N-terminal kinase (JNK) had no effect on cell death. Because epidermal growth factor (EGF) mediated ERK activation does not induce histone H3 phosphorylation in LLC-PK_1 cells, we attempted to define whether TGHQ induced EGF receptor (EGFR) activation is coupled to histone H3 phosphorylation. We compared the relative ability of TGHQ, H_2O_2 and EGF to activate EGFR and MAPKs. TGHQ and EGF induced rapid EGFR phosphorylation at both Y992 and Y1068, either of which could lead to ERK activation. In contrast, H_2O_2 did not induce EGFR phosphorylation at either of these two sites. EGF-mediated EGFR phosphorylation was more rapid than with TGHQ and subsequent ERK activation was also more rapid and more sustained with EGF. H_2O_2 induced ERK activation was also more intense and sustained than with TGHQ, but was not as rapid as EGF. Although AG1478, an inhibitor of EGFR, blocked ERK activation induced by TGHQ, EGF and H_2O_2, it failed to block either TGHQ or H_2O_2-mediated JNK or p38 MAPK activation, neither of which was induced by EGF. The data suggest that the coordinate activation of the ERK and p38 MAPK pathways is required for effective histone H3 phosphorylation in response to ROS-mediated DNA damage in LLC-PK_1 cells. (ESO7784, ES07247, DK59491)

311 DIFFERENTIAL MODULATION OF STRESS AND UBIQUITINATION SIGNALING PATHWAYS BY CADMIUM, H_2O_2, AND SERUM WITHDRAWAL IN CULTURED MOUSE FIBROBLASTS.

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Cadmium causes oxidative stress in cells by inducing lipid peroxidation, binding to protein thiols, and altering intracellular glutathione status. Oxidative stress can lead to structural changes in proteins, ubiquitination of the damaged proteins, and subsequent proteolysis of the ubiquitin-tagged proteins. Cadmium has been shown to affect the ubiquitin/proteosome proteolytic pathway, causing an accumulation of ubiquitinated proteins in mouse neuronal cells and in yeast. Cadmium induces apoptosis, but very little is known of the molecular signaling mechanisms involved. In this study cultured mouse fibroblasts were exposed to cadmium chloride ($CdCl_2$), hydrogen peroxide (H_2O_2), and serum deprivation for 1-24 hours. Proteins from whole cell extracts were separated by SDS-PAGE, and analyzed for

stress (p38, SAPK/JNK, and the cell survival regulator Akt) and ubiquitin signaling pathway responses using the indicated polyclonal antibodies. Fifty and 100μM $CdCl_2$ induced p38 and SAPK/JNK phosphorylation, and accumulation of poly-ubiquitinated proteins in a time-dependent manner, but very little increase in caspase-3 cleavage. $CdCl_2$ also activated Akt by 1 hour, with a diminishing response over time. Serum deprivation also resulted in p38 and SAPK/JNK phosphorylation, but less accumulation of poly-ubiquitinated proteins relative to $CdCl_2$, and marked caspase-3 cleavage or Akt activation. 200μM H_2O_2, despite clear morphological evidence of cytotoxicity by 4 hours, did not cause p38 phosphorylation, accumulation of ubiquitinated proteins, or caspase-3 cleavage. These results show the involvement of the ubiquitin/proteosome proteolytic pathway in cadmium-induced cytotoxicity. Efforts are currently underway to identify specific targets of cadmium-induced ubiquitination. Supported by NIH Grants P30 ES07033, T32 ES07032, R01 ES09601 and R01 ES10613-01, and EPA Grant R826886.

312 REGULATION OF CYP1A1 TRANSCRIPTION ELONGATION BY AH RECEPTOR THROUGH INTERACTION WITH P-TEF B.

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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that regulates most of the dioxin-induced toxic responses. Dioxin, as a classic ligand for the AhR, causes the AhR to translocate from its cytoplasmic residence into the nucleus where it binds to the xenobiotic response elements (XREs), which are the cis-acting element for the regulation of gene expression. We now report that AhR may directly control transcriptional elongation by interacting with the positive transcription elongation factor b (p-TEFb). We found that AhR directly associated with the cyclin T1 subunit of p-TEFb. P-TEFb regulates the transcription elongation by phosphorylating the C-terminal peptapeptide repeats (YSPTSPS) of RNA polymerase II (Pol II-CTD). Furthermore, expression of cyclin T1 by transient transfection in Hepa 1c1c7 cells caused marked enhancement of AhR-driven luciferase reporter gene, suggesting that elongation control by p-TEF-b is a rate-limiting step in AhR-regulated transcription. Intriguingly, we observed that dioxin treatment induced time-dependent differential phosphorylation of the serine 2 and serine 5 of Pol II C-terminal domain on the promoter region of cyp1a1, suggesting involvement of different kinases at different stage of transcription elongation. These results demonstrate that AhR exerts an elaborate control of transcription through regulating transcription elongation as well as formation of preinitation complex. Supported in part by NIEHS Grant ES09859.

313 INVOLVEMENT OF CASPASE-2 UPSTREAM OF MITOCHONDRIA DURING ETOPOSIDE-INDUCED APOPTOSIS.

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DNA damage induced by the cancer chemotherapeutic drug etoposide triggers the onset of a series of intracellular events characteristic of apoptosis. Among the early changes observed is the release of cytochrome *c* from mitochondria, although the mechanism responsible for this effect is unclear. In the present study, we demonstrate a role for caspase-2 in etoposide-induced cytochrome *c* release. Jurkat T-lymphocytes treated with an irreversible caspase-2 inhibitor, benzyloxycarbonyl-Val-Asp-Val-Ala-Asp-fluoromethyl ketone (z-VDVAD-fmk), or stably transfected with pro-caspase-2 antisense (*Casp-2/AS*) are refractory to cytochrome *c* release stimulated by etoposide. Experiments performed using a reconstituted cell-free system indicate that etoposide-induced cytochrome *c* release by way of caspase-2 occurs independently of cytosolic factors, suggesting that the nuclear pool of pro-caspase-2 is critical to this process. Apart from inhibiting cytochrome *c* release, undermining caspase-2 activity results in an attenuation of downstream events, such as pro-caspase-9 and -3 activation, phosphatidylserine (PS) exposure on the plasma membrane, and DNA fragmentation. Taken together, our data indicate that caspase-2 provides an important link between etoposide-induced DNA damage and the engagement of the mitochondrial apoptotic pathway.

314 DIFFERENTIAL SENSITIVITY OF LUNG AND BRAIN CELL LINES TO TETRAHYDROCANNABINOL-INDUCED MITOCHONDRIAL INJURY.

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Marijuana smoking produces numerous neuropsychologic and neuromotor effects without extensive brain cell injury or cell death. However, in the lungs marijuana smoking is associated with chronic bronchitis, inflammation, immunosuppression and loss of ciliated epithelial cells. We reported previously that exposure of pulmonary A549 cells to marijuana smoke or to 9-tetrahydrocannabinol (THC) suppressed apoptotic cell death and increased necrotic death (Sarafian et al., 2001, Toxicol. Appl. Pharmacology 174:264-272). THC exposure was found to be associated with a decrease in cellular ATP levels. Studies with the fluorescent probe, JC-1, revealed that THC caused a dose-dependent decrease in red fluorescence suggesting disruption of the mitochondrial membrane potential. Disruption of cell energetics could be responsible for the observed suppression of apoptotic death in A549 cells since cellular energetics must be maintained above critical permissive level for apoptosis to occur. To investigate this possibility, we have compared three different cell lines for sensitivity to THC. Unlike A549 cells, neuroblastoma N18TG2 cells are relatively resistant to THC-induced cell killing, while U87 glioma cells are killed by an apoptotic mechanism. JC-1 fluorescence studies revealed that 24 hr exposure to 6 microg/ml THC had a greater impact on mitochondrial membrane potential in A549 cells (61% decrease in red fluorescence) than in U87 (25 % decrease) or N18TG2 (4 % decrease)cells. Loss of cellular ATP was also enhanced in A549 cells. These changes showed a relationship to patterns of apoptotic and necrotic cell death which may, in part, explain the differing pathologies observed in tissues of marijuana smokers. (Supported by NIH R37DA030)

315 MECHANISM OF CANNABINOID-INDUCED APOPTOSIS IN CELLS OF IMMUNE ORIGIN.

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Previous studies from our laboratories have shown that ligation of cannabinoid receptor by delta 9 -tetrahydrocannabinol (THC) leads to the induction of apoptosis in normal and transformed immune cells. These data suggested that immunosuppression mediated by cannabinoids may result from induction of apoptosis and that cannabinoids can be used as novel anti-cancer agents. Here we show that JWH015, a CB2 specific agonist, exerts a similar effect on splenocytes and thymocytes *in vitro*. Splenocytes treated with JWH015 showed a decreased ability to proliferate in response to mitogenic stimuli. In addition treatment of both splenocytes and thymocytes with JWH015 led to the induction of apoptosis *in vitro*. Studies to determine the mechanism of action of JWH015 showed a decrease in mitochondrial membrane potential of thymocytes and splenocytes, suggesting the involvement of the mitochondrial pathway. Further mechanistic studies conducted in the human lymphoma cell line Jurkat showed that caspase 8-deficient and FADD-deficient cells were sensitive to decrease in the mitochondrial membrane potential thereby suggesting that the mitochondrial pathway was independent of death-receptor signaling. However, the caspase 8 deficient cells were partially sensitive to apoptosis thereby suggesting that death-receptor pathway may play a role in THC-induced apoptosis. Moreover, Western blot analysis of protein extracted from wild-type Jurkat cells treated with THC showed cleavage of both caspase 8 and caspase 9, thereby confirming the involvement of these two pathways. Together this study shows that cannabinoids induce apoptosis in cells of immune origin and that both the death receptor and the mitochondrial pathways are involved. (Supported by NIH grants ES09098, DA014885 and HL058641).

316 ARSENIC-INDUCED APOPTOSIS AND NECROSIS IN RAMOS B CELLS ARE DEPENDENT ON INTRACELLULAR CA2+ FLUXES.

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Arsenicals have significant anti-tumor activity in hematopoietic malignancies, but their mechanisms of action remain elusive. Our recent work indicated that efflux of Ca2+ from the endoplasmic reticulum and subsequent mitochondrial Ca2+ uptake in apoptosis induced by staurosporine and the cancer chemotherapeutic agent doxorubicin in human prostate cancer cells. In this study we characterized the effects of arsenic trioxide on apoptosis in the human Ramos B cell lymphoma. At clinically achievable concentrations (1-10 uM) arsenic trioxide induced delayed, caspase-independent depletion of the endoplasmic reticular Ca2+ pool, resulting in mitochondrial Ca2+uptake, cytochrome c release, and apoptotic cell death. However, higher concentrations caused immediate increases in cytosolic and mitochondrial Ca2+, cytochrome c release, and necrotic cell death. Importantly, a selective inhibitor of the mitochondrial Ca2+ uniporter (RU-360) inhibited mitochondrial Ca2+ uptake, cytochrome c release, and death in cells exposed to either high or low doses of arsenic, indicating that mitochondrial Ca2+ uptake functioned as a final common pathway for cell death under all conditions. Together, our data demonstrate that intracellular Ca2+ fluxes leading to increases in mitochondrial Ca2+ are centrally involved in arsenic trioxide-induced cytochrome c release and cell death.

317 ARSENITE INDUCED MITOTIC ARREST IS P53 DEPENDENT.

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SV40 transformed human fibroblasts are sensitive to arsenite induced cytotoxicity. Exposure of SV40 transformed human fibroblasts to low concentrations of arsenite induces mitotic arrest followed by apoptosis. In contrast, only a mitotic delay is induced by arsenite in normal diploid fibroblasts. One effect of SV40 T-antigen is to abrogate p53 function. Therefore, we investigated the potential for p53 to modulate the response to arsenite exposure using TR9-7 human fibroblasts in which p53 is under exogenous control (tet-off). Effects of high (p53hi), low (p53lo) and no (p53-) p53 expression on arsenite induced cytotoxicity and mitotic arrest were examined. TR9-7 p53hi cells grew very little and were relatively resistant to arsenite induced toxicity. In asynchronous populations of TR9-7 p53- cells, 5 μM arsenite caused an accumulation of cells arrested in mitosis indicated by an 3-fold increase in mitotic index and induced apoptosis as indicated by morphological criteria. Arsenite did not cause asynchronous TR9-7 p53lo cells to accumulate in mitosis. Arsenite exposure caused a delay of entry into mitosis in TR9-7 cells released from G2 synchronization regardless of p53 expression. However, TR9-7 p53lo cells exited from mitosis whereas TR9-7 p53- cells remain arrested long after p53lo cells had exited. These results suggest that p53 plays an essential role in preventing arsenite induced mitotic arrest but does not affect delayed exit from G2 and entry into M phase. (Supported by ES06460 and Kentucky Research Challenge Trust Fund)

318 COORDINATE REGULATION OF BLEOMYCIN-INDUCED APOPTOSIS BY P53 TARGET GENES IN THE MURINE LUNG.

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Chronic inflammation leading to pulmonary fibrosis is the unfortunate consequence of exposure to a variety of cancer chemotherapeutic agents and environmental pollutants. We recently demonsrated that the DNA damaging agent bleomycin, induced higher levels of apoptosis in $p53^{-/-}$ mice compared to wild-type controls. Here we used C57BL/6 mice deficient in p53 target genes to define the regulators of apoptosis *in vivo*. Intratracheal bleomycin instillation stimulated p53-dependent increases in Fas and Fas ligand (FasL) in $p53^{+/+}$ but not $p53^{-/-}$ lungs. Levels of bleomycin-induced apoptosis were significantly reduced (by 80%) in the lungs of FasL-deficient (*gld*) mice, confirming that Fas-FasL interactions accounted for almost all of the cell death observed on a $p53^{+/+}$ background. However, bleomycin only induced significant increases in Bax expression in $p53^{-/-}$ lungs. We speculate that DNA damage-induced Bax induction occurs in $p53^{-/-}$ mice to compensate for the loss of the Fas/FasL pathway. Thrombospondin-1 (TSP-1) is another p53 target gene that functions as an immunomodulator *via* its effects on TGF-β activation and the clearance of apoptotic cell debris. Bleomycin stimulated significant increases in TSP-1 expression in wild-type but not in $p53^{-/-}$ lungs, and TSP-$1^{-/-}$ mice displayed higher rates of apoptosis compared to controls. Furthermore, TSP-1-deficient macrophages failed to ingest apoptotic cells *in vitro*. Together, our results establish a novel role for p53-dependent increases in TSP-1 in the requirement for ingestion of apoptotic cell debris. Our data are also the first to establish that p53 can inhibit stress-induced increases in Bax expression.

319 TCDD-INDUCED LOSS OF MITOCHONDRIAL MEMBRANE POTENTIAL IN SPERMATOZOA IS INDEPENDENT OF FAS AND FASL.

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2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is known to have toxic effects on the male reproductive system. These include reduced fertility, reduced reproductive organ weight, reduction in sperm counts, and an increase in abnormal spermatozoa. However, the mechanism(s) through which TCDD exerts toxic effects on the testes are not fully understood. Recently, it has been shown that the Fas death receptor system is involved in TCDD-induced atrophy of the thymus. In addition, the Fas system is active in the testes, although the expression pattern of Fas and FasL remain controversial. As it has been shown that TCDD treatment does not result in a loss of either germ cells or Sertoli cells, we examined the effects of TCDD on spermatozoa. Male C57BL/6 mice were injected i.p. with TCDD suspended in corn oil. The expression of Fas was upregulated in the testes following a 24hr exposure to 50 μg/kg TCDD compared to vehicle controls. In addition, histological examination of the epididymus showed a reduction in spermatozoa in TCDD treated mice. Sperm quality was assessed by measuring loss of mitochondrial membrane potential ($\Delta\Phi_m$) using $DiOC_6(3)$ staining. Doses as low as 0.1 μg/kg TCDD resulted in a reduction in $\Delta\Phi_m$ in 17.5% of spermatozoa after a 24hr exposure. 42% ± 16.3% of spermatozoa from mice treated with 50 μg/kg for 24hr showed loss of $\Delta\Phi_m$. To determine if Fas/FasL interactions were involved in the effect of TCDD on spermatozoa, the $\Delta\Phi_m$ of FasL defective *gld* and Fas-deficient *lpr* mice was assessed after exposure to 50 μg/kg TCDD. 50.3% ± 17.1% of spermatozoa from *gld* mice and 61.5% ± 13.6% of spermatozoa from *lpr* mice showed loss of $\Delta\Phi_m$, suggesting that Fas and FasL are not involved in TCDD-induced loss of mitochondrial membrane potential in spermatozoa (Supported by NIH grants R01ES09098, F32ES011732, R21DA014885 and R01HL058641).

320 IDENTIFICATION OF CASPASE-INDEPENDENT APOPTOSIS IN EPITHELIAL AND CANCER CELLS.

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We recently reported that 50% of cisplatin-induced apoptosis in primary cultures of rabbit renal proximal tubule cells (RPTC) proceeded *via* caspase-independent mechanisms. The goals of this study were to determine if caspase-independent apoptosis was induced by toxicants other than cisplatin and in cell models other than RPTC. Cisplatin (50 μM), staurosporine (2 μM), vincristine (2 μM), and A23187 (10 μM) induced RPTC apoptosis after 24 hr as determined by 2-2.5-fold increases in annexin V staining in the absence of propidium iodide staining (a marker for oncosis). All toxicants induced 8-50-fold increases in caspase 3 activity, and caspase 3 activities were completely inhibited by the pan caspase inhibitor ZVAD-fmk (50 μM). However, ZVAD-fmk only decreased toxicant-induced RPTC annexin V staining 30-50%. Cisplatin and staurosporine also induced annexin V staining in the human epithelial cancer cell lines Caki-1 (kidney carcinoma), A549 (lung carcinoma), A172 (glioblastoma) and murine L1210 cells (lymphocytic leukemia). Cisplatin increased annexin V staining to 20 ± 1%, 24 ± 1%, 26 ± 1%, and 28 ± 2% in Caki-1, A549, A172 and L1210 cells, respectively (vs. 12 ± 1% in controls). Pretreatment with ZVAD-fmk did not reduce cisplatin-induced annexin V staining in L1210 cells, but reduced annexin V staining to 17 ± 2% in A549 cells, 10 ± 1% in A172 cells, and 13 ± 1% in Caki-1 cells. Staurosporine increased annexin V staining to 41 ± 1%, 58 ± 2%, 36 ± 2%, and 46 ± 3% in Caki-1, A549, A172 and L1210 cells, respectively. Pretreatment with ZVAD-fmk did not decrease annexin V staining in Caki-1, A549, and L1210 cells, but decreased annexin V staining in A172 cells to 31 ± 2%. Cisplatin- and staurosporine-induced caspase 3 activation was totally inhibited by ZVAD-fmk in all cell lines. These results suggest that a significant fraction of apoptosis induced by diverse toxicants in renal epithelial cells and in four different cancer cell lines is caspase-independent.

321 THE COMBINATION OF THE PROTEASOME INHIBITOR, PS-341, AND DOCETAXEL INHIBITS ANGIOGENESIS AND OVERCOMES CELL-CYCLE MEDIATED RESISTANCE IN PANCREATIC CANCER.

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Pancreatic carcinoma is the fourth most common cause of cancer-related death in the United States. Chemotherapy and irradiation are largely ineffective, and metastatic disease frequently develops even after potentially curative surgery. Therefore, more effective therapies are clearly needed for treatment of this disease. PS-341, a boronic acid dipeptide derivative, is a selective and potent inhibitor of the proteasome. Our previous studies demonstrated that PS-341 treatment results in an accumulation of cells in the G2-M phase of the cell cycle. Considering this phenomenon, we hypothesized that the combination of PS-341 with docetaxel may have synergistic anti-tumor activity. Two pancreatic cell lines, MiaPaCa2 and L3.6pl, were selected to test this hypothesis *in vitro* and *in vivo*. This drug combination was antagonistic in MiaPaCa2 cells and showed no difference from single treatment in L3.6pl cells *in vitro*. The antagonism resulted from a decreased percentage of mitotic cells due to stabilization of the cyclin dependent kinase inhibitors, p21 and p27, and cdc2 inhibition. Combination studies were also conducted *in vivo* using an orthotopic, nude mouse model. Surprisingly, tumor weight and volume were significantly reduced in both cell line models despite the cell-cycle mediated resistance observed *in vitro*. Further characterization of the tumors revealed a significant decrease in tumor endothelial cells and a reduction in vascular endothelial growth factor (VEGF) expression. The anti-angiogenic effect is cell-cycle independent and may provide an explanation for the reduction in tumor size. Our studies demonstrate that while the combination of PS-341 and docetaxel ex-

hibits cell-cycle related effects that render it ineffective *in vitro*, the anti-angiogenic effects of this drug combination appear to overcome this mechanism of resistance *in vivo*. Thus, PS-341 is a candidate for use in combination chemotherapy in the treatment of pancreatic cancer and warrants further investigation.

322 EVALUATION OF CELL CYCLE KINETICS IN P53 MOUSE EMBRYONAL FIBROBLASTS: EFFECTS OF METHYL MERCURY.

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Methyl mercury, a ubiquitous environmental contaminant, is a known potent teratogen selectively affecting the developing central nervous system. While a definitive mechanism for methyl mercury (MeHg) induced developmental neurotoxicity remains elusive, rats exposed *in utero* to low levels of MeHg appear to have reduced numbers of cerebellar cells without cell death. This suggests earlier toxicant interference with critical molecular signaling events controlling cell behavior i.e. proliferation. We have previously observed an accumulation of cells in G2/M following MeHg treatment (0, 2, 4, and 6μM) in asynchronous transgenic mouse embryonal fibroblasts (MEFs) independent of p21 or p53 genotype. The degree of inhibition is differential by genotype and dose indicating partial involvement of p21 and p53 but not a singular mechanism. Specifically, after 24h treatment with 2 μM MeHg, a statistically significant increase in the proportion of cells in G2/M was observed in (+/-) and (-/-) and the difference became significant in all genotypes at 4μM. We employed bivariate flow analysis and observed cyclin B1 downregulation in treated p53 (+/+) cells and induction in (-/-) cells in the 4N population. Taken together, this data suggests MeHg acts through a p53-dependent pathway in these cells but also causes G2/M accumulation through other as of yet undetermined mechanisms. To examine the role that cell cycle stage plays in MeHg toxicity, we synchronized p53 transgenic MEFs through 24h serum starvation and are following their progression through the cell cycle by BrdU-Hoechst flow cytometric analysis. Our data have shed light on the potential role that cell cycle signaling pathways play in mediating MeHg toxicity. This should greatly enhance our understanding of temporal and regiospecific MeHg neurodevelopmental susceptibility. Supported by NIH grants ES10613-01, ES07033, ES0-7032.

323 METHYL MERCURY INDUCES DIFFERENTIAL UBIQUITIN-CONJUGATED PROTEIN LEVELS IN P53 VARIANT MOUSE EMBRYONAL FIBROBLASTS.

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The ubiquitin-proteasome pathway is critical for the targeting and rapid intracellular degradation of many proteins associated with regulation of cell cycle progression, differentiation, and development. The accumulation of ubiquitinated proteins is usually attributable to a malfunction, inhibition, or overload of this pathway. Recent studies have suggested that various chemical stressors can disrupt critical cell cycle checkpoints, e.g. p53, *via* increased ubiquitination resulting in cytotoxicity. We examined such a potential mechanism for the environmental contaminant, methylmercury (MeHg), which causes neurological disorders and disruption of fetal neurodevelopment. Cultures of p53 transgenic mouse embryonal fibroblasts (MEFs) were treated for 0.5, 1, 2, 4, 8 and 24h with MeHg (0, 0.5, 2.5 μM) and compared to similar treatments with lactacystin, a potent proteosomal inhibitor. Cell extracts were prepared and ubiquitinated protein levels were visualized by western blot analysis using a polyclonal antibody to ubiquitin. Lactacystin induced the accumulation of ubiquitinated proteins in a time-dependent manner irrespective of p53 genotype. In contrast, MeHg treatment (2.5 μM) resulted in a saturated accumulation between 4-24h in the p53 (+/+) cells. In the (+/-) cells, an equivalent accumulation peaked at 4h and then declined while the (-/-) cells showed a minor response at early timepoints. MeHg-induced accumulation of ubiquitinated proteins suggests an inhibition of the proteasome by this agent which is qualitatively comparable to that observed with lactacystin. However, the significant disparity in the level of ubiquitinated proteins observed between both agents in the (-/-) suggests a p53-dependent mechanism for MeHg-induced disruption of proteosomal degradation. The toxicant-induced inhibition of this critical cellular function may help explain the aberrant effects associated with MeHg-induced cytotoxicity in neuronal populations of the developing fetal brain. Supported by NIH Grants ES10613-01, ES09601-02, ES07033.

324 TOXICITY OF METHYLMERCURY IN CO-CULTURES OF ASTROCYTES AND NEURONS.

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Methylmercury (MeHg) causes damage in selective areas of the brain. The mechanism of toxicity is not known although the selective loss of e.g. cerebellar granule cells has been known for a long time. It has been suggested that the loss of neurons might be the result of primary effects on astrocytes. We have incubated cerebellar granule neurons and astrocytes separately and in co-culture and used changes in MTT-reduction and LDH leakage as indicators of methylmercury toxicity. The primary cerebellar cultures were prepared from seven-day-old mice. Cortical astrocytes were prepared from one-day-old mice. The results obtained from these experiments are compared to those from cell lines (C6 glioma and IMR-32 neuroblastoma), using EC50 (concentration where 50% change occurs). The neuroblastoma cells were slightly more sensitive than the glioma cells (3 versus 5 μM). These numbers agree well with literature values. The cerebellar neurons demonstrated sensitivity similar to the C-6 glioma cells and were far more sensitive than astrocytes which proved to be considerably more resistant. Cortical astrocytes were slightly more sensitive than cerebellar astrocytes. When cerebellar astrocytes and neurons where incubated in separable co-culture using inserts the astrocytes became more sensitive to MeHg while neurons could tolerate a three-fold increase in MeHg concentration. Thus, astrocytes appear to reduce the sensitivity of neurons considerably indicating a possible protective role for astrocytes in methylmercury neurtoxicity.

325 EXPRESSION OF CALBINDIN D-28K CORRELATES WITH DECREASED METHYLMERCURY (MEHG) CYTOTOXICITY IN MYENTERIC PLEXUS NEURONS.

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MeHg causes selective neurotoxicity, particularly in cerebellar granule neurons. The exact mechanisms of MeHg-induced cytotoxicity are unknown, but MeHg elevates intracellular divalent cations including Ca^{2+}. Granule cells don't express the Ca^{2+} binding protein, calbindinD-28k -in contrast to adjacent Purkinje cells which express calbindinD-28k and are more resistant to MeHg cytotoxicity. Calbindin is thought to prevent neuronal death and buffer against elevations of $[Ca^{2+}]_i$. Neurons containing calbindin are more resistant to cell death associated with neurodegenerative diseases and neuronal cell lines transfected with calbindin are more resistant to glutamate excitotoxicity. We hypothesized that other neurons containing calbindinD-28k would also be resistant to MeHg cytotoxicity. To test this, guinea pig myenteric plexus neurons, some of which express D-28k, were exposed in culture to 2, 5 and 7 μM MeHg for 1 h. Viability was assayed 24 h later. Little or no cell death occurred in control or 2 μM MeHg groups. However, ~ 60% of cells died in the 5 and 7 μM MeHg groups. Two cell types remained at both 5 and 7 μM MeHg treatment groups, fibroblast type cells and cells immuno-reactive for calbindinD-28k. To explore the role of calbidinD-28k in buffering $[Ca^{2+}]_i$, cells were loaded with fura-2 and exposed to 2 μM MeHg. In prior studies, cells with greater resistance to MeHg-induced cytotoxcity have longer time-to-onset increases in fura-2 fluorescence. Myenteric plexus neurons exhibiting the distinct morphology of calbindinD-28k positive neurons had a biphasic increase in fura-2 fluorescence. The 1st increase occurred at 14.1 ±1.8 min, and the 2nd at 22.8 ±5.4 min. Similar times-to-onset of fura-2 fluorescence were seen previously in cerebellar Purkinje neurons, in response to MeHg. These data support the hypothesis that expression of calbindinD-28k provides enhanced resistance to the cation elevating and subsequent cytotoxic effects of MeHg. Supported by NIEHS grant ES03299.

326 EFFECTS OF PRE- AND POSTNATAL METHYLMERCURY EXPOSURE ON EXPRESSION OF EPHS AND EPHRINS IN THE MOUSE.

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Repulsive interactions mediated by the EPH tyrosine kinase receptors and their ephrin ligands guide several morphogenetic processes, including the establishment of proper topographical mapping of CNS pathways. Methylmercury (MeHg), a potent environmental neurotoxicant, induces morphological changes during brain development suggestive of neuronal guidance errors. The effects of maternal MeHg exposure on EPH and ephrin mRNA expression in brains of exposed offspring were examined in the C57 mouse. Pregnant mice were administered 2.5 mg/kg MeHg *via* gavage every other day from gestational day 14 until sacrifice of their pups on postnatal day 1, 5, 10, or 15. On days 1 and 5, whole brains were removed; on days 10 and 15, brains were dissected into cerebellum, hippocampus, and rest of brain (ROB). Samples were analyzed for EPH and ephrin mRNA expression using an RNase protection assay. In day 1 whole brains, EPHA3, A4 and ephrin-B3, B2, B1, A3 were decreased while on day 5, receptors A3, A4, A5, A6, A7, A8 and ligands B3, B2, A3 showed increased expression. Ephrin-A5 was significantly elevated in day 10 hippocampus while the other ligands and receptors were less sensitive to MeHg. Day 10 cerebellum samples showed a trend for decreased ligand expression. EPHA4 was most significantly decreased in day 15 cerebellum while the other receptors were decreased to a lesser extent. These data suggest that even slight perturbation of repulsive guidance molecules may play a role in the brain dysmorphogenesis caused by MeHg. (Supported by ES 05022, ES 07148, and ES 11256).

327 ALTERED APOPTOTIC GENE EXPRESSION IN WHOLE CEREBELLA OF MICE EXPOSED TO METHYLMERCURY *IN VIVO*: A cDNA MICROARRAY ANALYSIS.

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Human and animal exposure to methylmercury within the environment occurs mainly due to the consumption of fish. Methylmercury has been known since the mid-1900s to be a neurotoxicant and the resulting symptoms are well known; however, the molecular mechanisms of its toxicity have yet to be elucidated. Neurodegeneration of the cerebellum by means of apoptosis produces similar symptoms to those observed in methylmercury exposed individuals and therefore apoptosis is suspected of playing a role in methylmercury toxicity. In this study, the Panorama Mouse Apoptosis Gene Array (Sigma Genosys) containing 243 apoptosis-related genes was used to analyze gene expression in cerebella of 21 day old C57BL/6J, male and female mice that were exposed to a total of 5 mg/kg methylmercury over a 7 day period. Array results indicate an upregulation in several apoptosis-related factor genes such as TDAG8 and DAXX. In addition, upregulation was observed in caspase-11, caspase-14, and IL-1 RI. Decreased gene expression was observed in TRADD and DR-6. These data indicate that cells within the cerebellum of methylmercury exposed mice undergo apoptotic cell death and provide a basis for further investigation into the mechanism of methylmercury neurotoxicity. Supported by CERH Pilot Project funds to L.C.A. (NIEHS grant P30-EF09106).

328 REACTIVE OXYGEN SPECIES MEDIATE METHYLMERCURY-INDUCED NEUROTOXICITY IN ASTROCYTES: PROTECTIVE EFFECT OF ANTIOXIDANTS.

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Excessive reactive oxygen species (ROS) generation has been implicated as a causal factor in various neurodegenerative diseases. The present work examined the effect of methylmercury (MeHg) on ROS formation by monitoring 2', 7'- dichlorodihydro-fluorescein diacetate (H2DCF-DA) fluorescence in primary astrocytic cultures from neonatal rat cerebral cortex. MeHg, at 10 and 20 uM concentrations, induced a significant increase in ROS formation (10 uM, $p<0.01$; 20 uM, $p<0.001$). Additional studies demonstrated that both superoxide dismutase (SOD), an antioxidant enzyme and n-propyl gallate (PG), a free radical scavenger, were able to significantly attenuate the MeHg-stimulated ROS generation. A significant decrease in MeHg-induced ROS generation was also observed when the astrocytes were preincubated (3 hours) with arachidonyl trifluoromethyl ketone (AACOCF3, 20 uM, $p<0.05$), a specific inhibitor of cytosolic phospholipase A2 (cPLA2). In additional studies, preincubation (24 hours) of astrocytes with 100uM buthionine-L-sulfoxamine (BSO, a glutathione synthesis inhibitor), was found to significantly increase ($p<0.05$) ROS formation in MeHg treated astrocytes, compared to controls. In summary, the present study invokes ROS as potent mediators of MeHg cytotoxicity and support the theory that excessive ROS generation, at least in part, contribute to MeHg-induced neurotoxicity. (This work was supported by PHS grant NIEHS 07331).

329 CALCIUM HOMEOSTATIS AND REDOX STATUS ALTERATIONS IN SY5Y NEUROBLASTOMA CELLS EXPOSED TO INORGANIC MERCURY.

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Heavy metals found in contaminated environmental sites pose serious risks to human and animal health. Mercury toxicity, for example, produces moderate to severe central nervous system disorders. CNS toxicity associated with mercury involves organic forms of mercury that cross the blood-brain barrier; however, once internalized within the brain, specific toxicity is perpetuated by the disassociated inorganic form. It is suspected that inorganic mercury within the brain perturbs intracellular signaling pathways and interferes with normal cellular function. Studies suggest that calcium homeostasis and redox status may play a role in mercury toxicity and in the subsequent induction of genes responsible for cytoprotection. In this study, mercuric chloride was administered *in vitro* to SY5Y cells cultured in 96-well plates. Mercury exposure occurred for a period of 24 and 36 hours at concentrations of 0, 0.1, 1.0 and 10 μM $HgCl_2$. Subsequent analysis of intracellular calcium (Ca), glutathione (GSH), mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) was performed with the fluorescent dyes Fluo-4 AM, monochlorobimane (mBCl), tetramethylrhodamine (TMRE), and 5-6-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA), respectively. Fluorescence was detected with a microplate reader. Data indicate a significant dose dependent increase in Ca, GSH, MMP and ROS levels after 24 hours of exposure to $HgCl_2$, followed by recovery to control levels at 36 hours. It appears as though mercury initially caused changes in calcium homeostatasis and redox potential, but that other mechanisms within the cells provided a cytoprotective effect. These results provided a basis for further investigations involving the secondary effects associated with alterations in calcium and redox homeostasis.

330 BLOCKAGE OF IL-6 SECRETION IN GLIA BY LEAD AND MERCURY.

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Mercury (Hg) and lead (Pb) are among known neurotoxicants to the development and function of the central nervous system (CNS). IL-6 produced by astroglia protects neurons from damage in many progressive degenerative disorders. It was reported that IL-6 secretion was chaperoned by a 78 kD glucose-regulated protein (Grp78), which is an ER-chaperone protein and involved in protein folding, assembly and trafficking, and Pb can target Grp78 in astroglia. We hypothesize that Pb and Hg could target Grp78 and block IL-6 secretion from astroglia. In this report, we constructed an IL-6/EGFP chimera and transiently transfected rat C6 glioma cells and rat primary astroglia. IL-6/EGFP signal in transfected cultures exposed to Pb, Hg or anti-oligos against Grp78 was detected with a fluorescence microscope. Data of bioimage analysis showed that the retention of IL-6/EGFP in both astroglia and C6 cells transfected with IL-6/EGFP was at an undetectable level. However, the IL-6/EGFP signal in these cultures, while exposed to Pb or Hg (0-10 μM) for 24 hrs, apparently increased, suggesting that Pb and Hg could block IL-6 secretion from astroglia. Furthermore, when these transfected cultures were exposed to anti-oligos against Grp78, as expected, the retention of IL-6/EGFP inside cultures obviously increased. In order to identify the increase of IL-6/EGFP retention, we used ELISA to detect IL-6 level in the medium of non-transfected astroglia exposed to Pb (0-100 μM). Data showed that IL-6 level decreased with increased Pb concentration, suggesting that the increased retention results in part from a decrease of IL-6 secretion. These data, together with our previous studies, suggest that Pb and Hg probably impaired IL-6 metabolism by targeting Grp78 for their neurotoxicity.

331 NICOTINE REVERSES SPATIAL LEARNING DEFICITS IN PERINATALLY LEAD-EXPOSED RATS.

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The effect of nicotine on spatial learning performance in Morris Water Maze was investigated in perinatally lead-exposed rats. Rats were exposed to lead *via* maternal administration of 0.2% lead acetate in drinking water from gestational day 16 through weaning on postnatal day 21 (P21). Lead-exposed and age-matched controls were tested on reference and working memory from P28 through P40. Half the littermates in each group received intraperitoneal injections of nicotine hydrogen tartrate salt at dose of 1 mg/ml/kg once per day, 20 min prior to the start of behavioral session. The perinatal lead-exposure resulted in a 20% reduction of hippocampal ChAT activity, and a significant spatial learning and memory deficits, including an increased latency to find the hidden platform in reference memory (133%) and working memory (166%) trials, and a reduction of the swimming time (72%) and swimming distance (81%) in reference memory probe test. In the lead-exposed littermates that were given nicotine prior to behavioral session, the latencies to find the hidden platform in reference memory (108%) and working memory (106%) trials, and swimming time (89%) and swimming distance (96%) on probe tests, were not significantly different from those in control animals. Administration of nicotine to control animals by itself had no effect on any of the behavioral parameters. These results indicate that nicotine reverses the learning and memory deficits induced by lead-exposure without affecting spatial learning performance in normal animals. Reversal by nicotine of spatial learning deficits in lead-exposed rats supports the hypothesis of a causal link between cognitive impairments and lead-induced cholinergic deficit in the hippocampus. Supported by grant # ES06365 from NIEHS

332 ACUTE LEAD EXPOSURE INCREASES THE RESPONSE TO N-METHYL-D- ASPARTATE IN MIDBRAIN DOPAMINE NEURONS OF THE RAT.

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Previous *in vivo* studies have shown that, at clinically relevant blood levels, inorganic lead (Pb) caused a decrease in the number of spontaneously active dopamine (DA) neurons without causing a decrease in immunohistologically stained TH (+)

cell count. The direct effects of Pb on the electrophysiological properties of DA neurons is unknown. Using the whole cell patch clamp recording technique we studied the effect of acute Pb exposure on the NMDA-induced inward current of young dopamine (DA) neurons in the rat brain slice. Pb (10 uM) was applied *via* addition to a standard Krebs based perfusion solution. Following acute Pb exposure(5 minutes) the response to NMDA (30uM) increased by 59+/-23% in Krebs + lead acetate (n=6; $p<0.05$) and 57+/-10% in Krebs + lead atomic absorption standard solution (n=7; $p<0.005$). In 2 out of 15 neurons, Pb did not alter the NMDA (30uM)-induced inward current. These results indicate that acute 10uM Pb exposure has significant effects on excitatory glutamatergic neurotransmission within the substantia nigra.

333 DIFFERENTIAL MODULATION OF DOPAMINE AND GLUTAMATE RECEPTOR AGONISTS ON LEAD-INDUCED CHANGES IN NEUROTRANSMITTER RELEASE IN CULTURED CORTICAL AND HIPPOCAMPAL NEURONS.

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Low level lead (Pb) exposure has been repeatedly demonstrated to disrupt reinforcement-related behavior in the mesocorticolimbic system of the brain. Interaction between the dopaminergic and glutamatergic release mechanisms of the mesocorticolimbic system plays a major role in reinforcement-based behavior. In order to investigate the role of D_1/D_2 dopamine receptors, N-methyl D-aspartate (NMDA) and metabotropic glutamate (mGLU) receptors on the effects of Pb on dopaminergic and glutamatergic release mechanisms, primary cultures of cortical (FC) and hippocampal (HIP) neurons were used to test the hypothesis that Pb alters glutamate receptor-mediated dopamine release. This study used the relatively novel sequential high-performance liquid chromatography analysis of monoamines and amino acids to examine the modulatory effects of quinpirole (a D_2-like receptor agonist), SKF-38393 (a D_1-like receptor agonist), NMDA (an NMDA receptor agonist) and trans-ACPD (a mGLU receptor agonist) on dopamine (DA) and glutamate (GLU) release in Pb-exposed primary cultures of FC and HIP neurons. Exposure to 1 to 1000 nM Pb for periods from 1 hour to 24 hours produced time-dependent and neuron-specific effects on DA and GLU release. Cortical neurons exposed to 100 nM Pb released increased levels of DA in response to 20 mM K^+, 100 μM SKF-38393 and 50 μM trans-ACPD but released decreased levels in response to 100 μM NMDA compared to control. However, similarly exposed hippocampal neurons released lower levels of DA in response to 20 mM K^+, 1000 nM quinpirole, 100 μM NMDA, and higher levels of DA in response to 50 μM trans-ACPD. These findings suggest that Pb may change DA release by modulating ionotropic GLU receptors in FC neurons and metabotropic GLU receptors in HIP neurons. Supported by ATSDR Cooperative Agreement # U50/ATU 398948.

334 LOW LEVEL LEAD ENHANCES GLUTAMATE TRANSPORT BY ASTROCYTES.

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Low-level lead (Pb) exposure in children is associated with disturbances in learning, memory and behavior. Glutamate, an excitatory amino acid neurotransmitter, is responsible for initiating long-term changes in the strength of synaptic connections, such as long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD are understood to form the basis of memory processing. The concentration of glutamate in the synaptic cleft, available for triggering EPSPs and initiating changes in synaptic communication, is determined by the amount of presynaptic release, the rate of presynaptic reuptake and the magnitude of astroglial glutamate uptake. The purpose of this study was to examine the effects of Pb on Na^+-dependent glutamate uptake by astrocytes. Astrocytes were grown and maintained in DMEM in 24 well plates. Astrocyte cultures were washed and treated in Krebs HEPES buffer solution for 30 min with the protein kinase c (PKC) activator phorbol myristate acetate (PMA) (10 nM) or with Pb^{2+} (10^{-9} to 10^{-6} M maintained with 5F-BAPTA as the divalent cation buffer). Astrocyte glutamate uptake was measure by addition of ^{3}H-glutamate to the treatment solutions. Uptake was stopped and cultures were washed and cellular content of ^{3}H-glutamate was measured and normalized to protein content of cells. Glutamate uptake in the absence of Na^+ (choline Krebs) was subtracted from total uptake in normal Na^+-containing Krebs to determine Na^+-dependent uptake. PMA treatment resulted in a 60% increase in Na^+ dependent glutamate uptake while Pb^{2+} treatment resulted in a University-shape dose response with a maximal 60% increase in glutamate uptake at 10^{-8} M. These data indicate that the activity of the glutamate transporter in astrocytes in regulated by PKC and that Pb^{2+} at very low concentrations and most likely by acting on PKC can also increase glutamate transport in astrocytes. Increased glutamate uptake by astrocytes may alter the dynamic glutamate concentration present in synapses and necessary for the induction of changes in synaptic strength and thus alter the ability of lead-exposed individuals to learn.

335 LOW LEVEL LEAD DISRUPTS PKC-MEDIATED REGULATION OF EVOKED GLUTAMATE RELEASE FROM HIPPOCAPAL PRESYNAPTIC NERVE TERMINALS.

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Low-level lead (Pb) exposure is associated with learning and memory deficits in children and laboratory animals. Glutamate, an excitatory neurotransmitter, plays a central role in the production long-term changes in the strength of synaptic connections, such as long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD are thought to form the electro-physiological basis of memory processing. Control of presynaptic glutamate release is essential to the expression of both LTP and LTD. The purpose of this study was to examine the effects of Pb on the evoke release of glutamate from presynaptic nerve terminals. Presynaptic nerve terminals (synaptosomes) were isolated from young rat hippocampi using a Percoll gradient method. Synaptosomes were loaded with ^{3}H-glutamate and superfused with oxygenated Krebs HEPES buffer. Synpatosomes were treated for 30 min with or without Pb^{2+} buffered with 5F-BAPTA to set the free Pb^{2+} concentrations in the superfusion solutions. Superfusate was collected to measure the fractional release of ^{3}H-glutamate from synaptosomes. Evoked release was initiated by depolarization in 25 mM K^+. Potentiation of glutamate release from nerve terminals was induced with the PKC activator, phorbol myristate acetate (PMA)(10 nM), added to the superfusion buffer for the 6 min preceding K^+ depolarization. PMA treatment potentiated the evoked release of glutamate to twice that of control. Pb treatment at 10^{-8} M enhanced the evoked release of glutamate by nearly 40% but inhibited the PMA-induced potentiation of glutamate release by 50%. These results indicate that Pb, at very low concentrations, can disrupt the regulation of depolarization-evoked glutamate release from hippocampal presynaptic nerve terminals. Pb-induced disruption of the regulation of presynaptic glutamate release could contribute to the reduction in LTP and LTD induction described in Pb exposed rats and the learning deficits found in low level Pb intoxicated children.

336 THE APP PROMOTER RESPONDS TO PB EXPOSURE IN TRANSFECTED PC12 CELLS.

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The predominantly sporadic nature of Alzheimer's disease (AD) and the occurrence of neurodegenerative processe in the aging brain suggest that the environment may play a role in the development of AD. AD is characterized by excessive deposits of aggregated beta-amyloid peptides (Aβ), which are snippets of a larger protein, the β-amyloid precursor protein (APP). Therefore, any agent, which results in the overproduction of APP, would also elevate the formation of Aβ, eventually leading to the neuropathological changes of AD. The regulatory region of the APP gene contains elements recognized by the transcription factor Sp1, which is essential for the activation of the APP gene. Exposure to lead (Pb) has been previously shown by us to induce Sp1 activity. To test the hypothesis that Pb may induce APP gene expression, we transfected PC12 cells with the human APP promoter linked to a reporter gene (luciferase). The responsiveness of the promoter was tested over time in the presence of nerve growth factor (NGF) and low levels of Pb. We found that the presence of Pb stimulated APP promoter activity in a time and dose-dependent manner suggesting that Pb exposure may be a potential risk factor for the promotion of amyloidgenesis.

337 ZINC-INDUCED CHANGES IN HSP70 DISTRIBUTION AND ACTIN CYTOSKELETON IN CULTURED CULTURED CHOROID PLEXUS.

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Previously, our laboratory showed latent stimulation and thermoprotection of choline transport by Zn preconditioning in cultured choroid plexus epithelium. Our present objective is to investigate MAP kinase (MAPK) regulation of the concomitant thermoprotection of actin networks and altered HSP70 distribution by Zn preconditioning. Cultured choroidal epithelial cells were incubated with 10 μM $ZnCl_2$ for 1.5h (37C) and recovered 0-6h in Zn-free medium/5% NuSerum before severe heat shock (SHS, 45C, 1h). After fixation, cells were probed for actin with

TRITC-labeled phalloidin and immunostained for HSP70 using FITC-labeled secondary antibody. Epifluorescence imaging of control cells showed modest peri-nuclear HSP70 accumulation and colocalization of HSP with microfilaments. SHS markedly reduced microfilaments but greatly intensified perinuclear accumulation of HSP70. Inhibition of p38 MAPK by SB203580 during SHS resulted in complete disappearance of microfilaments; an ERK pathway inhibitor, PD98059 had no effect. Zn exposure modestly increased peri-nuclear HSP70 accumulation and microfilament formation, but also induced migration of filaments to the cell cortex. During recovery, bundling of microfilaments and peri-nuclear HSP70 staining progressively increased; colocalization of HSP70 with microfilaments was more pronounced. Immunoblot analysis indicated increased and sustained phosphorylation of ERK-1/2 with Zn exposure and subsequent Zn-free recovery that was attenuated by PD98059. Zn preconditioning enhanced thermotolerance of the actin cytoskeleton against SHS; complete protection was observed at 3-6 h recovery. Neither MAPK inhibitor modified Zn-induced changes in actin networks or HSP distribution; however, PD98059 attenuated thermotolerance. These data suggest 1) responses to SHS are mediated in part by p38 MAPK, 2) HSP70 may colocalize with actin filaments, and 3) ERK-1/2 may regulate thermoprotection of actin networks and HSP70 redistribution induced by Zn preconditioning. ES10439; NS39452

338 EFFECT OF Zn ON THE ENERGY STATUS OF THE C6 GLIOMA AND THE HEPG2 CELLS.

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Zn has been implicated in mediating a number of neurological injuries and disorders including global ischemia, epilepsy, Parkinson's and Alzheimer's diseases. In other tissues, the level of Zn could change in respond to physiological or toxicological status. The mechanism of action of Zn is not well understood. In the nervous system, studies have shown that the toxic action of Zn may be related to its ability to interfere with energy metabolism. The present study was designed to examine the action of Zn on cellular energy metabolism in the C6 glioma and the HepG2 cells. In the C6 glioma cells, toxicity of Zn (0, 0.6, 0.9 and 1.2 mM) was measured by its effect on decreasing mitochondrial activity following a 3-hr exposure. The LC_{50} of Zn was 0.8 mM. Morphological observation showed that as the level of Zn increased, an increasing amount of cytosolic Zn containing granules (zincosomes) were visualized by TSQ staining. At [Zn]=1.2 mM, cells appeared apoptotic with a birefringent nucleus. Only a very small amount of Zn can be visualized near the nucleus. Cellular ATP, ADP, and AMP were measured by isocratic RP-HPLC. Results revealed that Zn caused a decreased in total cellular adenylate nucleotides (ATP+ADP+AMP). The ratio of ATP:ADP:AMP also varied from 0.82:0.07:0.11 at control to 0.88:0.07:0.05 after exposure to 1.2 mM Zn. The marked decrease in AMP resulted in a significant increase in cellular energy charge potential (ECP) from 0.85±0.007 to 0.92± 0.008. The cellular lactate level remained unchanged. This is consistent with an energy state that could inhibit glycolysis. On the other hand, the HepG2 cells respond to Zn differently. While increasing the level of Zn caused a decrease in total adenylate nucleotide level and cell viability in this cell line, cellular ECP calculated from the levels of ATP:ADP:AMP decreased from 0.77 to 0.67. These findings indicated that the nervous system and the hepatic system respond differently to the action of a high concentration of Zn.

339 STRUCTURE-ACTIVITY RELATIONSHIP AMONG ORGANOTINS IN A MODEL OF NEURONAL DIFFERENTIATION AND PROGRAMMED CELL DEATH.

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The organotins are used as heat stabilizers in PVC pipes and as marine biocides. Human exposure to monomethyltin (MMT) and dimethyltin (DMT) can occur in the water supply as a result of leaching from PVC pipes. Here we compared the effects of MMT, DMT, and TMT on neurite outgrowth and cell viability, endpoints relevant for developmental neurotoxicity, using PC12 cells. The dose-response curve for the effects of TMT on neurite outgrowth was steep with 2 μM TMT having no significant effect while 4.0 μM TMT inhibited NGF-induced neurite outgrowth by approximately 80%. Higher concentrations of TMT further inhibited neurite outgrowth. DMT also inhibited neurite outgrowth at low micromolar concentrations although not as dramatically. NGF-stimulated neurite outgrowth was inhibited by approximately 6% in the presence of 2.0 μM DMT. Higher concentrations of DMT resulted in progressively greater inhibition of neurite outgrowth. MMT did not have any significant effects on NGF-stimulated neurite outgrowth. Cell viability following organotin treatment was measured by trypan blue exclusion. Treatment with 6.0 μM TMT increased the percentage of non-viable cells from 7% to 16%. Treatment with 10 μM DMT increased the percentage of non-viable cells to approximately 18%. Higher concentrations of DMT caused a further increase in the percentage of non-viable cells. MMT had no significant effect on cell viability at any concentration tested. The cell death induced by TMT was apoptotic as indicated by increased DNA fragmentation while that induced by DMT was not. Our results will be correlated with neurotoxic effects observed following organotin administration *in vivo* to determine whether this type of *in vitro* assay can be used as a predictor of *in vivo* toxicity when evaluating a family of compounds. Because human exposure to MMT and DMT occurs as a mixture, we will also evaluate the effect of MMT and DMT mixtures on neurite outgrowth and cell viability. This abstract does not reflect EPA policy.

340 THE NEUROTOXICANT TRIMETHYLTIN STIMULATES APOPTOSIS VIA OXIDATIVE STRESS, CASPASE ACTIVATION AND P38 PROTEIN KINASE.

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Acute exposure to the tri-substituted organotin trimethyltin (TMT) causes neuronal degeneration in the hippocampus, amygdala, pyriform cortex, and neocortex. Developmental exposure to TMT impairs later learning and memory. Despite extensive efforts elucidating neuropathological changes and behavioral deficits following TMT exposure, little work has examined the molecular signaling mechanisms that lead to these changes. The present study demonstrates that TMT impairs neurite outgrowth and cell viability in an *in vitro* model of neuronal differentiation utilizing NGF-primed PC12 cells. Similar doses of TMT were required for both of these effects suggesting that inhibition of neurite outgrowth might be a secondary consequence of decreased cell viability. The increase in cell death is paralleled by a decrease in cell body size, an increase in DNA fragmentation, activation of caspase-9, and cleavage of the caspase substrate poly-ADP ribose polymerase (PARP) suggesting that TMT induces apoptosis. Pharmacological inhibition of caspase activity, p38 stress-responsive protein kinase activity, or oxidative stress prevented TMT-induced cell death. Inhibition of JNK, another stress-responsive protein kinase, or PARP, a DNA repair enzyme thought to be involved in some forms of necrosis, did not prevent TMT-induced cell death. This work provides the first evidence for a TMT-initiated apoptotic pathway involving oxidative stress, caspase activation, and p38 activation. This abstract does not reflect EPA policy.

341 BEHAVIORAL IMPAIRMENTS FOLLOWING TRIMETHYLTIN-EXPOSURE ARE LINKED TO ALTERATIONS IN PSA-NCAM EXPRESSION.

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Trimethyltin (TMT) has been shown previously to produce temporal changes in polysialated neural cell adhesion molecule (PSA-NCAM) expression in the mouse hippocampus, with maximal reduction occurring 24hrs and partial recovery observed 7d after treatment. The current studies sought to link these alterations in PSA-NCAM expression with established TMT-induced impairments in learning. Male BALB/c mice, 6-9 weeks old, were treated with 2.25 mg/kg TMT or saline i.p. and observed 24, 96, and 168hrs after injection for motor activity and trained on both the visible and hidden version of a water maze. PSA-NCAM levels, as determined by Western Blot, were maximally reduced at 24hrs post-treatment, a time point which revealed impairments on the hidden, but not visible platform version of the maze. At 96hrs post-treatment, TMT exposed mice showed a persistent alteration in cortical and hippocampal PSA-NCAM expression, and significant impairment in water maze acquisition. However, when TMT was administered after maze navigation was acquired, increased escape latencies were observed only the first few trials, indicating the post-translational modification of NCAM protein is necessary for acquisition and consolidation, but not maintenance and storage of a learned task. Finally, by 168hrs post-TMT, both PSA-NCAM expression and acquisition of the water maze had returned to control levels. In addition, the 5HT1a agonist buspirone was administered to TMT and saline-treated animals 96hrs post injection, and animals were tested for changes in motor activity and time spent in the lighted side of a light/dark chamber. Buspirone differentially reversed deficits in motor activity and produced an anxiolytic effect in TMT-treated mice. These results demonstrate a transient and reversible TMT-induced reduction in PSA-

NCAM levels which is linked to learning and plasticity in the mouse; and furthermore links these effects to alterations in 5HT1a sensitivity. (Supported by NIH/EPA ES11256, NJ Governor's Council on Autism and F32ES11729-01)

342 EFFECTS OF 7-NITROINDAZOLE ON KAINATE-INDUCED SEIZURES, NO AND ENERGY METABOLITES IN RAT BRAIN REGIONS.

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This investigation was undertaken to determine whether the nNOS inhibitor 7-nitroindazole (7-NI) prevents kainate (KA, 15 mg/kg, sc)-induced seizures and related changes in NO and high-energy phosphates (HEP) in rat brain regions (cortex, amygdala, and hippocampus). After a single injection of KA, the onset of seizures appeared within 30-45 min, progressed to full seizure activity within 60 min, and lasted more than 2 hr. Rats were sacrificed using head-focused microwave irradiation and brain regions were analyzed for citrulline (determinant of NO) and HEP. Time course studies revealed significant increases in NO, preceding the seizures and depletion of HEP (ATP and PCr) and their metabolites. The maximum changes by KA in NO and HEP occurred 2 hr post-injection. A single dose of 7-NI (50 mg/kg, ip) 30 min prior to KA delayed the onset of seizures by 15-20 min and significantly prevented an increase in NO and a decrease in HEP in all three brain regions. 7-NI given 30 min before and 30 min after KA injection further delayed the onset of seizures and prevented induced changes in NO and HEP. These results suggest that NO (derived from NOS) is involved in depletion of HEP in brain caused by KA-induced seizures.

343 ELEVATED BLOOD BETA-CARBOLINE ALKALOID CONCENTRATION IN ESSENTIAL TREMOR PATIENTS: A CASE-CONTROL STUDY.

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The etiology and pathogenesis of essential tremor (ET) are not well understood. Although beta-carboline alkaloids are normal body constituents, they are potent tremor-producing chemicals that are naturally present in the food chain. The objective of this study was to explore the hypothesis that high concentrations of beta-carboline alkaloids are associated with ET. One hundred cases and 100 controls were frequency matched on age, gender, and ethnicity. Harmane and harmine in whole blood were extracted and their concentrations were quantified by high performance liquid chromatography, blinded to clinical information. The mean log blood concentration of harmane was higher in cases than controls (0.72 +/- 0.53 g^{-10}/ml vs. 0.51 +/- 0.64 g^{-10}/ml, means +/- SEM, p = 0.01). A non-parametric test on non-transformed data (median harmane = 5.21 g^{-10}/ml in cases and 2.28 g^{-10}/ml in controls) confirmed this difference (p = 0.005). The mean log blood concentration of harmine was 0.20 +/- 0.48 g^{-10}/ml in cases and 0.10 +/- 0.65 g^{-10}/ml in controls (p = 0.20). Log harmane concentrations were stratified based on the median value; 62% of cases vs. 39% of controls had a high log harmane concentration (p = 0.001). Mean log harmane concentration was similar in the cases with (0.74 +/- 0.58 g^{-10}/ml) and without (0.71+/- 0.50 g^{-10}/ml) an affected relative (p = 0.83). These data indicate that blood concentrations of harmane were elevated in cases with and without a family history of ET, further suggesting a possible role of environmental risks in the etiology of ET. (Support in part by NIH RO1 NS39422, P30 ES09089 and RO1 ES08146)

344 LEARNING IMPAIRMENT CAUSED BY INFUSION OF A TOXIN PRODUCED BY *PFIESTERIA PISCICIDA* INTO THE HIPPOCAMPUS OF RATS.

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Pfiesteria piscicida, an estuarine dinoflagellate, which has been shown to kill fish, has also been associated with neurocognitive deficits in humans. With a rat model, we have demonstrated the cause-and-effect relationship between Pfiesteria exposure and learning impairment. In several studies, we have replicated the finding in Sprague-Dawley rats that exposure to fixed acute doses of Pfiesteria caused radial-arm maze learning impairment. In the current study, hydrophilic toxin (Pftx) isolated from cultures of P. piscicida zoospores recloned from cultures toxic to fish was administered by bilateral infusion into the ventral hippocampus of Sprague-Dawley rats (N=9) caused persisting learning impairment relative to vehicle infused controls (N=7) on a repeated acquisition procedure in which new learning is assessed each session over weeks. Both control and Pftx-treated groups showed learning, however, the Pftx-treated group learned more slowly. There was a significant Pfiesteria x trial interaction ($p<0.01$). The Pftx-treated rats had significant deficits during the early phase of learning. Tests of the simple main effects of Pftx on performance at each trial showed significant impairments on Trial 1 ($p<0.05$) and Trial 2 ($p<0.01$). The Pftx-treated rats were not incapable of learning and the Pftx-induced impairment was overcome during the later part of the session with no differences detected for Trials 3-5. The Pftx-induced deficit showed no sign of diminishing over the 6 weeks after a single infusion. Pftx treatment did not have a significant effect on response latency. This model has demonstrated the cause-and-effect relationship between Pfiesteria toxin exposure and learning impairment and specifically that the ventral hippocampus was critically involved. (Supported by NOAA and Duke University)

345 *IN VIVO* BASAL AND AMPHETAMINE-STIMULATED STRIATAL DOPAMINE (DA) RELEASE IS SIMILAR IN ADULT SPONTANEOUSLY HYPERTENSIVE (SHR), WISTAR-KYOTO (WKY), AND SPRAGUE-DAWLEY (SD) MALE RATS.

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Nigrostriatal alterations are proposed to partially underlie the hypertension and hyperactivity exhibited by the SHR relative to the WKY. Certainly, *in vitro* studies of striatal DA release provide substantial support for this hypothesis. Few studies, however, examine *in vivo* release and fewer still compare these two strains (SHR and WKY) to other common rodent strains. Linthorst et al. (1991) described decreased *in vivo* basal DA levels in the SHR but Inada et al. (1992) reported no strain differences. Here, *in vivo* microdialysis measured basal and d-amphetamine (AMPH)-stimulated striatal DA, homovanillic acid (HVA), and 3, 4-dihydroxyphenylacetic acid (DOPAC) in unanesthetized 19-week-old male SHR, WKY, and SD rats. Basal levels were measured for 60 minutes after which time, each rat was injected ip with 2 mg/kg AMPH and samples collected for the subsequent 200 minutes. There were no significant differences in basal levels although DOPAC was slightly decreased in the WKY relative to the SD. AMPH treatment altered DA, DOPAC, and HVA to a similar extent in all strains; thus, there were no strain differences. Similarly, area under the curve for DA release did not differ between strains. Previous testing of these subjects indicated mildly elevated blood pressure and only moderate hyperactivity in the SHR (see Ferguson & Cada, in press). Thus, the current results do not support hypotheses of nigrostriatal alterations in the SHR, at least as measured by *in vivo* microdialysis. Further, although there was a substantial body weight difference (≅ 100 g) between the SD and the other two strains, basal and AMPH-stimulated DA release was quite similar.

346 TESTOSTERONE REVERSES THE EFFECTS OF ETHANOL ON NITRIC OXIDE SYNTHASE IN THE CORTEX OF CASTRATED RATS.

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Previous results obtained in our lab have indicated that testosterone reverses ethanol-induced spatial memory deficit in castrated rats. The present study was conducted to investigate the effects of ethanol and testosterone on the activity of nitric oxide synthase (NOS) in specific brain regions of castrated rats. Male Sprague-Dawley rats (150-200g) were used in this study. Animals were castrated under halothane anesthesia and allowed to recover for a period of 15 days. Animals were randomly assigned to 4 groups. The animals were administered either ethanol (3 g/Kg as a 22.5% w/v solution orally), testosterone (2 mg/Kg, S.C.), a combination of ethanol (3g/Kg) and testosterone (2 mg/Kg), or saline (control). The animals were dosed daily at 9:00 AM for 7 consecutive days. One hour after the last injection, the animals were sacrificed by decapitation, and their brains were immediately flash frozen in liquid nitrogen and kept at -70 C until analysis. NOS activity was measured in the cortex, hippocampus, hypothalamus and midbrain using C14 Arginine and NOS detect assay kits (Stratagene, Ca). Ethanol administration resulted in a significant increase in NOS activity in the cortex of castrated rats

(38.3% increase). Testosterone did not significantly alter cortical NOS activity in the cortex. However, when testosterone was concurrently administered with ethanol, it completely reversed ethanol-induced increase in NOS. Ethanol did not produce any significant alteration of NOS activity in other brain regions tested. The present findings may in part explain the reversal of ethanol-induced memory deficit by testosterone as well as the sex difference in ethanol effects. (Supported by NIAA grant T31 AA 07561 and by NIH grant RR30320)

347 12 WEEK EXPOSURE TO CARBONYL SULFIDE PRODUCES BRAIN LESIONS AND CHANGES IN BRAINSTEM AUDITORY (BAER) AND SOMATOSENSORY (SEP) EVOKED POTENTIALS IN FISCHER 344N RATS.

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Carbonyl sulfide (COS) is a chemical intermediate in the production of pesticides and herbicides, is a metabolite of carbon disulfide, is produced by the combustion of organic material, and is found occurring in nature. COS was included in a Toxic Substances Control Act request for data, and has been listed as a Clean Air Act hazardous air pollutant. To examine possible neurotoxicity of COS, male and female Fischer 344N rats were exposed by inhalation to 0, 200, 300, or 400 ppm COS for 6 h/day, 5 days/week, for 12 weeks. After the final exposure, the animals were allowed to recover for about 42 days. Subjects were surgically implanted with screw electrodes over the forelimb/facial and hindlimb/tail somatosensory cortex and the cerebellum, and allowed to recover for one week. Unanesthetized animals were placed in a restrainer and presented with electrical stimuli (1, 2, 3 mA biphasic square wave presented at 0.9 Hz) to the ventral caudal tail nerve to record somatosensory potentials (SEPs). Auditory stimuli consisted of rarefaction clicks and pure tone pips (4, 16, 64 kHz presented at 5.6 Hz) using three intensities (50, 65, 80 dB SPL; 65, 70, 80 dB SPL for 64 kHz). Exposure to COS produced a lesion in the lateral frontal/parietal cortex in a subset of the 400 ppm group. In the animals with the lesion there was an increase in SEP amplitude recorded from the forelimb/facial (but not the hindlimb/tail) somatosensory cortex. Exposure to COS decreased BAER amplitudes (peak P_3 and P_4 region), and histopathology confirmed the presence of brainstem lesions in the superior olivary complex and inferior colliculus. Thus, exposure of rats to COS resulted in changes in auditory and somatosensory physiology and produced brain lesions in regions associated with the neural generators of these physiological measures. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

348 THROMBIN PRECONDITIONING PROVIDES NEUROBEHAVIORAL PROTECTION AGAINST A UNILATERAL 6-HYDROXYDOPAMINE LESION.

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Pretreatment with thrombin has been shown to protect against both ischemic and hemorrhagic stroke models. Here, thrombin preconditioning (TPC) was evaluated for protection against neurobehavioral deficits elicited by 6-hydroxydopamine (6-OHDA). In a blinded study, three groups of male Sprague-Dawley rats (250-300g) were administered either 6-OHDA (n=6), saline+6-OHDA (n=5), or thrombin (1 unit/50mL saline)+6-OHDA (n=5). Animals were anesthetized with pentobarbital (50mg/kg, ip.), followed by pargyline and desipramine (50 and 25mg/kg, ip.). After 30 min, the animals were given a stereotaxic injection of 10µg of 6-OHDA (2.5µg/µL at 0.5µL/min), containing 0.02% ascorbic acid, onto the medial forebrain bundle. Three days prior to administration, the preconditioning groups received 50mL of saline or thrombin, 1mm dorsal to 6-OHDA site. Behavioral deficits were assessed at intervals before 6-OHDA injection and for 3 weeks after. Vibrissae-elicited placing was measured for both sides and the percentage of unsuccessful placings determined. A forelimb asymmetry score was calculated by observing the use of the left, right or both forelimbs, during rearing. The animals turn preference on escaping a corner was also determined. TPC or saline injection alone did not induce a behavioral deficit. After 6-OHDA injection, deficits in the saline group were not significantly lower than in rats given 6-OHDA alone. The TPC group exhibited a significantly ($p<0.05$) lower placing deficit on days 3 (12±15% vs. 63±14%; mean±SE) and 21 (11±11% vs. 55±12%) than the 6-OHDA group. The asymmetry score for TPC was significantly ($p<0.05$) lower than the 6-OHDA group on days 3 (30±8% vs. 80±4%), 7 (25±10% vs. 46±7%) and 21 (6±5% vs. 44±15%). The corner test deficit was less ($p<0.05$) in the TPC group than either of the 6-OHDA and saline+6-OHDA groups at 7, 16, and 21 days. The results indicate that TPC provides protection from 6-OHDA induced behavioral deficits. Mechanistic analysis of TPC-induced protection may identify novel protective mechanisms for Parkinson's disease.

349 PROSTAGLANDIN H SYNTHASE (PHS)-DEPENDENT OXIDATIVE STRESS AND DNA DAMAGE IN LONG-TERM AMPHETAMINE-INITIATED NEURODEGENERATION AND FUNCTIONAL DEFICITS.

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Reactive oxygen species (ROS) are implicated in amphetamine-initiated neurodegeneration, but the mechanism is unclear. Here, we show that 3, 4-methylenedioxyamphetamine (MDA) and methamphetamine (METH) cause immediate PHS-dependent CNS oxidative DNA damage, with related long-term structural and functional changes. CD-1 female mice were treated with low and high doses of MDA (10 or 20mg/kg), METH (5 or 10mg/kg) or their saline vehicle. The brains were isolated 1, 3 or 6 hr following drug administration, microdissected and analyzed for oxidized DNA, determined by 8-oxoguanine formation. Within 1 hr, compared to saline controls, both low and high doses of MDA caused a 2- to 3-fold elevation in DNA oxidation in the striatum ($p<0.001$), substantia nigra ($p<0.001$) and brainstem ($p<0.007$), with delayed elevations in the hippocampus at 3 hr ($p<0.001$) and in the cortex at 6 hr ($p<0.04$). A similar pattern of increased DNA oxidation was seen for METH. Increased DNA oxidation was not apparent in the cerebellum. MDA and METH dose- and time-dependent DNA oxidation in multiple brain areas was dependent upon the level of PHS-1 expression, and pretreatment with the PHS inhibitor acetylsalicylic acid (ASA) reduced MDA-initiated DNA oxidation in all affected regions ($p<0.05$). Degeneration of dopaminergic neurons occurred in the striatum within 18 hr of MDA and METH administration, with increased neurodegeneration after 1 wk, and motor coordination impairment, assessed by the rotarod test, was evident 2 and 3 wk after MDA ($p<0.04$) and METH ($p<0.001$) administration respectively, consistent with a 2-fold greater enhancement of striatal DNA oxidation by MDA ($p<0.009$). This is the first direct evidence for temporal and spatial differences in PHS-catalyzed amphetamine bioactivation and CNS oxidative DNA damage related to structural and functional consequences, which may provide mechanistic insights into amphetamine-related neurodegeneration and neurodegenerative diseases (Support: CIHR).

350 NEUROPROTECTION AGAINST STEREOSELECTIVE 3, 4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA, ECSTASY)-INITIATED OXIDATIVE STRESS AND DNA DAMAGE IN PROSTAGLANDIN H SYNTHASE-1 (PHS-1) KNOCKOUT MICE.

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MDMA generates reactive oxygen species (ROS), which can oxidatively damage cellular macromolecules, causing altered CNS cell function or death. We previously showed that MDMA and related amphetamines are substrates for PHS-catalyzed bioactivation to free radical intermediates that lead to DNA oxidation *in vitro* (Tox. Sciences. abstr. No. 1752, 2001). Here, we used PHS-1 knockout mice to determine *in vivo* neurodegenerative contribution of PHS-catalyzed bioactivation of MDMA. Wild-type [+/+] PHS-1 normal, and heterozygous [+/-] and homozygous [-/-] PHS-deficient, knockout female and male mice were treated with racemic MDMA (rac-MDMA), (+)-MDMA, (-)-MDMA isomer (4x10 mg/kg) or their saline vehicle. Brains were isolated 1 hr following the last drug administration, microdissected and analyzed for oxidized DNA, determined by 8-oxoguanine formation. Within 1 hr, rac-MDMA ($p<0.04$) and (+)-MDMA ($p<0.002$) caused respective 2.8- and 2.4-fold elevations in DNA oxidation in the striatum of +/+ PHS-normal mice, whereas the nontoxic (-)-MDMA isomer was comparable to saline. In contrast, rac- and (+)-MDMA-initiated DNA oxidation seen in the +/+ mice was reduced 45% and 33% in +/- PHS-deficient mice ($p=0.057$, $p=0.06$) and 57% and 46% in -/- PHS-deficient mice ($p<0.05$, $p<0.01$), respectively. A similar gene dose-dependent pattern was seen in the brainstem and substantia nigra, except that the latter was sensitive to (+)-MDMA for all genotypes, suggesting increased susceptibility ($p<0.05$). DNA oxidation was not enhanced in the cerebellum, cortex or hippocampus, although the latter two regions may experience delayed damage (Proc. Soc. Neurosci. Abstr. No. 809.3, 2002). The stereoselective nature of oxidative DNA damage and PHS-1 gene dose-dependence provide the first direct *in vivo* evidence for PHS-catalyzed bioactivation of MDMA to a free radical intermediate that initiates DNA oxidation, which may contribute to the mechanism of MDMA neurotoxicity. (Support: CIHR)

351 PERIPHERAL BENZODIAZEPINE RECEPTOR RESPONSE TO DEMYELINATION IN THE MOUSE BRAIN: ASSOCIATION WITH GLIAL CELL TYPES.

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The peripheral benzodiazepine receptor (PBR) has been used as a sensitive marker of gliosis and inflammation associated with chemical-induced neurotoxicity. We have previously shown using a model of cuprizone-induced demyelination that PBR expression is increased in a region, dose, and time-dependent manner consistent with the degree of demyelination (Chen & Guilarte, Toxicology. Sciences. 66(1-S); 211, 2002). In the present study, we report the association of the PBR response to cuprizone-induced demyelination with specific glial cell types, i.e. microglia and astrocytes. Adult male C57BL/6J mice were continuously fed a 0.2% (w/w) cuprizone-mixed powder diet and sacrificed at 2, 3 and 4 weeks of treatment. [3H]-(R)-PK11195 autoradiography was performed to quantitatively measure PBR levels in selected brain regions. Glial fibrillary acidic protein (GFAP) and CD 11b (Mac-1) immunohistochemistry were used as specific markers of activated astrocytes and microglia, respectively. Increased PBR levels in the cerebellum deep nuclei, hippocampus, frontal, temporal, and entorhinal cortex in cuprizone treated mice appeared to be associated with activation of both microglia and astrocytes. However, increased PBR expression in the corpus callosum, striatum, thalamic regions, and intermediate white layer superior colliculus was mostly due to activation of microglia. In general, microglia were anatomically more closely associated with increased PBR expression in demyelinated brain regions, with astrocytes providing a background contribution. This study supports the notion that in some brain regions microglia are primarily responsible for the increased PBR signal in this model of demyelination but astrocytes also contribute to the overall PBR response. [Supported by grant # ES07062 to TRG]

352 MORPHOMETRIC ANALYSIS OF γ-DIKETONE AXONOPATHY IN RAT SPINAL CORD.

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Quantitative morphometric analysis of peripheral nerves from 2, 5-hexanedione (HD) intoxicated rats showed that axon atrophy was the primary neuropathological feature, whereas neurofilamentous swellings were a minor component related to lower daily dose-rates (Tox Appl Pharmacol 165: 127-140, 2000). In this study we used analytical morphometrics to measure axonal changes in nerve roots and white matter tracts of lumbar spinal cord from rats intoxicated at either 400 mg/kg/d (x8, 13, 18 or 22 days) or 175 mg/kg/d (x42, 70, 85 or 99 days). Results show that at the higher HD dose-rate extensive axon atrophy occurred in both ascending (gracile, cuneate, spinocerebellar) and descending (corticospinal, rubrospinal) tracts. As an early (day 8) consequence of HD intoxication, axon area decreased significantly (30-40%) and was accompanied by reductions in myelin. Axon and myelin perimeters were also reduced, although to a lesser extent (20-25%). Neither g-ratio nor the index of circularity were affected. Analyses of ventral and dorsal roots revealed similar initial quantitated atrophic changes in axons and myelin. As exposure to the 400 mg/kg rate continued (days 13-22), the magnitude of this initial atrophy response did not change in any area examined. Regardless of region or experimental time-point, no axon swellings were observed. At the lower dose-rate (175 mg/kg/d), atrophy was the earliest(42 and 70 days) and most prominent axon alteration noted in the spinal cord tracts and nerve roots examined. As intoxication continued (85 and 99 days), both atrophy and giant axonal swellings were frequently noted. Our combined results now suggest that axon atrophy is the hallmark feature in PNS and CNS, and that corresponding functional consequences might be a pathophysiologically relevant effect involved in diketone neurotoxicity. Supported by NIEHS grant ESO7912-06.

353 ACUTE NEUROTOXIC EFFECTS OF INHALED PERCHLOROETHYLENE ON PATTERN VISUAL EVOKED POTENTIALS AS A FUNCTION OF EXPOSURE AND ESTIMATED BLOOD AND BRAIN CONCENTRATION.

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Previous experiments have shown the effects of acute inhalation exposure to trichloroethylene (TCE) and toluene are related to the target tissue concentration at the time of testing. The current studies examined exposure to another volatile organic compound, perchloroethylene (PERC), for comparison to the other solvents. A physiologically- based pharmacokinetic (PBPK) model was developed for ad-lib fed (350-450 g) adult male Long-Evans rats. Rats were exposed by inhalation to 0, 1, 000, 2, 000, 3, 000 or 4, 000 ppm PERC and tested at exposure durations (including 0.5, 1, 1.5 or 2 hr) yielding C x t products up to 4000 ppm-hr. Exposure conditions yielding C x t products greater than 4000 ppm-hr were not tested. Steady-state pattern-elicited visual evoked potentials were recorded during exposure sessions. 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 PERC reduced the amplitude of the frequency-double spectral component (F2) at all air concentrations tested, including those projected to produce concentrations as low as 17.7 mg/l in blood and 97.1 mg/l in brain (1000 ppm for 0.5 hr). The actions of PERC were qualitatively similar to those measured previously for toluene and TCE. (This abstract does not necessarily reflect EPA policy).

354 DURATION ADJUSTMENT OF ACUTE EXPOSURE GUIDELINE LEVEL (AEGL) VALUES FOR TRICHLOROETHYLENE (TCE) USING A PHYSIOLOGICALLY- BASED PHARMACOKINETIC (PBPK) MODEL.

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AEGL recommendations are developed for 10 min, 30 min, 1 hr, 4 hr, and 8 hr exposure durations that are designated as concentrations above which acute exposures may cause notice- able discomfort including irritation (AEGL-1), irreversible health effects or impaired ability to escape (AEGL-2), and life threatening health effects or death (AEGL-3). Standard procedure for setting AEGL values across durations when applicable data are unavailable involves empirical modeling. We employed an alternative approach in which adverse outcomes appropriate for AEGL-1, 2, or 3 level effects were selected from the literature, then a PBPK model was used to predict the arterial blood concentrations (Ca) associated with those outcomes. Finally, the model was used in a boot- strap approach to estimate the atmospheric concentration sufficient to produce the same Ca levels at each exposure duration. This approach yielded Ca values of 3.93-4.78 mg/l for AEGL-1 (depending on which of two outcome studies was used), or 18.3 mg/l for AEGL- 2. AEGL-3 analyses are currently in progress. This approach has several advantages including that exposure duration adjustments are based on production of the same target tissue dose, and should therefore be more likely to provide similar degrees of toxicity, or toxicity protection, than are models based on external dose factors. In addition, the parameters of the PBPK model can be adjusted based on known or measurable physiological parameters, to predict toxicity in a variety of situations such as across species, ages, levels of physical exertion, or other factors. (This abstract does not reflect EPA policy).

355 SENSITIVE HISTOLOGICAL INDICATORS OF DAMAGE REVEAL TREATMENT WITH SUPRAPHYSIOLOGICAL LEVELS OF CORTICOSTERONE AND HIGH DOSAGES OF KAINIC ACID PRODUCE LIMITED HIPPOCAMPAL DAMAGE IN A STRAIN OF MICE RESISTANT TO KAINATE NEUROTOXICITY.

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The high levels of glucocorticoids that accompany stress are believed to increase the sensitivity of the hippocampus to excitotoxic injury but this issue has received little examination utilizing mouse strains reported to be resistant to this type of neurotoxicity. We have previously utilized standard histological methods and GFAP ELISA to examine the interaction of supraphysiological levels of corticosterone and high doses of kainic acid on the integrity of the hippocampus in "kainate-resistant" C57BL/6J mice. No overt cell loss was evident in KA-treated mice but GFAP elevation indicated damage was present. Cort alone produced no damage, and was unable to exacerbate the injury produced by KA. Here, we used histological stains sensitive to damage (cupric-silver and Fluoro-Jade) to further characterize the interactions between supraphysiological levels of Cort and KA. C57BL/6J mice were implanted with 35 or 100 mg corticosterone pellets (68 mg/kg/d and 192 mg/kg/d respectively). After seven days, mice were injected with 35 mg/kg kainic acid and were allowed to recover for an additional seven days. These stains revealed moderate damage to hippocampal neurons caused by KA. In mice treated with Cort+KA, significant neuronal damage was observed in cortex and cerebellum; however, limited damage was seen in hippocampus. No damage profiles were observed in mice treated with Cort alone. These data suggest high physiological levels of corticosterone may attenuate hippocampal damage caused by certain neurotoxicants in mice.

356 METAL AND PESTICIDE INTERACTION: EFFECTS OF ALUMINIUM CHLORIDE AND ACEPHATE EXPOSURE FOR SHORT TERM AND SUB CHRONIC DURATION ON RAT BRAIN SEROTONINERGIC SYSTEM.

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Metals and pesticides are suspected etiological agents in neurodegenerative disorders such as Parkinson's disease. The present investigation aims to study changes in the serotoninergic system of rat brain following exposure to aluminium chloride and the organophosphorous pesticide acephate individually and in combination and to study 1] short term and sub chronic duration specific changes and 2] brain region specific changes following toxicant exposure. Male Wistar albino rats were divided into four groups and each group of animals were orally dosed individually water, sub-chronic doses of aluminium chloride (320 mg/kg), acephate (178 mg/kg) and a 1:1 combination (145 mg/kg and 178 mg/kg) respectively for 0, 4, 14 and 60 days. Significantly increased 5-HT level observed in the brain regions following aluminium exposure suggests aluminium deactivating 5-HT system by decreased release and subsequent breakdown of 5-HT. Decreased 5-HT level was observed in cerebral cortex, hippocampus (60 days) and in cerebellum after 4 and 60 days of exposure suggest inhibitory effect of aluminium on 5-HT system due to withdrawal of cholinergic input. 5-HIAA level changes correlate with 5-HT level changes. Acephate exposure decreased 5-HT level in brain regions at the different durations studied. A short-term increase in 5-HIAA levels was observed in olfactory lobe and midbrain after 14 days of treatment. Acephate exposure produced activation of the serotoninergic system, resulting in decrease of the monoamine levels and its metabolite. Exposure to combination of the compounds for 60 days results in decreased serotonin level and its metabolite 5-HIAA, but produced no change in serotonin level in olfactory lobe, striatum, hypothalamus and pons brain regions. The results reveal that the vulnerability of the brain regions following exposure to the combination of compounds are brain region specific and are able to recover from the toxic effect of the combination of toxicants.

357 DEMONSTRATION OF MURINE NEUROTOXIC RESPONSES TO 1, 2-DIACETYLBENZENE PREPARATORY TO TOXICOGENOMIC CHARACTERIZATION.

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The aromatic gamma-diketone 1, 2-diacetylbenzene (1, 2-DAB), but not 1, 3-diacetylbenzene, forms blue-colored polymeric protein adducts and induces proximal, neurofilamentous axonal swellings in motor and, to a lesser extent, sensory nerve cells of Sprague-Dawley rats (Kim et al., Toxicol. Appl. Pharmacology 177: 121-131, 2001). Toxicogenomic responses in rat spinal cord are reported at this meeting (Kim et al.). We assessed whether these chromogenic and neurotoxic responses are also seen in C57BL/6N mice, the animal of choice for toxicogenomic studies. Male 10-week-old mice (~25g) were treated i.p. daily or every other day with 30 (low), 50 or 70 (high) mg 1, 2-DAB/kg body weight (n=4/dose) for up to 3 weeks. Animals lost body weight and developed limb muscle spasms and abnormal gait in proportion to total 1, 2-DAB dose. Tissues, including brain, spinal cord, and peripheral nerves, were stained blue after two high-dose injections. Light microscope examination revealed grossly swollen axons most prominently in lumbar anterior horn and proximal ventral roots after one week of treatment with higher doses of 1, 2-DAB. Spinal tracts showed beginning pathological changes after 3 weeks of 50 mg/kg every other day. Ventral root nerve fibers with swollen axons sometimes showed edema between ruptured layers of myelin. Ultrastructural examination of swollen axons revealed clumps of maloriented 10 nm neurofilaments and excessive numbers of mitochondria and other organelles. These findings demonstrate qualitative similarities between the chromogenic and neurotoxic responses of mice and rats treated with 1, 2-DAB, although the former appears to be less susceptible to this agent. Mice treated with single doses of 50-70 mg/kg 1, 2-DAB and equivalent doses of its non-neurotoxic isomer are suitable to define genomic and proteomic signatures that precede induction of aromatic gamma-diketone axonopathy. Supported by NIEHS grants ES10338 & ES11384, and Oregon's Worker Benefit Fund.

358 SUB-ACUTE SARIN EXPOSURE LEADS TO NEUROPATHOLOGICAL AND NEUROCHEMICAL CHANGES IN THE RAT BRAIN: DOSE-RESPONSE RELATIONSHIPS.

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We previously reported that acute exposure to an LD50 dose of sarin induces early neuropathlogical changes in the adult rat brain. In the present study, we investigated the effects of a single exposure to sarin on the adult rat forebrain after 7 days. Adult male rats were exposed to sarin by a single intramuscular injection at doses of 1, 0.5, 0.1, and 0.01x LD50. Seven days after the treatment, both sarin-treated and vehicle-treated (controls) animals were analyzed for: (i) plasma butyrylcholinesterase (BChE) activity; (ii) brain acetyl cholinesterase (AChE) activity, (iii) m2 muscarinic acetylcholine receptor (m2 mAChR) ligand binding; (iv) histopathological changes in the brain using H & E staining, TUNAL and ssDNA immunostining and microtubule-associated protein (MAP-2) and glial fibrillary acidic protein (GFAP) immunoassaying, . Animals treated with 1x LD50 sarin, exhibited a significant decrease in forebrain and brainstem AChE and m2 mAChR ligand binding. Whereas, animals treated with 0.5X LD50 exhibited a significant decrease in forebrain and brainstem m2 mAChR ligand binding; a diffuse neuronal cell death; a significant reduction of healthy (or surviving) neurons; a typical apoptotic (TUNAL-positive) cell, detection of ssDNA; a reduced MAP-2 immunoreactivity, and increased GFAP expression. Further, degenerating neurons were infrequent in animals treated with 1X LD50 compared to animals treated with 0.5X LD50. In contrast, neither the 0.1X or 0.01X LD50 treatment exhibited significant changes in the above brain regions. Collectively, the above results indicate that, sarin causes neuronal degeneration in many regions of the brain which is clearly dose-dependent, and exacerbated with time.(Supported in part by a grant from the US Army Medical Research and Materiel Command under DAMD 17-98-8027).

359 COMPARATIVE EFFECTS OF WEEKLY EXPOSURES TO ANATOXIN-A AND NICOTINE ON THE OPERANT PERFORMANCE OF RATS.

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Anatoxin-a is a nicotinic-receptor agonist produced by several genera of cyanobacteria. We have previously shown (Jarema et al., 2002) tolerance to the effects of nicotine on discrete-trial operant behavior (repeated acquisition) when the drug was administered at weekly intervals. The current experiment was undertaken to extend this finding to free-operant performance and to directly compare the effects of weekly nicotine and anatoxin-a exposures. Adult male Long Evans rats were maintained at 350 g body weight and trained to perform under a multiple variable-ratio 30-response variable-interval 30-sec schedule of food reinforcement. Once performances had stabilized, rats were divided into groups of eight that received saline vehicle or a dose of nicotine (0.125 - 1.8 mg/kg) or anatoxin-a (50 - 250 mcg/kg). Treatments were administered s.c. (1 ml/kg) 5-min prior to 45-min sessions. When initially administered, both compounds decreased response rates and reinforcement rates in both components of the multiple schedule. Substantial tolerance developed to the disruptive effects of both compounds with subsequent weekly administrations. These results indicate substantial similarity in the behavioral effects of anatoxin-a and nicotine with both acute and weekly administration. This abstract does not necessarily reflect USEPA policy.

360 TEA EPIGALLOCATECHIN 3-GALLATE PREVENTS MPTP-INDUCED PARKINSON'S DISEASE THROUGH THE INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE EXPRESSION IN MICE.

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The toxicity of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is mediated by oxidative stress, especially by nitric oxide (NO) in animal models of Parkinson's disease (PD). Inhibition of excessive NO production in the brain produces a neuroprotective effect against PD induced by MPTP. Green tea containing high levels of (-)-epigallocatechin 3-gallate (EGCG), which has been known to inhibit inducible NO synthase (NOS), was administered to test whether EGCG attenuates MPTP-induced PD in mice through the reduction in the expression of NOSs. Both tea and the oral administration of EGCG prevented the loss of tyrosine hydroxylase (TH)-positive cells in the substantia nigra and of TH activity in the striatum. These treatments also preserved striatal levels of dopamine and its metabolites, 3, 4-dihydroxyphenylacetic acid and homovanillic acid. Both tea and EGCG decreased expressions of nNOS in the substantia nigra. Also tea pus MPTP and EGCG puls MPTP treatments decreased expressions of nNOS at the similar levels of EGCG treatment group. Unexpectedly, the expression of iNOS was not detectable in substantia nigra. Therefore, the preventive effects of tea and EGCG on the MPTP-mediated PD can be explained by the inhibition of nNOS in the substantia nigra.

361 FERTILITY & GENERAL REPRODUCTION TOXICITY STUDY OF A SURROGATE MURINE ANTI-CD11A ANTIBODY IN MICE.

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CD11a is a subunit of lymphocyte function antigen-1, a β2 integrin expressed on leukocytes involved in intercellular interactions necessary for immunologic responses and inflammation. Raptiva™ is a humanized anti-human CD11a mono-

clonal antibody in development for psoriasis. Due to the binding specificity of Raptiva to human and chimpanzee CD11a, a surrogate antibody, muM17 (a chimeric rat/mouse anti-mouse CD11a antibody), was developed for nonclinical safety assessment studies. In a fertility and general reproduction toxicity study, CD-1 male and female mice were administered vehicle or muM17 (3, 10, or 30 mg/kg) subcutaneously once a week. Previous studies evaluating the suitability of muM17 as a surrogate antibody for Raptiva determined the 3 mg/kg/wk muM17 dose as approximately equivalent to the clinical dose of Raptiva. Male mice received a total of 8 doses beginning 28 days prior to cohabitation and finishing 1 week after the end of cohabitation. Female mice received a total of 4 doses beginning 15 days prior to cohabitation until 1 week after confirmed mating. Male mice were euthanized after completion of the cohabitation period, and cesarean sections were performed on females on Day 11 of gestation. Toxicokinetic analysis confirmed exposure to muM17 in treated animals, and no antibodies to muM17 were detected. There were no muM17 treatment-related mortalities or effects on clinical observations or body weight. No treatment-related effects on mating, fertility, sperm motility or sperm density were noted in any males. In females, treatment with muM17 did not affect weight gain or estrous cycling, and the average numbers of corpora lutea, implantations, and viable/nonviable embryos did not differ among the groups. In summary, the fertility and general reproductive no observable effect level (NOEL) for muM17 was greater than 30 mg/kg/wk, and inhibition of the interaction between CD11a and ICAM-1 did not appear to adversely effect fertility or reproduction.

362 *IN UTERO* THROUGH LACTATIONAL EXPOSURE TO ETHINYL ESTRADIOL CAUSES ANOMALY OF THE FEMALE EXTERNAL GENITALIA AND LOSS OF REPRODUCTIVE CYCLICITY OF SD IGS RAT.

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Endocrine-active chemicals may affect fetuses and neonates more seriously than adults. To evaluate the efficacy of an "*in utero* through lactational exposure" protocol and to estimate the optimal endpoints, 0, 0.5, 5, and 50 μg/kg/day of ethinyl estradiol (EE) was administered by gavage to pregnant Crj: CD (SD) IGS BR rats from gestational day 7 to day 18 after delivery. Clinical signs, body weight and physical development of the offspring were recorded. In addition, reproductive performance of the offspring at 14-20 weeks old was evaluated by checking their copulation indices and examinations at the caesarian section. Furthermore, at least 7 females / group were maintained until 6 months old and their cyclicity was examined followed by necropsy and histopathological examination of the genital tract. As a result, the dams showed no abnormalities. As to the offspring, cleft phallus was observed in the female at 50 μg/kg. Although the retardation of body weight gain of both sexes at 50 μg/kg was observed, it did not cause any secondary deleterious effects. Sex cycle prior to mating and reproductive performance of the EE-exposed offspring did not differ to those of the control rats. However, at 6 months old, abnormal cyclicity, mostly persistent estrus, was observed in 6/8 of the female at 50 μg/kg whereas 8/8, 6/7 and 9/9 at 0, 0.5 and 5 μg/kg respectively, showed normal cyclicity. Histopathologicaliy, the rats with persistent estrus showed absence of corpora lutea and follicular cysts in the ovary. These findings are similar to those in aged rats. In conclusion, cleft phallus was observed in the female at 50 μg/kg, the dose which did not induce major adverse effects. Furthermore, although their fertility was confirmed, later examination disclosed their loss of reproductive cyclicity. These suggest the study protocol we employed is effective and observation of the cyclicity at 6 months old or later may be useful.

363 *IN UTERO* AND LACTATIONAL EXPOSURE TO 1R4F CIGARETTE SMOKE: EFFECTS ON NEONATAL DEVELOPMENT, GROWTH AND NEUROBEHAVIOR IN THE OFFSPRING RATS.

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Potential pre-and postnatal effects of 1R4F cigarette smoke were examined using male and female Sprague-Dawley rats exposed 2 hrs/day 7 days/wk by nose-only inhalation at total particulate matter (TPM) concentrations of 150, 300, or 600 mg/m3 (males: 4-wks prior to and during mating, females: 2-wks prior to mating, during mating, and through lactation day 20). Sham controls received filtered air to simulate exposure conditions, while shelf controls were untreated. During lactation and until weaning (post natal day: PND 61), the offspring rats were weighed, examined for gross signs of toxicity and evaluated for neurobehavioral effects. Biomarker analysis indicated smoke concentration related increases in blood COHb, nicotine and cotinine in exposed parental animals. Nicotine and cotinine were found in the pup blood during the lactation period. Characteristic cigarette smoke-related rodent respiratory tract histopathological changes were noted in all exposed parental groups. During gestation, maternal toxicity was indicated at smoke concentrations of 300 and 600 mg TPM/m3, where total maternal body weight gain was significantly ($p \leq 0.05$) decreased compared to sham controls. Smoke-related effects in the offspring rats were noted at 600 mg TPM/m3, where pup body weights were significantly decreased at birth (~17%), at weaning (~18%) and at PND 61 (~12%). At 300 mg TPM/m3, significantly reduced pup weights were noted at birth but were consistent with sham controls thereafter. No adverse effects on developmental landmarks, age at vaginal patency or preputial separation, motor activity, acoustic startle response or learning and memory were observed in the offspring rats. This study indicates that maternal exposure to toxic levels of cigarette smoke during gestation, reduces birth weight, and retards growth in the rat. Cigarette smoke does not appear to produce developmental or neurobehavioral effects under the conditions of this test.

364 DEVELOPMENTAL EFFECTS OF ZINC CHLORIDE IN RATS.

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Zinc is frequently found in the environment and at hazardous waste sites in several National Priority List (NPL) sites. The primary objective of our study was to evaluate the effects of zinc chloride in neonatal and maternal mammalian models. Rats were randomly assigned to one of four treatment groups (25/group/sex) to receive either 0.00; 7.5, 15.0 and 30 mg/kg by oral gavage. Rats were dosed during precohabitation (11 weeks), cohabitation (3 weeks); gestation (3 weeks); lactation (3 weeks) periods. Selected tissues from the reproductive tract (testes, seminal vesicles, ovaries, uterus) liver, brain, kidney, bone, spleen, and pituitary were evaluated histopathologically and for zinc analysis. Daily clinical observations and feed consumption and body weights were recorded. The F_1 pups (2 males and 2 females/litter) were randomly selected for neonatal evaluations including body weights, land mark developments, surface rightening reflex, and negative geotaxis. There was a significant difference (SD) in body weight of male and female pups versus their respective controls. Males exposed to the high dose showed a trend toward earlier incisor eruption. Zinc hastened incisor eruption in females which was SD when control was compared to the high dose group. Eye opening in both male and female pups was significantly shortened in all zinc treated groups (SD between high dose and control). There was no difference in testicular decent. Zinc chloride caused a significant increase in anogenital distance in male pups. There was a trend toward hastening vaginal opening, but there were no SD between the zinc treated groups. There were no differences in the pup rightening reflex on postnatal days 2-5. Male rats appear to be more affected by zinc exposure at the mid and high dose levels than their female siblings. Based on these data zinc can affect neonatal development of rats.

365 MULTIGENERATIONAL EFFECTS OF ZINC CHLORIDE ON THE REPRODUCTIVE PERFORMANCE OF CD-1 MICE.

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Zinc chloride ($ZnCl_2$) has been found in significant levels at many hazardous waste (NPL) sites.The major objective was to determine the effects of low doses of $ZnCl_2$ on reproductive performance in a mammalian species. Mice were exposed during puberty, cohabitation, gestation, and lactation. Mice (25/group/sex) were exposed to either 0.00, 0.78, 1.56, or 3.125 mg/kg $ZnCl_2$ daily by oral gavage until terminated. Males were sacrificed after cohabitation and females after lactation. The F_1 parents were randomly selected from the F_1 pups and the reproductive cycle was repeated. The F_1 generation was exposed to $ZnCl_2$throughout their prenatal and postnatal life. Reproductive parameters evaluated were mean litter size, pup viability index, pup litter weight, live births, clinical signs, fertility index, post partum dam body weights, food consumption and parental body weights. There were no significant differences (SD) in the F_0 male and female body weight gain or food consumption. There were trends toward a decrease in MCV and MCH in both F_0 and F_1 mice suggestive of early phases of anemia associated with zinc exposure. Control groups in both generations had the highest number of total pups and live pups per liter, whereas, the highest and the mid dose groups had the fewest number of total pups. The fertility index was higher in the F_0 generation compared to the F_1 generation in all $ZnCl_2$ treated groups. Abnormal nursing and nesting behaviors were observed in the mid and high dose groups in both generations, but were more prominent in the F_1 generation. The latter maternal behavior was characterized by hyperactivity, irritability, absence of nest building, failure to allow pups to nurse

and killing of pups. Parental mortalities occurred in all $ZnCl_2$ treated groups in both generations. These data suggest that $ZnCl_2$ adversely affects some reproductive parameters when mice were exposed to oral doses of either 0.78, 1.5, and 3.125 mg/kg/day. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU3922660032).

366 REPRODUCTIVE EFFECTS OF ZINC CHLORIDE IN SPRAGUE-DAWLEY RATS.

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Zinc is commonly encountered in the environment and is a frequent contaminant at over 700 National Priority List (NPL) sites. The major objective of our study was to evaluate the effects of zinc chloride in a mammalian model. Selected doses were derived from earlier studies that established maximum tolerated dose (MTD). Rats were randomly assigned to one of four treatment groups (25 /group/sex) to receive either 0.00; 7.5, 15.0 and 30 mg/kg/day by oral gavage. Rats were dosed during precohabitation (77 days), cohabitation (21 days), gestation (21 days), and lactation (21 days) periods. Males were removed after cohabitation, weighed, and terminated for pathologic evaluation. The testes, seminal vesicles, ovaries, uterus, liver, brain, kidney, bone, spleen, and pituitary were evaluated for histopathological and zinc analysis. Clinical observations were made throughout the day, weekly feed consumption, weekly and biweekly body weights (gestation period) were maintained throughout the study. There were no significant differences (SD) in the hematological and clinical chemistry values, or organ-body weight ratios. The control group had the highest implantation efficiency, which was SD from the low dose group. There were no SD in the length of gestation, the total number of pups born, the mean number of live births and the pup litter weights at days 0, 4, 7, 14, and 21. There were dose related trends toward decreased numbers of implantation sites and decreases in survival indices on postnatal days 0, 4, 7, 14, and 21. Similar trends toward decreased live birth index and weaning index were also noted in zinc treated groups. Based on these data, although mild, zinc chloride decreased the reproductive performance in rats when they were exposed to doses as low as 7.5 mg/kg/day.

367 CROSS-FOSTERING STUDY WITH ATOSIBAN IN CD RATS TO ELUCIDATE THE ROLE OF MATERNAL EFFECTS.

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Atosiban, an oxytocin antagonist, has been demonstrated safe for the treatment of imminent preterm labour. In a peri- and post-natal study in rats there was an effect on neonatal survival at a high dosage of 300 mg/kg/day. Whilst considered attributable to a transient effect on milk production, this was not demonstrated unequivocally. This cross-fostering study was designed to investigate the aetiology of this effect. Atosiban was administered subcutaneously to 40 rats from Day 15 to 20 of gestation at a dosage of 300 mg/kg/day. Controls received the vehicle. Gestation length, duration of parturition, number of pups born and live birth index were unaffected. Within one hour of completion of parturition, litters were standardised to five per sex and cross-fostered either between or within groups. At birth, and within 2 hours of cross-fostering, the percentage of lactating treated females was markedly reduced. Prior to cross-fostering, litters from atosiban-treated females had the highest incidence of litters not being fed. Atosiban-treated litters cross-fostered to control females showed a marked increase in the percentage of pups fed; this was not the case for treated litters fostered to treated females. Control litters cross-fostered to atosiban-treated females showed a marked decrease in the percentage of pups fed. Offspring viability was reduced in both control and treated litters fostered to atosiban-treated females. Offspring bodyweight on Day 0 showed no effect of maternal treatment, but following cross-fostering weight gain to Day 7 was lower for litters fostered to atosiban-treated females. Bodyweight gains for treated litters fostered to control females were marginally greater than those recorded for control litters fostered to control litters. It was concluded that there was a clear effect on milk letdown in the atosiban-treated dams, leading to an indirect effect on neonatal survival; offspring were not directly affected.

368 A FIVE GENERATION REPRODUCTIVE TOXICITY ASSESSMENT OF THE SOY ISOFLAVONE GENISTEIN IN CD SPRAGUE-DAWLEY RATS.

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Genistein, the major isoflavone found in soy products, interacts with estrogen receptors (ER), with preference for ER-β, and with multiple other biochemical targets, depending on dose. An assessment of the toxicology of genistein is important in light of high levels of human exposure from the intake of soy infant formula and dietary supplements. The toxicity of dietary genistein (0, 5, 100, and 500 ppm) has been evaluated in a unique multigeneration design using NCTR CD rats. Serum levels of genistein achieved by these doses were previously shown to span the range expected from human consumption of soy products. The parental generation was exposed to genistein in a soy- and alfalfa-free diet (Purina 5K96) from 28 days prior to mating until necropsy at PND 140, the age at which all generations were terminated. The F_1 and F_2 generations were exposed continuously from conception, F_3 from conception until wean, and F_4 was not exposed to genistein. In the continuously exposed generations, body weight gain in females was depressed ≥ 10% at 500 ppm. A linear trend toward decreasing litter size was observed in the F_1-F_3 generations and mean litter size was lower than the control in F_2 (by 13% at 100 ppm and 33% at 500 ppm) and F_3 (by 17% at 500 ppm). Time and/or body weight at vaginal opening were accelerated at 500 ppm in F_1-F_3 and also at 100 ppm in F_1. There were significant small (5-7%) decreases in female anogenital distance at 100 and 500 ppm, with results dependent on the method of adjustment for body weight. Microscopic evaluation revealed treatment effects only in the male mammary gland (alveolar and/or ductal hyperplasia) and kidney (mineralization). These effects were most evident in the F_1 and F_2 generations, suggesting the importance of exposure during early development and the apparent reversibility of the effects. Thus, for data analyzed to this point, possible adverse reproductive effects of genistein under these exposure conditions were generally modest.

369 MULTI-GENERATION REPRODUCTION STUDY OF AMMONIUM PERFLUOROOCTANOATE IN RATS.

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Ammonium perfluorooctanoate (APFO) is a surfactant used primarily as an aid in processing various fluoropolymers, and perfluorooctanoate has been identified in human sera from the US at very low concentrations (mean 6 ppb). The potential reproductive toxicity of APFO was studied using current EPA OPPTS 870.3800 guidelines. Male and female Sprague-Dawley rats were dosed orally with 0, 1, 3, 10, or 30 mg/kg/day APFO. P rats (~ 6 weeks old) were dosed at least 70 days prior to mating and until sacrificed (after mating for males; after weaning for females). F1 rats were dosed similarly, beginning at weaning. The F2 pups were maintained through 22 days of lactation. Reproductive parameters evaluated in P and F1 rats included estrous cycling, sperm number and quality, mating, fertility, natural delivery, and litter viability and growth. Day of sexual maturation (F1) and anogenital distance (F2) were also determined. Feed consumption, body weight gain, selected organ weight, gross pathology and appropriate histopathology were collected. No effects on mating or fertility were discovered. P and F1 males experienced significant toxicity (decreased body weight and organ weight changes) even at the lowest dose tested. P females had reduced body weights and organ weight changes at 30 mg/kg/day, and F1 females had reduced body weight gains at 30 mg/kg/day and reduced pituitary weights at 3 mg/kg/day and higher. The 30 mg/kg/day F1 pups had decreased birth weight and viability; however, F2 pups at 30 mg/kg/day did not show a loss in viability. Sexual maturation was delayed in F1 (both sexes). Estrous periods per 21 days were increased in F1 females; however, this is likely an artifact of the evaluation method, as an evaluation of raw estrous cycling data did not indicate significant abnormalities. At the doses tested, APFO did not cause specific effects on reproductive capacity, but did produce toxicity and delayed sexual maturation. The NOAEL for reproduction was > 30 mg/kg (P and F1) based on lack of effect on mating, fertility, and natural delivery.

370 A COMBINED REPEATED DOSE TOXICITY STUDY AND REPRODUCTION/DEVELOPMENTAL SCREENING STUDY IN SPRAGUE-DAWLEY RATS WITH ACETOPHENONE (OECD GUIDELINE NO. 422).

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This study consisted of a repeated dose toxicity test combined with a reproduction/developmental screening test conducted following OECD Guideline No. 422. The purpose was to: 1.) provide initial information on the repeated dose systemic toxicity of acetophenone - including potential neurological effects, and 2.) serve as a screening study for potential reproductive and developmental effects in male and female rats. Upon completion of a range finding study, acetophenone was administered for a minimum of 28 days *via* oral gavage once daily to 4 groups of 10 male and female rats per group at 0, 75, 225 and 750 mg/kg/day for the toxicity phase and once daily for a minimum of 14 days through day 3 of lactation *via* oral gavage to 4 groups of 10 female rats per group at 0, 75, 225, and 750 mg/kg/day for the reproduction/developmental phase. The male rats from the toxicity phase were used

to breed the reproduction phase females. There was no mortality in the toxicity study. Mean forelimb grip strength and motor activity were decreased in the high dose males. Predose and postdose salivation was noted in both the 225 and 750 mg/kg/day groups. Body weights and food consumptions were lower in the high dose group. Hematology and coagulation parameters were unremarkable; however, the cholesterol levels were increased in the high dose animals. The 75 mg/kg/day dose level was determined to be the NOAEL for systemic toxicity while the 225 mg/kg/day dose level was determined to be the NOAEL for neurological effects. There were no parental deaths in the reproduction study; however, the live birth index, pup survival during the lactation phase and mean pup body weights were decreased in the high dose group. Hence, the 225 mg/kg/day dose level was considered a NOAEL for reproductive effects. However, the mating and fertility indices and mean gestation length appeared unaffected at dosage levels up to 750 mg/kg/day.

371 DOSE ADDITIVITY OF ATRAZINE AND BROMODICHLOROMETHANE IN CAUSING PREGNANCY LOSS IN F344 RATS.

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Atrazine (ATRZ), a widely used herbicide, and bromodichloromethane (BDCM), a disinfection by-product found in drinking water, have both been shown to cause pregnancy loss, i.e., full-litter resorption (FLR), in F344 rats. Although chemically quite different, both ATRZ and BDCM have similar modes of action; ATRZ- and BDCM-induced pregnancy loss are associated with reduced levels of luteinizing hormone (LH) and progesterone during the LH-dependent period of gestation. ATRZ and BDCM co-exist in drinking water; thus, we sought to evaluate their cumulative effect on pregnancy maintenance using a dose-additivity model. Each agent was administered alone at near-threshold doses and in combination at one-half of the near-threshold doses. If synergistic, the two sub-threshold doses would combine to cause a greater-than-threshold response. Both agents were administered by gavage (BDCM in 10% alkamuls EL-620, then ATRZ in 1% methylcellulose) on gestation days 6-10. Each agent was administered alone at 40 mg/kg/d (LOEL=50 mg/kg); whereas the two were administered together at 20 mg/kg/d each. Dams were allowed to deliver and litters were examined on postnatal days 1 and 6. Uteri of nonparous females were stained with 10% ammonium sulfide to detect FLR. Alone or in combination, the agents were maternally toxic, causing weight loss after the first dose. As expected, 40 mg/kg of each chemical was a near-threshold dose for causing FLR; low rates (5-6%; 1 affected of 18-19 dams) were seen for each chemical alone. In combination, no FLR was seen (n=19). Thus, in this study, ATRZ and BDCM clearly lacked synergy in their ability to cause pregnancy loss in F344 rats. Although we did not assess the possibility of antagonism, these results are consistent with the default risk-assessment assumption of dose additivity for agents with the same mechanism. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

372 EXPOSURE PARAMETERS FOR DELAYED PUBERTY AND MAMMARY GLAND DEVELOPMENT IN LONG-EVANS RATS EXPOSED *IN UTERO* TO ATRAZINE.

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Prenatal exposure to the herbicide atrazine (ATR) was found to delay vaginal opening (VO) in the offspring of Long-Evans (LE) rats. Our preliminary studies suggested that ATR exposure also delayed mammary development in LE female offspring. To evaluate ATR exposure parameters for pubertal and mammary gland development delays, we asked whether ATR-induced delays were strictly dam-mediated (via milk) or a direct effect (transplacental) on the pups. Timed-pregnant LE rats (N=20/group) were gavaged gestational days 15-19 with 100 mg ATR/kg body weight (BW) or vehicle (controls, C). On PND1, half of all litters were cross-fostered, creating 4 treatment groups; C-C, ATR-C, C-ATR, and ATR-ATR (birth-milk source, respectively). BW was compared on PND4, PND22, day of VO, and PND60. Pup BW was significantly reduced on PND4 for all groups exposed to ATR vs. C-C, but was no longer different among the dose groups at weaning. On PND40 and 60 serum luteinizing and prolactin levels did not vary between groups. Serum thyroid stimulating hormone was slightly elevated in the ATR-ATR group on PND60, but not PND40 ($p<0.05$). A significant delay in VO and increase in VO BW was seen only in the litters receiving milk from ATR-treated dams (MeanVO±SE, N>8 litters/group); C-C 33.3±0.6d, ATR-C 33.8±1.1d, C-ATR 35.1±0.9d, and ATR-ATR 37.0±0.7d. Following developmental scoring, mammary glands of female offspring (2/dam) in groups ATR-C, C-ATR, and ATR-ATR displayed significant delays in epithelial development on PNDs4, 33, and 40. However, at all ages examined, ATR-ATR offspring exhibited the least developed glands. Our data suggests that the delay in VO of *in utero* ATR-exposed offspring is mediated *via* the dam [milk], and does not appear to be influenced by offspring BW or direct exposure to ATR *in utero*. However, delays in mammary gland development appear to be the result of direct and/or suckling exposure parameters. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy; Supported by NHEERL-DESE, USEPA CT826513, and NSF, No. HRD-9978874)

373 EFFECTS OF PERINATAL EXPOSURE OF FIVE PUTATIVE ENDOCRINE DISRUPTING CHEMICALS (EDCS), METHOXYCHLOR, GENISTEIN, DIISONONYLPHTHALATE 4-NONYLPHENOL AND BISPHENOL A, ON ENDOCRINE/REPRODUCTIVE SYSTEMS IN RATS.

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Methoxychlor (MXC, 24, 240, 1200 ppm), genistein (GEN, 20, 200, 1000 ppm), diisononylphthalate (DINP, 400, 4000, 20000 ppm), 4-nonylphenol (NP, 60, 600, 3000 ppm) or bisphenol A (BA, 60, 600, 3000 ppm) were given to maternal rats from gestational day 15 to postnatal day (PND) 10 to assess their perinatal exposure effects on offsprings. Soybean-free diet was used as a basal diet. Organ weights at PND 21, onset of puberty, estrous cyclicity, gonadotrophin-immunopositive index (IPI) in pituitary at PND 21 and 77, and histological changes at PND77 were assessed as well as the size of sexually dimorphic nucleus of preoptic area (SDN-POA). In terms of MXC, DINP and GEN studies, expression of GABA transporter-1 (GAT-1), an estrogen responsive gene, was analyzed in medial preoptic area (MPOA) at PND10 using microdissection and real-time RT-PCR techniques. Females exposed to 1200 ppm MXC showed accelerated onset of puberty, irregular estrous cyclicity, histological changes such as multifollicular ovaries, hyperplasia in endometrium, vaginal mucosa and anterior pituitary at PND77, and decrease in LH-IPI at PND21 and increase in FSH- and PRL-IPIs at PND77. Females of 240 ppm MXC also showed increased PRL-IPI at PND77. Males of 1200 ppm MXC showed delayed onset of puberty and decreased LH-, FSH- and PRL-IPIs at PND21. DINP at 20000 ppm caused very slight degeneration of spermatocytes and Sertoli cells at PND77. The sizes of SDN-POA did not alter at any doses of chemicals examined. GAT-1 levels in male MPOAs decreased with DINP at 20000 ppm, and also showed a dose-related decreasing tendency with MXC. GEN, BA and NP did not affect any endocrine parameter examined. Results suggest that maternal exposure to MXC and DINP affects reproductive system of offsprings by disrupting brain sexual differentiation.

374 THE INDUCTION OF OXIDATIVE STRESS IN VARIOUS BRAIN REGIONS OF RATS FOLLOWING SUBCHRONIC EXPOSURE TO TCDD.

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In this study, TCDD was administered to various groups of female Sprague-Dawley rats, p.o, at doses of 0, 10, 22 and 46 ng/kg/day for 90 day. The animals were then sacrificed and their brains were removed and dissected into various regions including, cerebral cortex (Cc), hippocampus (H), mid brain (MB) and cerebellum (C). Production of superoxide anion (SA) and lipid peroxidation (LP), as well as the activities of various antioxidant enzymes, including superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) were determined in those regions. The results of the studies indicate that TCDD caused dose dependent increases in the production of SA and LP in the H and Cc with no significant changes in these biomarkers observed in the MB and the C. While dose dependent elevations in SOD activities were observed in C and MB, dose dependent suppressions in the activities of this enzyme were observed in the Cc and H. Catalase activities on the other hand underwent dose dependent elevations in the C and were significantly elevated in the H and Cc of rats treated with the lowest dose of TCDD. However, Catalase activities were suppressed in a dose dependent manner in H and Cc of rats treated with doses higher than 10 ng of TCDD/kg/day and were not significantly changed in MB of rats treated with any of the doses of the compound. While patterns similar to those of catalase were observed in GSH-Px activities in C, Cc and H of the treated rats, GSH-Px activities in MB underwent dose-dependent elevations. The results suggest that oxidative tissue damage is significantly induced in the Cc and H but not in the MB and C of brains after exposure to subchronic doses of

TCDD. Also, modulation of activities of various antioxidant enzymes in those regions by various doses of TCDD may contribute to the production of, or protection against tissue damage observed in those regions. (Supported by NIH/NIEHS grant ES11048).

375 FCCP-INDUCED TOXICITY AND OXIDATIVE STRESS IN RHABDOMYOSARCOMA CELLS.

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Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) is a mitochondrial respiratory chain uncoupler which depletes cellular ATP. The objective of this investigation was to assess FCCP potential to induce cytotoxicity, oxidative stress (OS) and OS relevant gene expression. FCCP at concentrations up to 150 μM produced minimal toxicity in rhabdomyosarcoma derived human cell line (RD) (≤ 20% cell death) after 10 h incubation. As expected, FCCP dramatically depleted cellular ATP in RD in a concentration-dependent manner. Flow cytometric analysis indicated FCCP induced apoptosis after 4 h. Biochemical markers of OS were affected by FCCP. Intracellular superoxide production was elevated by FCCP at concentrations ≥ 10 μM after 1 h. FCCP induced a concentration-dependent increase in lipid peroxidation in RD after 1 and 4 h. FCCP (50 μM — 150 μM) markedly decreased cellular reduced glutathione (GSH) and increased oxidized glutathione (GSSG) after 10 h. A panel of OS related genes were examined by RT-PCR in RD after FCCP exposure. Genes examined included: CYP1A1, heme oxygenase–1, DNA topoisomerase 2A, p21, γ-glutamyl-cysteinyl ligase regulatory subunit, thioredoxin reductase and glutathione reductase. FCCP significantly altered expression of these genes. As an example, heme oxygenase–1 expression was induced 30 fold by 150 μM FCCP. Our study suggests that high concentrations of the mitochondrial uncoupler, FCCP, in addition to depleting cellular ATP induces apoptosis and oxidative stress in RD.

376 ROLE OF OXIDATIVE STRESS IN THE MODIFICATION ON RAT UTERINE CONTRACTION BY 2, 2'-DICHLOROBIPHENYL.

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Previously, we showed that polychlorinated biphenyl (PCB) mixtures and several lightly chlorinated ortho-substituted PCB congeners modify contractions of uterine strips *in vitro*. The present study characterizes the *in vitro* effects of 2, 2'-dichlorobiphenyl (2, 2'-DCB) on spontaneous oscillatory uterine contractions and examines the role of oxidative stress in 2, 2'-DCB-induced modification of uterine contraction. Uterine strips from gestation day 10 pregnant rats were suspended in isometric muscle baths and exposed to 30, 60 or 100 mM 2, 2'-DCB. Over 150 min of exposure, 2, 2'-DCB increased the frequency of oscillation, decreased the average peak amplitude per contraction, and decreased the syncronization of contractions as measured by the percent of contractions that returned to baseline (completed contractions). These 2, 2'-DCB-induced changes reversed spontaneously and the time to recovery was directly related to 2, 2'-DCB concentration. The most dramatic effect was an increase of contraction frequency to 1288.5% at 110 min, a decrease in the amplitude to 10.5% at 140 min, and a decrease in the completed contractions to 10.9% at 140 min after 100 mM 2, 2'-DCB exposure. Pre- and co-treatment with the antioxidants 100 mM ascorbic acid or 100 mM glutathione ethyl ester did not alter the uterine response to 2, 2'-DCB. In response to pre- and co-treatment with a-tocopherol, 100 mM a-tocopherol had no effect whereas 200 or 400 mM a-tocopherol delayed the onset of 2, 2'-DCB-induced modification of uterine contraction. When uterine strips were treated with 100 mM a-tocopherol or 50 mM deferoxamine mesylate after a 1-h exposure to 100 mM 2, 2'-DCB, the 2, 2'-DCB-induced modifications were reversed within 1 h. In contrast, treatment with 100 mM ascorbic acid or 100 mM glutathione ethyl ester after a 1-h exposure to 100 mM 2, 2'-DCB did not alter the response to 2, 2'-DCB. This study suggests that 2, 2'-DCB modifies uterine contraction by a mechanism that is at least partially dependent on oxidative stress.

377 NITROTYROSINE AND SPLENIC TOXICITY OF ANILINE.

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Splenic toxicity of aniline is characterized by vascular congestion, hyperplasia, fibrosis and development of a variety of sarcomas in rats. However, the mechanisms of this selective splenic toxicity are not well understood. Previously we observed that aniline exposure causes oxidative damage to spleen. To further explore the oxidative mechanism of aniline toxicity, we evaluated the role of nitric oxide. Nitric oxide reacts with superoxide anion to form peroxynitrite, a powerful oxidant which converts the tyrosine residues of proteins to nitrotyrosine (NT). Therefore, aim of this study was to establish the role of nitric oxide through the formation and localization of NT in the spleen of rats exposed to aniline. Male SD rats were given 1 mmol/kg/day aniline hydrochloride in water by gavage for 7 days, while the controls received water only. Immunohistochemical analysis for NT showed an intense staining in the red pulp areas of spleen from aniline-treated rats, localized in macrophages and sinusoidal cells. Occasionally mild NT immunostaining was also evident in the white pulp areas. To further characterize the nitrated proteins, Western blot analyses of the post-nuclear fraction of the spleens showed major nitrated proteins with molecular weights of 18 kD, 30 kD and 49 kD. Immunohistochemical analysis of inducible nitric oxide synthase (iNOS) also showed increased expression in the red pulp of the spleens from aniline-treated rats; the cellular localization was similar to nitrated proteins. These studies suggest that oxidative stress in aniline toxicity also includes aberration in nitric oxide production leading to nitration of proteins. Functional consequences of such nitration will further elucidate the contribution of nitric oxide to the splenic toxicity of aniline.

378 NITRIC OXIDE MEDIATES INCREASED SUSCEPTIBILITY TO DOPAMINERGIC DAMAGE IN NURR1 DEFICIENT MICE.

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Mutation in Nurr1 gene, a member of the nuclear receptor superfamily, causes selective agenesis of dopaminergic neurons in the midbrain. Reduced expression of Nurr1 increases the vulnerability of mesencephalic dopamine neurons to dopaminergic toxins. We evaluated the role of nitric oxide as a possible mechanism for this increased susceptibility. Increased expression of neuronal nitric oxide synthase and increased 3-nitrotyrosine were observed in the striatum of Nurr1 deficient mice as compared to the wild type. Increased cytochrome C activation and the consecutive release of Smac/DIABLO were also observed in Nurr1 deficient mice. Furthermore, an induction of active Caspase-3 and p53, cleavage of poly-ADP(ribonuclease) polymerase and reduced expression of bcl-2 were observed in Nurr1 deficient mice. Methamphetamine significantly potentiated the increase of these markers in Nurr1 deficient mice as compared to the wild type. The present data, therefore, suggest that nitric oxide plays a role as a modulating factor for the increased susceptibility of the dopaminergic system in Nurr1 deficient mice. We also report that this increased neuronal nitric oxide synthase expression and increased nitration in Nurr1 deficient mice led to the activation of apoptotic cascade *via* differential alterations in the DNA-binding activity of transcription factors responsible for the propagation of growth arrest as well as apoptosis.

379 COCAINE INDUCES A DOSE DEPENDENT ALTERATION IN GENE EXPRESSION OF APOPTOTIC CASCADE IN PC12 CELLS.

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Cocaine is a widely used drug of abuse and psychostimulant which acts on the central nervous system by blocking the dopamine re-uptake sites. PC12 cells, a rat pheochromocytoma clonal line, which in the presence of nerve growth factor (NGF), multiply and differentiate into competent neurons that can synthesize, store and secrete the neurotransmitter dopamine (DA). In the present study, we evaluated the effect of increasing doses of cocaine on the expression of immediate early genes (IEGs), c-fos and SP-1 and a closely related nuclear factor, NF- 24 h after exposure to cocaine (50, 100, 250, 500, 1000, 2500 M) in NGF-differentiated PC12 cells. Cocaine (50-500 M) resulted in significant induction of the expression of c-fos, SP-1 and NF-. However, higher concentrations of cocaine (1000 & 2500 M) resulted in the down-regulation of these expressions after 24 h. To further understand the role of dose-dependent changes in the mechanisms of cell death, we evaluated the protein expression of apoptotic markers. A dose-dependent increase in the expression of caspase 9 and 3 was observed upto 500 uM of cocaine. However, the higher dose did not show any expression. We also evaluated the effect of increasing doses of cocaine on dopamine concentration and the expression of dopamine transporter. A significant dose-dependent decrease in the concentration of dopamine as well as the expression of dopamine transporter was observed 24 h after the exposure of PC12 cells to cocaine. Therefore in the present study, we report that cocaine has both upstream and downstream regulatory actions on some IEGs and nuclear factors, which can regulate the mechanism of cell death and these effects on gene expression, are independent of its action on the dopaminergic system.

380 REPERFUSION DERIVED OXYGEN RADICAL DAMAGE IN ATLANTIC MENHADEN, *BREVORTIA TYRANNUS.*

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Atlantic Menhaden, *Brevortia tyrannus*, serve a role as primary consumer in the estuaries of the mid-Eastern Atlantic coast in the US. These fish serve as a basic component of food webs for game fish as well as an economic resource (as a source of polyunsaturated oils). Hundreds of thousands of these fish die in kills unassociated with any specific etiology in the New, Neuse, and Tar-Pamlico River systems on the coast of North Carolina each year. Epizootic ulcerative syndrome (EUS), frequently associated with these kills (5-95‰), is seen during seasonal outbreaks, but has a complex pathogenesis and uncertain primary etiology. Due to the fact that menhaden are often trapped in estuarine hypoxic zones, this experiment was designed to test the hypothesis that reperfusion derived oxyradicals, driven by hypoxia, causes skin and/or muscle injury which may lead to EUS. A system has been designed which sparges oxygen from tank water *via* a foam fractionator and nitrogen source. Hypoxic treatments (1.4 +/- 0.091 mg/L) for 48 hours were followed by rapid resumption of normoxic conditions. Sampling occurred at 0, 24, 48, and 60 hours. Lipid peroxidation, 8-hydroxy-2'-deoxyguanisine formation, low molecular weight antioxidant status, total oxyradical scavenging capacity (TOSC), and histology were selected as endpoints to assess oxygen radical damage. Histology and lipid peroxidation showed no significant increases in cellular damage beyond background post-treatment. These data suggest that Atlantic Menhaden are highly resistant to reperfusion derived oxyradical damage.

381 DFP-INDUCED OXIDATIVE STRESS IN SKELETAL MUSCLES OF RATS.

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This study was undertaken to determine the role of oxidative stress as determined by effects on energy metabolism and lipid peroxidation in the skeletal muscles of rats acutely intoxicated with diisopropylphosphorofluoridate (DFP, 1.5 mg/kg, sc). F2-isoprostanes (F2-IsoPs), specific markers of reactive oxygen species (ROS)-induced lipid peroxidation, and citrulline (determinant of nitric oxide, NO and nitric oxide synthase, NOS), markers of reactive nitrogen species (RNS), were determined in skeletal muscle. Control values for F2-IsoPs varied from 0.963 to 1.270 ng/g, for citrulline 331.2 to 451.2 nmol/g, for ATP 3.42 to 4.91 mmol/g, and for phosphocreatine (PCr) 6.18 to 9.63 mmol/g. Treated rats developed onset of toxicity signs (tremors) within 5-10 min, which progressed to maximal severity (convulsions and fasciculations) within 60 min. At this time point, acetylcholinesterase activity was reduced by 90-96%, Severe fasciculations induced by DFP caused significant increases in F2-IsoPs (156%), increases in citrulline (272-288%), and declines in high-energy phosphates (ATP and PCr) (30-43%). Findings revealed that the increase in NO and NOS was greater than the decrease of ATP and PCr. The data suggest that the DFP-induced muscle hyperactivity produces oxidative stress by excess generation of ROS and RNS, causing depletion of energy metabolites and increased lipid peroxidation, leading to muscle fiber damage/death (Supported by NIH grant ES04597-W-D Dettbarn and KSTC 2002-RCG).

382 LOW DOSE OXIDATIVE INSULT PROMOTES APOPTOSIS IN DOPAMINERGIC CELLS VIA CASPASE-3 DEPENDENT PROTEOLYTIC ACTIVATION OF PKCδ: RELEVANCE TO ENVIRONMENTAL FACTORS AND PARKINSON'S DISEASE.

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Oxidative stress has been recognized as an important cell death mechanism in many degenerative conditions including Parkinson's disease. Recently, we demonstrated that dopaminergic toxins, including 1-methyl-4-phenylpyridinium (MPP^+) (Kaul *et al.*, 2001), dieldrin (Kitazawa *et al.*, 2001), and MMT (Anantharam *et al.*, 2002) initiate oxidative stress and subsequently promote caspase-3 dependent proteolytic activation of pro-apoptotic protein kinase Cδ in mesencephalic dopaminergic neuronal (N27) cells. This study was designed to examine the effects of a low dose oxidative insult on apoptotic signaling in N27 dopaminergic neuronal cells. Significant cell death was observed in dopaminergic cells within 4 hrs of exposure to 100 μM H_2O_2, as demonstrated by a live-dead cell fluorescence assay. Caspase-3 activity increased two-fold within 30 min of H_2O_2 exposure and continued to increase time-dependently over the next 4 hrs. H_2O_2 induced a dose-dependent proteolytic cleavage of PKCδ (72-74 kDa) into a 41-kDa catalytic and a 38-kDa regulatory subunit; the cleavage was completely attenuated by caspase-3 inhibitor Z-DEVD-FMK (50 μM), indicating that it was mediated by caspase-3. Quantification of DNA fragmentation by ELISA revealed that low dose H_2O_2 also induces cellular apoptosis in a time dependent manner over a 4 hr period. Furthermore, pretreatment with MnTBAP (a SOD mimetic-5 μM), rottlerin (a PKCδ specific inhibitor-2 μM) and Z-DEVD-FMK (caspase-3 specific inhibitor-50 μM) significantly attenuated H_2O_2-induced apoptosis in dopaminergic cells. Together, we demonstrate for the first time the rapid induction of an apoptotic pathway involving caspase-3 mediated PKCδ proteolytic cleavage in dopaminergic cells by low dose oxidative insult.*(Supported by NIH grant ES 10586)*

383 OXIDATION OF 4-HYDROXY-2-NONENAL BY SUCCINIC SEMIALDEHYDE DEHYDROGENASE (ALDH5A).

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4-Hydroxy-trans-2-nonenal (HNE) is a cytotoxic aldehyde product of lipid peroxidation that is implicated in the progression of several neurodegenerative disorders such as Alzheimer disease and Parkinson disease. Previous studies have implicated the mitochondrial detoxification of aldehydes in the development of AD. Numerous studies have demonstrated the oxidation of HNE in liver and kidney mitochondria but have not identified the aldehyde dehydrogenases (ALDH) responsible for this oxidation. Two potential ALDH enzymes, ALDH2 and succinic semialdehyde dehydrogenase (ALDH5A) are found in mitochondria. In this study, we examined the oxidation of HNE to 4-hydroxy-2-nonenoic acid (HNEAcid) in isolated rat brain mitochondria in order to determine if HNEAcid formation occurs and the enzymes responsible. HNEAcid was generated from HNE in lysed mitochondria with an apparent Km of 20 μM HNE and a Vmax of 4 nmol/min/mg protein. Dose response curves were then established to compare HNEAcid formation, ALDH2 activity, and SSADH activity using the fungicide, benomyl, a potent ALDH2 inhibitor. Benomyl (3 μM) completely inhibited ALDH2 activity. However, HNEAcid formation and SSADH activity were inhibited only 30%, suggesting that SSADH may be responsible for oxidizing HNE. To test this hypothesis, recombinant rat SSADH (rrSSADH) was expressed as a poly-histidine tagged fusion protein in E.coli. Following purification by nickle-agarose chromatography, a protein of approximately 55 kDa with a Km of 3.3 μM for succinic semialdehyde with a Vmax of 20 μmol/min/mg was obtained, similar to native enzyme. rrSSADH oxidized HNE with a Vmax of 1.2 μmol/min/mg and a Km of 50 μM. Other alkenals such as 2-nonenal, 2-hexenal, and 4-hydroxyhexenal had lower rates of oxidation. These data demonstrate that the oxidation of HNE in rat brain mitochondria, in part, is due to SSADH activity.

384 MOTOR NEURONS FAIL TO UPREGULATE METALLOTHIONEIN DURING OXIDATIVE STRESS: A SOURCE OF VULNERABILITY TO DISEASE?

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Amyotrophic lateral sclerosis (ALS) is a disease characterized by selective degeneration of motor neurons leading to paralysis and death. Excitotoxicity and oxidative stress contribute to the preferential vulnerability of motor neurons relative to spared neuronal populations. Approximately 1-2% of ALS cases can be attributed to dominantly inherited mutations in the gene encoding Cu/Zn-superoxide dismutase (SOD1). Although disease does not result from loss of dismutase activity, but from a toxic gain of function, evidence from transgenic mouse and human studies indicates oxidative stress is a contributing factor. Metallothioneins are a group of non-enzymatic, low molecular weight, metal-binding proteins that are cytoprotective through sequestration of reactive oxygen species and metal ions. Transgenic mice expressing a disease-associated mutant form of human SOD1 on a MT-I/II null background died significantly earlier than those expressing MT at normal levels (Nagano et al. 2001), suggesting a protective role for MT in motor neuron diseases. We have examined the constitutive expression of MT isoforms MT-I/MT-II and MT-III in motor neurons of dissociated murine spinal cord cultures and induction by oxidative stress, excitotoxicity or expression of mutant SOD1. MT-I and MT-II were not detected in motor neurons by immunocytochemistry (DAKO:E9), either under basal conditions or following treatment with paraquat (LD50 = 10mM for 48 hrs), glutamate (LD50 = 20mM for 48 hrs) or zinc chloride (LD50 = 30mM for 48 hrs). MT-I/II expression also remained below detection in motor neurons microinjected with G93A mutant human SOD 1 expression vector. However, MT was expressed in glial cells and increased levels were measured by Western blot following zinc chloride treatment. MT-III was expressed in motor neurons, but also failed to upregulate with treatment. It is concluded that the failure to upregulate MT isoforms during oxidative stress, coupled with low levels of reduced glutathione, may contribute to the vulnerability of motor neurons in disease. supported by CIHR.

385 THE USE OF PROTEOMIC ANALYSIS TO ASSESS OXIDATIVE INSULT, AND PROTECTION BY DEHYDROEPIANDROSTERONE, USING CELL MODEL SYSTEMS.

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Oxidative stress has been implicated in a number of chronic disease states including cardiovascular disease, rheumatoid arthritis, vascular dementia, and Alzheimer's disease. Uptake of oxidised low density lipoprotein (LDL) by monocytes leads to the formation of foam cells, which initiates the formation of atherosclerotic plaques and vascular dementia. Oxidative insult upon neuronal cells has also been linked to plaque formation in Alzheimer's disease. Dehydroepiandrosterone (DHEA) is the most abundant steroid in human circulation, with levels naturally decreasing with age to less than 20% of those found in young adults. It has been shown to protect against free radical insult in the brain and can protect against LDL oxidation. In order to increase knowledge of the effects of oxidative insult on cellular adaptation and survival, and to evaluate the effects of DHEA in this process, the protective properties of DHEA against oxidative insult were assessed in two cell model systems. Human neuroblastoma SH-SY5Y were exposed to hydrogen peroxide (50, 100μM) after pre-treatment with DHEA (3, 10, 30μM). DHEA reduced oxidative insult and returned cell viability to control levels. Protein expression, assessed by colloidal gold staining on 2D-electroblotted samples, was seen to increase after insult. This was seen to decrease upon DHEA pre-treatment. LDL, prepared from human plasma, was exposed to oxidative insult (2, 2'-azobis(amidinopropane)dihydrochloride [AAPH] 50mM) in the presence or absence of DHEA (3μM). Uptake by U937 monocytes of oxidised LDL was increased significantly compared to uptake of native LDL, but was reduced to control levels by co-incubation with DHEA. Following uptake of oxidised LDL U937 moncytes showed an increase in protein expression, both quantitatively and qualitatively, which returned to control levels when DHEA was used as a protective agent. Proteomic analysis demonstrates the complexity of the adaptive response to oxidative stress and the nature of protective effects of DHEA.

386 DETECTION OF CARBONYLATION AND 3-NITROTYROSINE AS PROTEIN OXIDATION BIOMARKERS.

M. Grant and H. Griffiths. *Life and Health Sciences, Aston University, Birmingham, West Midlands, United Kingdom.* Sponsor: E. Lock.

Reactive oxygen species (ROS) lead to protein oxidation, where ROS can arise from endogenous processes, e.g. glycoxidation and lipid peroxidation, or from xenobiotic metabolism or radiation. Furthermore, in the absence of ROS specific scavenging, nitric oxide can combine with the superoxide anion to form the potent reactive nitrogen species (RNS), peroxynitrite, which can further cause post-synthetic protein modification through nitration. The resultant formation of carbonyl groups and 3-nitrotyrosine on proteins represent stable changes that can be utilized as biomarkers of oxidative stress. The antioxidant hypothesis suggests that dietary supplementation with antioxidants may decrease disease progression. We have reported previously, that dietary vitamin C protects against immunoglobulin oxidation, over 10 weeks *in vivo* ($P<0.01$). However, the nature of protection afforded by dietary vitamin C against other plasma proteins has not previously been investigated. Therefore, the purpose of this work was to assess the use of 2D electrophoresis and Western blotting as a sensitive and reproducible method for the investigation of protein oxidation and nitration. Carbonyls were detected following derivatisation on the electroblot using 2, 4-dinitrophenylhydrazine. Analysis of plasma from subjects receiving vitamin C (400mg/day for 10 weeks) showed a differential pattern of nitration and carbonylation pre- and post-supplementation. Immunoglobulins showed significant oxidation that can be ameliorated by vitamin C supplementation and intensity of immunoglobulin staining for carbonyl groups correlated with ELISA determination of immunoglobulin carbonyl groups. Concurrent cellular experiments, in the neuroblastoma SH-SY5Y cell line, using vitamin C (100μM) to prevent oxidant (100μM H_2O_2) induced apoptosis, have also been examined for nitrotyrosine and protein carbonyl groups. Colloidial gold staining of Western blots showed differences in protein expression when insulted cells were protected with vitamin C.

387 METABOLISM OF 4-OXONONENAL BY HUMAN CLASS 2 ALDEHYDE DEHYDROGENASE.

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Class 2 aldehyde dehydrogenase (ALDH2) catalyzes the oxidation of the lipid peroxidation product 4-hydroxynonenal (4-HNE) to 4-hydroxynonenoic acid, resulting in inactivation of 4-HNE as an electrophile (i.e. Michael acceptor). Structurally analogous to 4-HNE is 4-oxononenal (4-ONE), a recently discovered product of lipid peroxidation, which is more reactive than 4-HNE towards protein nucleophiles. The goal of this work was to determine whether 4-ONE is a substrate or inhibitor of human ALDH2 (hALDH2). Kinetic analyses were performed using hALDH incubated with NAD and various concentrations of 4-ONE. At low concentrations of 4-ONE (≤ 10 mM), hALDH2 catalyzed the oxidation of 4-ONE to 4-oxononenoic acid (4-ONA) with a yield of 20 mol 4-ONA produced per mol of enzyme. However, subsequent analysis of hALDH2 activity using propionaldehyde as a substrate revealed that both 4-ONE and the oxidation product, 4-ONA, inhibited hALDH2 activity. The reactivity of 4-ONA toward glutathione was measured and found to be comparable to that of 4-HNE, indicating that the 4-ONA oxidation product is a reactive electrophile. Matrix-assisted laser desportion/ionization time of flight mass spectrometery analysis of tryptic peptides revealed covalent modification of hALDH2 by 4-ONE and 4-ONA that was NAD-dependent. These data indicate that hALDH2 catalyzes the oxidation of 4-ONE to 4-ONA; however, 4-ONA is also a reactive electrophile that like 4-ONE is capable of covalently modifying and inhibiting hALDH2. (Supported by grants NIH/NIAAA R01AA09300 and NIH/NIEHS R01ES09410 (DRP); and PHS 5T32AA07464 and NIH/NIEHS F32ES011937 (JAD)).

388 PROTEIN AND PEPTIDE CROSS-LINKING BY 4-OXONONENAL.

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Previous work demonstrated that the lipid peroxidation product 4-oxononenal (4ONE) covalently modifies peptides and proteins. Reaction of proteins/peptides with 4ONE *via* Michael addition results in an adduct with two carbonyls that can react with amines to yield protein cross-links. The purpose of this study was to determine whether 4ONE cross?links proteins and to identify residues involved in cross-linking. Ovalbumin (OVA) was reacted with various concentrations of 4ONE ranging from 10 μM to 1 mM under reducing and non-reducing conditions. The products were analyzed using SDS-PAGE. In a separate experiment, OVA was treated with concentrations of the 4ONE-glutathione conjugate ranging from 20 μM to 1 mM under reducing conditions and analyzed *via* SDS-PAGE. Combinations of peptides containing one or more nucleophilic residues (i.e. Arg, Cys, His, and Lys) were reacted with 4ONE under reducing and non-reducing conditions, and the products analyzed by mass spectrometry. Cross-linking of OVA was apparent for protein treated with ≥50 μM 4ONE under non?reducing conditions. Protein cross?linking was observed for OVA modified by ≥10 μM 4ONE when treated with a reducing agent. Furthermore, cross-linking of OVA occurred for protein treated with ≥100 μM of the 4ONE-glutathione conjugate. Reaction of 4ONE with peptides resulted in new products with masses corresponding to a peptide dimer plus one molecule of 4ONE. The peptide dimers consisted of one peptide with a Cys residue cross?linked to a peptide with a Lys residue. These data indicate that low concentrations of 4ONE (i.e. 10 μM) can induce protein cross-linking and that the reaction occurs *via* Michael addition of 4ONE with Cys followed by reaction of the adduct with a primary amine (i.e. Lys), yielding a Schiff base. Furthermore, results of this study indicate that glutathione conjugation of 4ONE does not result in an inactive product. (Supported by grants NIH/NIAAA R01AA09300 and NIH/NIEHS R01ES09410 (DRP); and PHS 5T32AA07464 and NIH/NIEHS F32ES011937 (JAD)).

389 REGULATION OF C-FOS PHOSPHORYLATION AND AP-1 ACTIVATION BY OXIDANTS IN CARDIOMYOCYTES.

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Cardiomyocytes develop hypertrophy during the process of heart failure. We have found that oxidants cause cardiomyocytes to enlarge within 4-5 days. Within 30 minutes of oxidant exposure, a number of signaling molecules are activated including phosphoinositide 3-kinase (PI3K), p70 S6 kinase and three branches of MAPKs (ERKs, p38 and JNKs). Oxidative stress also results in AP-1 transcription factor activation after 2 hrs in cardiomyocytes. A known component of AP-1 that responds to oxidative stress is c-fos protein. Measurement of c-fos protein by Western blots showed an increase in the protein level at 1-2 hrs after 100 mM H2O2 treatment. Interestingly, c-fos protein showed posttranslational modification as shown by slower migrating bands on Western blots. Using the type 2 serine/threonine phosphatase (PP2A), we are able to shift the intensity of the upper band to the lower band of c-fos in the nuclear extract, suggesting that c-fos is phosphorylated in cardiomyocytes by H2O2 treatment. The cytosolic extracts show 3 bands of c-fos protein which were abolished following incubation with PP2A. With immunoprecipitated c-fos protein from the cytosol, dephosphorylation with PP2A did not cause a loss of the c-fos protein, suggesting the possibility that phosphorylation of c-fos

protein protects it from being degraded. Using pharmacological inhibitors, we found that JNKs but not PI3K, p70S6K, ERKs or p38 are likely the upstream regulators of c-fos phosphorylation. In conclusion, we show a novel mechanism of AP-1 regulation by c-fos phosphorylation.

390 GENERATION OF OXYGEN FREE RADICALS BY PEROXISOMICINE A1.

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Dimeric anthracenones have been isolated from toxic plants of the genus Karwinskia. T-514 (Peroxisomicine A1) is one of these anthracenonic compounds which has been demonstrated that exhibit selective toxicity *in vitro* on tumor cells. At present, Peroxisomicine A1 is being assessed like anticancer drug in patients. A redox cycling mechanism has been suggested for anticancer activity and cytotoxicity of a number of drugs used in tumor therapy. Some of these drugs (anthracyclines) are structurally related to Peroxisomicine A1. In general these compounds induced oxygen free radicals formation. Activation of these compounds to reactive intermediates provided a rational explanation for their cytotoxic properties. The present study was undertaken to assess the production of oxygen free radicals by Peroxisomicine A1 *in vitro*. In addition the oxygen free radicals inhibition by antioxidant enzymes was also evaluated. The results showed that Peroxisomicine A1 readily form oxygen free radicals which were inhibited in presence of Superoxide dismutase and Catalase.

391 ROS-INDUCED HISTONE H3 PHOSPHORYLATION DOES NOT INVOLVE SITES NORMALLY ASSOCIATED WITH MITOTIC CHROMOSOMAL CONDENSATION.

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Treatment of renal proximal tubular epithelial (LLC-PK1) cells with the reactive oxygen species (ROS)-generating toxicant, 2, 3, 5-*tris*-(glutathion-S-yl)hydroquinone (TGHQ) causes extensive DNA damage and increases the phosphorylation of histone H3. This increase is concurrent with premature chromatin condensation, which may contribute to cell death. Phosphorylation of histone H3 at serines 10 and 28 contributes to the appropriate condensation of chromosomes during mitosis. These same residues can be phosphorylated by mitogen- and stress-activated kinase (MSK1) in response to treatments with UV-B and 12-*O*-tetradecanoylphorbol-13-acetate. To identify the role of MSK1 in the cellular events following TGHQ treatment, LLC-PK1 cells were transfected with wild-type or dominant-negative MSK1. [^{32}P] ortho-phosphoric acid labeled cells expressing wild-type or dominant-negative MSK1 showed no change in histone H3 phosphorylation after TGHQ treatment compared with empty-vector transfected cells. Accordingly, neutral red analysis showed no change in cytotoxicity between wild-type MSK1, dominant-negative MSK1, and empty-vector transfected cells. To determine if S10/S28 were involved in the increase in histone H3 phosphorylation after TGHQ treatment, Western analysis was performed using commercial antibodies for histone H3 phosphorylated at S10 or for histone H3 phosphorylated at S28. When LLC-PK1 cells were treated with TGHQ, both antibodies showed a decrease in the phosphorylation of histone H3 at S10/S28 compared to untreated cells. We conclude that the increase in histone H3 phosphorylation observed in cells undergoing ROS-induced cell death is not due to phosphorylation of S10 or S28, but perhaps a novel site responsive to ROS-induced DNA damage. (ES07784, ES07247, DK59491)

392 BIOLUMINESENT MEASUREMENT OF OXIDATIVE STRESS.

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Transgenic mice expressing the luciferase gene under the control of the Heme oxygenase (HO-1) promoter (HO-1-luc) were used to measure oxidative stress. HO-1, also known as heat shock protein HSP32, is exquisitely sensitive to a very diverse set of stimuli and agents that cause oxidative stress and cellular toxicity. We previously reported HO-1-luc activity measured *in vivo* by the Xenogen IVISTM, low light imaging system, in response to compounds known to produce oxidative stress. Here we examined correlations between HO-1-luc expression, HO-1 protein levels and chemical toxicity. Effects of cadmium chloride (CdCl2)(3.7 mg/kg), doxorubicin (15 mg/kg) and thioacetimide (300 mg/kg) were studied from 6-48 hours in male and female mice. The greatest increase in HO-1-luc was observed in response to CdCl2 and lower responses were observed for doxorubicin and thioacetimide. To assess other markers of toxicity, selected tissues were harvested and examined. Histopathological changes indicated mild to severe tissue damage in response to all treatments. HSP70 protein levels, catalase activity and circulating enzyme levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) were measured and found to reflect expected toxicity profiles. Western blot analysis demonstrated that the increase in endogenous HO-1 protein levels agreed temporally with HO-1-luc activity. These results suggest that the HO-1-luc transgenic mouse can be used as a model for *in vivo* screening of new and unknown compounds for their potential toxic effects.

393 CIGARETTE SMOKE AND ARSENIC SYNERGISTICALLY INCREASE DNA OXIDATION IN THE LUNG.

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Epidemiological evidence has indicated that cigarette smoking and arsenic exposure act synergistically to increase the incidence of lung cancer. Oxidative damage of DNA has been linked to cancer. Our hypothesis is that aerosolized arsenic and cigarette smoke work synergistically to decrease both reduced and total glutathione and increase DNA oxidation in the lung. To test this hypothesis male Syrian golden hamsters were exposed to room air (control), aerosolized arsenic trioxide (3 mg/m^3 for 30 minutes), cigarette smoke (5 mg/m^3 for 30 minutes), or both smoke and arsenic. Exposures were for 5 days/week for 5 and 28 days. Animals were sacrificed one day after the last exposure. In the 28 day group, glutathione was determined from the lungs of animals exposed to arsenic and/or cigarette smoke using HPLC. Results are reported as nmoles/mg lung tissue. In this same group, DNA oxidation (8-oxo-2'-deoxyguanosine (8-oxo-dG)) was quantified from genomic DNA extracted from lungs of animals exposed to arsenic and/or cigarette smoke using HPLC. In the 5 day group, the lungs were processed for immunohistochemistry. An antibody against 8-oxo-dG was used to analyze DNA oxidation in the lung using confocal microscopy. Our results show that in the 28 day group there was a significant increase in DNA oxidation, and a significant decrease in both the reduced and total glutathione levels in the combined arsenic/smoke group when compared with arsenic or smoke alone. In the 5 day group, while glutathione levels were decreased in the combined exposure group, total lung 8-oxo-dG was not significantly increased. However, in the five day exposures, we found that the combined arsenic/smoke-exposure group showed an increase in nuclear staining of the 8-oxo-dG over that of controls. This effect was seen mostly in the small airways. (Supported in part by NIEHS Grant P30 ES06694).

394 FEMALE MICE TRANSGENIC FOR MITOCHONDRIAL-DIRECTED CATALASE HAVE ALTERED GLUTATHIONE REDOX CYCLE ENZYME ACTIVITIES COINCIDENT WITH TISSUE SPECIFIC CATALASE EXPRESSION.

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Oxidative stress is thought to contribute to many diseases associated with aging including cancer, atherosclerosis, diabetes mellitus, and chronic neurological disorders such as Parkinson's and Alzheimer's diseases. In lower organisms, it has been shown that overexpression of antioxidant enzymes leads to increased scavenging of reactive oxygen species and a prolongation of life. We wished to determine if this also applied to mammals. Accordingly, we have created transgenic mice which overexpress human catalase in mitochondria (mCAT mice), with exceptionally strong overexpression in heart and skeletal muscles. Two independently derived mCAT founder lines were found to have increased expression of catalase in cardiac and skeletal muscle, and cells cultured from these animals displayed increased resistance to oxidative insult. We wished to determine what effect overexpression of catalase in mitochondria has on the activity of another system used by cells for scavenging hydrogen peroxide, namely, enzymes of the glutathione redox cycle. Brains, spleens, liver, heart, skeletal muscle, kidney and lungs from male and female mCAT transgenic mice and non-transgenic litter mates of different ages were assayed for glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase activities. GPx and GRx enzyme activities were increased in skeletal muscle of female mCAT mice, but no significant differences from controls were noted in other organs examined from both sexes. In addition, no significant age-related changes in activity were noted in the activity of any of these enzymes. These changes suggest co-regulation of these two hydrogen peroxide scavenging systems. Supported by NIH grants P42ES04696, P30AG01571, and P30ES07033.

395 LIPID PEROXIDATION IN FATHEAD MINNOW GILLS.

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This study was carried out to evaluate whether in vitro exposure of isolated whole gills to the heavy metal lead (Pb) results in lipid peroxidation injury. Isoprostanes (iPs) were used as a marker in this study; viz., immunoreactive 8-iso-PGF2α (iPF2α-III). Isolated gills of *Pimephales promelas* (fathead minnow) were incubated in 10 ml of oxygenated modified Cortland's saline medium. Two experimental designs were used to determine the time course of the release of iPs from control gills (no Pb present): 1) sampling small aliquots (200 µl) of medium at 20 min intervals without medium replacement, and 2) complete replacement of the medium at successive 20 min intervals by rapidly decanting and adding fresh oxygenated Cortland's saline. These two experimental designs yielded dramatically different results. For example, when small aliquots were removed at 20 min intervals, virtually all of the iP release occurred during the initial 20 min, whereas when all of the medium was replaced every 20 min, the cumulative release of immunoreactive 8-iso-PGF2α increased almost linearly over the 100 min observation period. The ability of Pb to cause oxidative stress was examined in similar experiments wherein medium was collected at only one time point (after 60 min of incubation). Gills incubated with Pb(NO3)2; either 12, 60 or 120 µM, for 60 min exhibited a dose-dependent reduction in the ratio of reduced to oxidized glutathione (GSH/GSSG). At the highest levels of iP release, tissue levels of total glutathione (GSH + GSSG) tended to correlate negatively with iP release. The results indicate that exposure of isolated whole gills to Pb results in direct oxidative damage to gill membranes. In addition, we conclude that teleost gills are exquisitely sensitive to lipid peroxidation injury as determined by measurement of iP release. SUPPORTED BY NIEHS GRANT 1R15ES09434 01A1

396 FREE RADICAL FORMATION IN THE RAT KIDNEY INDUCED BY CYCLOSPORIN A: PREVENTION BY DIETARY GLYCINE, RENAL DENERVATION, AND GREEN TEA POLYPHENOLS.

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BACKGROUND: Cyclosporin A (CsA) causes vasoconstriction and nephrotoxicity. Here, we investigated the role of hypoxia and free radical production in renal injury caused by CsA. **METHODS:** Rats were treated daily with CsA (25 mg/kg, i.g.) for 5-21 days. Pimonidazole, a hypoxia marker, was injected 2 h after CsA. POBN (α-(4-pyridyl 1-oxide)-*N*-tert-butylnitrone) was injected 3 h after CsA to trap free radicals. **RESULTS:** CsA doubled serum creatinine and decreased glomerular filtration rates (GFR) by 65%. Pimonidazole adduct binding in the kidney was increased 3-fold by CsA, providing physical evidence for tissue hypoxia. Dietary supplementation of glycine (5%), an inhibitory neurotransmitter, blunted hypoxia caused by CsA. Moreover, CsA increased POBN/radical adducts 4-fold in the urine but did not alter levels in the serum. Administration of CsA with ^{12}C-DMSO produced two radical species in urine, one with hyperfine coupling constants similar to the 4-POBN/methyl radical adduct, a product of hydroxyl radical attack. CsA given with ^{13}C-DMSO produced a 12-line spectrum, confirming the formation of hydroxyl radicals. Increases in free radical production and decreases in GFR were attenuated by glycine, renal denervation and dietary green tea polyphenols (0.1%) which are potent free radical scavengers. CsA infusion for 30 min increased efferent renal nerve activity 2-fold. Dietary glycine totally blocked this phenomenon. **CONCLUSION:** Taken together, these data support the hypothesis that CsA causes renal injury, at least in part, by increasing renal nerve activity resulting in hypoxia-reoxygenation. Moreover, CsA nephrotoxicity can be prevented effectively by dietary glycine which causes vasodilatation thus minimizing hypoxia/reperfuion and by free radical scavenging green tea polyphenols.

397 EFFECTS OF ORAL EXPOSURE TO METHYL *TERTIARY*-BUTYL ETHER ON REACTIVE OXYGEN SPECIES IN SPECIFIC RAT BRAIN REGIONS.

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Methyl tertiary-butyl ether (MTBE) is added to gasoline to boost octane and enhance combustion, thereby reducing carbon monoxide and hydrocarbon emissions. However, it has become a contaminant of surface water and groundwater due to its high solubility in water. MTBE is neurotoxic and has been shown to affect motor activity in laboratory animals. The mechanism by which MTBE exerts this toxicity is still unclear. Oxidative stress has been shown to play a major role in the neuronal cell death associated with neurodegenerative diseases such as Parkinsons disease, which is clinically manifested as motor impairment. The present study was conducted to evaluate the effects of sub-acute oral exposure to MTBE on the levels of Reactive Oxygen Species (ROS) and the activity of Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione Reductase (GR) as well as Glutathione (GSH) levels. Male Sprague-Dawley rats (n=8/group) were used in this study and were orally exposed to MTBE in drinking water (0, 150, 300, 900, 1200, 2400, and 3600 ppm for 14 days). At the end of the 14 days, animals were sacrificed by decapitation, their brains removed and the levels of ROS and antioxidant activities were measured in the prefrontal cortex and hippocampus. There were no significant differences among groups in body weight gain and absolute brain weights MTBE exposure resulted in a significant ($p < 0.01$) increase of ROS in the cortex at all dose levels except the 150 ppm, but caused an increase of ROS levels in the hippocampus only at the 1, 200 and 3, 600 ppm dose levels ($p < 0.01$ and $p < 0.05$, respectively). MTBE also augmented SOD, CAT, GR, GPx, and GSH activity in the cortex and hippocampus, the regions associated with motor activity and behavior. These results clearly show that MTBE not only increases ROS levels but also induces an impairment of antioxidant enzymes which indicates oxidative stress. These findings suggest that MTBE-induced oxidative stress may in part explain its effects on locomotor activity. (Supported by NIH Grant RR03020).

398 DOXORUBICIN INHIBITS FERRYLMYOGLOBIN-DEPENDENT LIPID PEROXIDATION.

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Doxorubicin (DOX) and other anticancer anthracyclines can cause cardiomyopathy attributed to oxyradical- and iron- dependent lipid peroxidation. However, we have shown that DOX can both facilitate formation and diminish reactivity of non heme iron-centered lipid oxidants; moreover, chain-breaking antioxidants seem to prevent anthracycline-induced cardiomyopathy in laboratory animals but not in patients. Little is known on the effects of DOX on heme iron-derived lipid oxidants. Myoglobin (Mb) is the most abundant source of heme iron in the heart. Hydrogen peroxide (H_2O_2), generated through the redox cycling of DOX, oxidizes Mb to an oxoferryl species (Mb^{IV}) that promotes lipid peroxidation; therefore, we evaluated whether DOX diminished the reactivity of Mb^{IV} the same way it diminished that of non heme-iron derived oxidants. Mb^{IV} was formed *in vitro* by reacting H_2O_2 with metmyoglobin (Mb^{III}). Optical spectroscopy showed that DOX reduced Mb^{IV} to Mb^{III} with an apparent second order rate constant of 1.3×10^3 M^{-1} s^{-1}. Comparisons between DOX and other approved or investigational anthracyclines showed that DOX reduced Mb^{IV} through a hydroquinone moiety in its tetracyclic ring. DOX did not promote complete reduction of Mb^{IV} to $Mb^{II}O_2$, but limited reduction of Mb^{IV} to Mb^{III} was sufficient to prevent peroxidation of arachidonic acid to thiobarbituric acid-reactive substances. DOX inhibited lipid peroxidation also when Mb^{IV} was formed in cytosolic fractions derived from human myocardial biopsies, a model developed to predict the biochemical mode of action of anthracyclines in clinical settings. These results argue against an involvement of lipid peroxidation in anthracycline-induced cardiotoxicity, as they confirm that DOX can inactivate lipid oxidants possibly formed in the heart during the course of chemotherapy. Pharmacologic measures to prevent anthracycline-induced cardiomyopathy should therefore be reappraised and targeted to biochemical events other than lipid peroxidation.

399 OXYGEN TOXICITY: A METABOLIC DISEASE.

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Prolonged exposure of mammals to increased partial pressures of oxygen (hyperoxia) causes progressive damage to the lung. It has been suggested that hyperoxic damage may derive from enhanced formation of reactive oxygen species (ROS) and mitochondria are probably the major source of this reactive oxygen. We hypothesized that if mithochondrial metabolism were responsible for enhanced ROS production under hyperoxia, then cells with impaired mitochondrial respiration might be relatively resistant to increased oxygen tensions. To test this general idea, we produced rho0 HeLa cells which lack mitochondrial DNA, mitochondrial membrane potential and respiration by long term exposure to ethidium bromide (EB). Under both normoxia and hyperoxia (80% O2), these cells exhibited greatly decreased ROS production as assessed by (1) dichlorofluorescein (DCFH) oxidation, (2) dihydroethidium oxidation, (3) ROS-mediated suppression of aconitase activity and (4) cell survival under hyperoxia. The ROS generation is largely mitochondrial because the protonophoric uncoupler, carbonyl cyanide m-chloro phenylhydrazone (CCCP) reduced ROS production under both normoxia and hyperoxia by >80%.

As a control for ancillary EB DNA damage, we also re-introduced normal mitochondria to these rho0 cells *via* platelet fusion and found that ROS production and cell survival once again resembled that of the wild type Hela cells. Finally, we grew HeLa cells in the presence of chloramphenicol (which disrupts the synthesis of mitochondrial inner membrane proteins and suppresses both respiration and mitochondrial ROS production). Under 80% O2, these cells were quite resistant to hyperoxic damage and death. Overall, these results support the concept that interactions between respiring mitochondria and O2 are primarily responsible for hyperoxic cell damage. These observations further suggest that hyperoxic damage might be averted through the use of pharmacological agents which preferentially diminish mitochondrial respiration.

400 COMPARISON OF COMPOUND CLASSIFICATIONS DETERMINED FROM STRUCTURAL ALERTS FOR RODENT CARCINOGENICITY EXTRACTED FROM DIFFERENT DATABASES.

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Toxicity endpoints reported in various sources vary widely. A major challenge in data analysis, especially with regard to developing predictive models, is dealing with disparate forms of data, ranging from continuous numerical values to categorical responses. For example, although the compound set having rodent carcinogenicity data in the NTP database is a complete subset of the CPDB database, the latter uses TD50 as a measure of carcinogenicity potency of a compound, whereas NTP reports nominal degrees of evidence. Structure alerts are extracted from the CPDB dataset using TD50 values as the response variable. Certain compound classes that correlate strongly with TD50 were identified for descriptive usage: therapeutic categories, fungicide, pesticide, etc. The same analysis was performed using the nominal 0/1 NTP response data. The structure alerts mined from these two data sources were compared for rodent models. Structural alerts were applied to then build prediction models.

401 DEVELOPMENT OF A DATABASE FOR QUANTITATIVE MODELING OF AGE-RELATED CARCINOGENICITY STUDIES.

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In support of the California Childrens Environmental Health Initiative (AB 2872), we have developed a relational database consisting of data extracted from published animal cancer bioassays that utilized exposures early-in-life. To date, we have identified over 1200 cancer bioassays with early-in-life exposure data on over 40 different compounds or substances representing different mechanisms of action that range from direct-acting genotoxic carcinogens to endocrine disruptors. These substances include alkylating agents, polycyclic aromatic hydrocarbons, hormones and hydrazines. Based on available developmental information on several species including rats, mice, hamsters, gerbils and non-human primates, the following four exposure windows were defined for each species: prenatal, postnatal, juvenile and adult. Strict selection criteria have been developed for inclusion in the database. The publication must contain data on approximate age at dosing, age at death/sacrifice, and tumor incidence data. The study must be on a single chemical or mixture, contain a concurrent control group, have a study length greater than 40 weeks and report a survival rate greater than 60% (except for early death due to neoplasms). Results of the literature review will be presented along with the methods of literature identification, rationale for including experiments and conventions adopted in summarizing and standardizing the extracted data. In addition, a complete description of the database structure and function will be demonstrated. This database is being used to support quantitative analysis of differential responses to cancer induction by age at exposure and to identify trends in tumor response related to specific chemicals, classes of compounds, and common modes of action. Such analyses may help to determine if an early-life susceptibility is anticipated in the absence of specific compound data.

402 DIET OPTIMIZATION AND BODY WEIGHT GROWTH RATE IN RATS - EFFECTS ON SURVIVAL, TUMOR INCIDENCE AND HISTOPATHOLOGY.

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Appropriate study designs to assure adequate survival of rats in chronic studies have been the topic of much discussion in the toxicology community. One way to enhance survival is to moderately restrict feed and, therefore, growth (diet optimization). A possible alternative is the use of rats which are smaller and slower-growing and should, therefore, have enhanced survival. This poster presents data on survival, tumor incidence and histopathology for rats using these two alternatives and compares these data to data from rats using the traditional (ad libitum feeding) protocol. 200 Charles River Sprague-Dawley rats (IGS) were divided into 2 groups of 100 (50/sex). One group received food ad libitum (AL) and one group (Optimization - Opt) received mild to moderate diet restriction (75 to 90% of ad libitum group). Data were compared for these 2 groups and for a group of 240 (120/sex) ad libitum fed Harlan Spargue-Dawley rats, which are smaller and grow more slowly than the Charles River rats. Survival was similar for the Harlan and AL rats (24-28% for males and 46-53% for females) and clearly higher for the Opt group (84% for males and 80% for females). Dietary optimization decreased the incidence of pituitary tumors (adenomas of the pars distalis) in both sexes and of mammary gland tumors (adenocarcinomas) in females when compared to ad libitum fed IGS rats. Incidence of these neoplasms in Harlan rats was similar to that seen in Opt rats. [Pituitary Tumors: Males: Opt: 10%, Harlan: 13%, AL: 36%; Females: Opt: 48%, Harlan: 34%, AL: 82%] [Mammary Gland Tumors (Females): Opt: 24%, Harlan: 18%, AL: 34%]. Dietary optimization also reduced the incidence and severity of microscopic lesions in the liver and kidney, when compared to ad libitum fed IGS rats.

403 TOXICITY EVALUATION OF A LIPOSOME-BASED FORMULATION OF SN38 IN MICE.

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SN38 is an active metabolite of CPT-11, an anti-neoplastic agent, prescribed for the treatment of colorectal cancer. SN38 contributes to the anti-tumor activity and toxicity of CPT-11. SN38 is 100-1000 fold more potent than CPT-11 *in vitro*. Due to unavailability of a suitable solubilizing agent, SN38 has not been exploited for therapeutic use as an anti-cancer agent. In general, the liposome encapsulation of anti-cancer drugs reduces the toxicity and thus enhancing the therapeutic efficacy. A liposome-based formulation of SN38 (LE-SN38) was developed at NeoPharm using proprietary NeoLipidTM Technology to overcome some of the problems associated with SN38. Significant increased *in vitro* cytotoxicity and therapeutic efficacy of LE-SN38 vs. CPT-11 against various tumor models, prompted us to evaluate the toxicity of LE-SN38 in CD2F1 mice. The acute dose toxicity data suggested a maximum tolerated dose of 37 and 46 mg/kg in males and females respectively. The multiple dose toxicity (i.v.x5 daily) study suggested a tolerated dose of 5 and 7.5 mg/kg in males and females respectively. The hematology and clinical chemistry results showed reversible toxicity. At higher doses the lesions were observed in small intestine, liver, lung and spleen. However, the liver was predominantly affected organ in the male animals. These lesions were greatly reduced or absent on day 20. Phase I clinical trials of LE-SN38 are planned.

404 THE LACK OF CARCINOGENICITY IN A 26-WEEK INTRAVENOUS STUDY WITH S-303-TREATED MOUSE RED BLOOD CELLS IN C57BL/6TAC-TRP53TML HETEROZYGOTE MICE.

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The INTERCEPT Blood System for Red Blood Cells has been shown to inactivate a broad range of viruses, bacteria, and leukocytes in red blood cells (RBC) using the compound S-303 in an *ex vivo* process. S-303 was designed to react rapidly with nucleic acid and then decompose by an independent hydrolytic step. After addition to RBC, S-303 intercalates into nucleic acids, forms covalent DNA and RNA cross-links, and hydrolyzes to a negatively charged unreactive decomposition product, S-300. An indwelling Compound Adsorption Device (CAD) integral to a 35-day RBC storage container is used to reduce levels of residual S-300 by ~10-fold. The study evaluated the potential oncogenicity of S-303-treated mouse blood components (RBC and plasma) with and without CAD exposure when administered IV at 20 mL/kg, to groups of 20 mice/sex for 26 weeks. The treatment regimens ranged from 1 mM S-303-treated RBC with CAD exposure once weekly (~10 times the clinical dose) to 1 mM S-303-treated RBC without CAD exposure once per week + 1 mM S-303-treated mouse plasma without CAD treatment twice per week (~100-fold the clinical dose). Chronic transfusions for sickle cell or thalassemia patients occur at an approximate frequency of once monthly. Therefore, the doses used in the study were 40-to 1200-fold the clinical exposure based on dose and frequency. A positive control group received p-cresidine by daily oral gavage at 400 mg/kg. Mice were observed for evidence of mortality and moribundity. Body weight and food consumption were measured during the study. After 26 weeks of treatment, mice were bled for hematologic evaluations, euthanized, and evaluated macroscopically. Tissues from all mice were processed for histopathologic evaluation. The positive control caused urinary bladder transitional cell hyperplasia and transitional/squamous cell carcinoma, findings consistent with previous 2-year mouse bioassay data for this compound. No S-303-related toxicities or evidence of S-303-induced carcinogenicity were observed.

405 THE FAILURE OF CHLOROFORM ADMINISTERED IN THE DRINKING WATER TO INDUCE RENAL CELL CANCER IN THE F344/N RAT.

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Chloroform (TCM) has been demonstrated to be a renal carcinogen in the male Osborne-Mendel rat when administered either by corn oil gavage or in drinking water. We examined the ability of TCM in the drinking water to enhance renal tubular cell (RTC) cancer in the Male F344/N rat. Animals (78/group) were exposed to concentrations of 803 ± 5 or 1592± 21 mg/L TCM in the drinking water for 100 weeks. Distilled water served as the vehicle control; lead acetate, 2000 mg/L, was the positive control. A complete necropsy was done at 100 weeks. Water consumption did not differ among the groups. Mean daily doses of 53, 126, and 132 mg/kg were calculated for the low and high dose TCM and lead acetate respectively. Mean body weight was significantly depressed by TCM treatments. No RTC tumors were found in the control or in the TCM groups. Lead acetate increased ($p<0.05$) the incidence of RTC carcinoma (12.8%), adenoma (10.3%) and hyperplasia (100%) compared to 7.7% for hyperplasia in the control. No RTC hyperplasia was seen in the THM groups. The high TCM dose increased the prevalence (% of animals with a lesion) of hepatocellular neoplasia (carcinoma and adenoma) 17.5% vs 5.1% ($p<0.05$) and marginally enhanced the prevalence of combined preneoplastic and neoplastic tumors (20.5% vs 7.7%, $0.05<p<0.1$). The numbers of combined neoplasms (0.18 vs 0.05). Combined preneoplastic and neoplastic lesions (0.23 vs 0.05) were also increased ($p<0.05$). RTC proliferation at 100 weeks was depressed by 132 mg/kg/day TCM. TCM and lead acetate significantly depressed the severity of RTC hyaline droplets. These results are similar to those recently described (George, MH et al. 2002, Internat. J. Toxicol., 21, 219-230) in which bromodichloromethane (BDCM) in the drinking water of male F344/N rats failed to induce RTC and colon cancer which had been reported in a corn oil gavage. BDCM did, however, increase hepatocellular neoplasia. (This is an abstract of a proposed presentation and does not reflect the views of the EPA)

406 EFFECTS OF BISPHENOL A, AN ENDOCRINE DISRUPTOR, ON HEPATOCARCINOGENESIS AND REPRODUCTIVE SYSTEM PARAMETERS IN A MEDIUM-TERM LIVER BIOASSAY.

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Bisphenol A (4, 4-isopropylidene-2-diphenol, BPA) is a high-production volume chemical used in manufacture of polycarbonate plastics. It is of great concern as an example of an estrogenic xenobiotic and the present study was performed to investigate its potential influence on rat hepatocarcinogenesis in a medium-term liver bioassay system. In addition, its influence on male accessory organs and spermatogenesis was examined. A total of 78 rats were divided into 6 groups. The rats in groups 1 to 4 were given a single i.p. injection of DEN (200 mg/kg body weight) dissolved in saline to initiate hepatocarcinogenesis. Groups 5 and 6 received a saline injection instead of DEN. After 2 weeks, the rats in groups 1 and 5 were maintained on basal diet without BPA. Animals in groups 2-4 and 6 received diet containing 25, 250, 2000 and 2000 ppm BPA, respectively, for the remaining experimental duration of 6 weeks. All rats were subjected to two-thirds partial hepatectomy at week 3 and survivors were sacrificed under ether anesthesia at week 8 for examination of livers immunohistochemically for glutathione S-transferase placental form (GST-P)-positive foci. Significant retardation of body weight gain was observed in rats treated with 2000ppm BPA. No modifying potential was evident in terms of numbers or areas of GST-P-positive foci. Furthermore spermatogenesis was not affected in any of the treated groups. These results demonstrate that BPA lacks initiating or modifying potential for liver carcinogenesis in our medium-term bioassay system.

407 INACTIVITY OF 2-HYDROXYESTRADIOL, 4-HYDROXYESTRADIOL, ESTRIOL, AND 4-HYDROXYESTRONE AS MAMMARY CARCINOGENS IN THE ACI RAT.

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The ACI rat, an inbred cross between August and Copenhagen Irish strains, is a valuable model in the study of factors modulating susceptibility to mammary carcinogenesis. ACI rats are highly susceptible to mammary carcinogenesis when estrogen levels are continuously elevated. This study investigated whether metabolites of estradiol were more or less effective than estradiol as complete carcinogens in this animal model. Adult female, ovary-intact ACI rats were implanted subcutaneously with pellets composed of either cholesterol vehicle or cholesterol (18 mg) and 2 mg estradiol, 2-hydroxyestradiol, 4-hydroxyestradiol, estriol, or 4-hydroxyestrone. The rats were monitored for mammary tumor incidence, markers of estrogen action, and general health. Rats treated with estriol had significantly reduced body weight compared to all treatment groups with the exception of estradiol. The food consumed per body weight of rat did not differ between the estriol and vehicle treated rats, demonstrating that this effect is not due to appetite suppression. The study was ended after 36 weeks of treatment. Rats treated with estradiol had 73% incidence of palpable mammary tumors, but none of the animals treated with the vehicle or estradiol metabolites had palpable mammary tumors. In some experimental models where estrogen shows toxicity, the proposed mechanism of toxicity has involved primary metabolites of estradiol. However, in this ACI rat model in which estradiol induces mammary tumors, the metabolites prove to be ineffective at mammary tumorigenesis. (Supported by ES05022, ES07148)

408 THE ARYL HYDROCARBON RECEPTOR (AHR) REGULATES PROSTATE TUMOR PROGRESSION IN THE TRAMP MOUSE.

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The AhR is a signal transduction protein that binds chlorinated dioxins, other xenobiotics, and putative endogenous ligands and mediates their effects on gene expression in various organs including prostate. We previously demonstrated that the AhR is necessary for normal prostate development. We have now interbred AhR null mutant mice with transgenic adenocarcinoma of the mouse prostate (TRAMP) mice to test the hypothesis that the AhR signaling pathway can affect prostate cancer development. TRAMP mice express SV40 T antigen under control of a rat probasin promoter to develop prostate cancer. TRAMP mice of each *Ahr* genotype were necropsied at 105, 140, 175 and 210 days of age to determine the incidence of macroscopic poorly differentiated prostate tumors (CaP). Mice died from CaP as early as 105 days and with much greater frequency at 175 days of age and older. The incidence of CaP in $Ahr^{+/+}$ TRAMP mice was 0/1, 1/15, 3/15 and 1/23 at 105, 140, 175 and 210 days of age, respectively. In TRAMP mice lacking one *Ahr* allele ($Ahr^{+/-}$), the incidence of CaP was much higher (3/20, 14/45, 16/37 and 8/31, respectively) than when both alleles were present. An even higher CaP incidence was observed in $Ahr^{-/-}$ TRAMP mice, which lack both *Ahr* alleles (1/4, 9/17, 8/10 and 3/4, respectively). These differences do not appear to be due to *Ahr*-dependent differences in TRAMP transgene expression because (1) large T antigen was comparably detectable, by immunohistochemistry, in prostatic epithelial cells of mice of each *Ahr* genotype, and (2) mice of each *Ahr* genotype exhibited comparable incidences and degrees of prostatic intraepithelial neoplasia. These results demonstrate that the AhR signaling pathway regulates the progression phase of prostate cancer development, and suggest that *Ahr* may be a tumor suppressor gene. (Supported by NIH grant ES 01332).

409 TIME TO FATAL TUMOR IN P53 KNOCKOUT MICE.

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P53 knockout mice were generated by gene targeting strain129 mice at Baylor University. Heterozygous P53 +/- F1 mice were crossbred to generate homozygous P53 -/- and P53 +/+ mice. More than 400 animals were bred and divided into 3 groups. We analyzed time to death and tumor type data with parametric and non-parametric statistical models. Only a limited number of critical events ($1 < n <= 10$) are possible in the chain affecting the cell-cycle in these animals. Malignant lymphoma, soft tissue sarcoma and various carcinomas cluster at different time intervals of their life spans. The homozygous P53- /- died mostly from a thymic lymphoma in less than nine months versus one year typical of heterozygotes. About half of this lymphoma resulted from a loss-of-heterozygosity. Soft tissue sarcoma time-to-death was similiarly affected by P53 genetic status. Osteogenic sarcoma was lower among homozygous recessives versus heterozygotes. Carcinoma frequency was low in both homozygous and heterozygous P53 knockout mice.

410 26-WEEK VALIDATION STUDY OF THE Tg.rasH2 MOUSE TEST SYSTEM USING THREE CARCINOGENS.

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The Tg.rasH2 mouse carries multiple copies of the human protooncogene c-Ha-ras linked to its endogenous promoter/enhacer and is recommended for genotoxic and non-genotoxic carcinogen identification. Short term carcinogenicity assays con-

ducted using this model are acceptable alternatives to traditional carcinogenicity testing as described in the ICH S2B document. With this in mind and anticipated increases in the use of the Tg.rasH2 model in the US, we performed a 26-week validation study in which the effects of three carcinogens were examined. Two groups of ten animals per sex and one group of nine animals per sex were administered N-methyl-N-nitrosourea (MNU) (single i.p. dose, 75 mg/Kg), urethane (three i.p. doses at two day intervals, 1000 mg/Kg), and p-cresidine (i.g. daily for 26-weeks, 400 mg/Kg), respectively. One other group of ten animals per sex was administered corn oil for 26 weeks by daily oral gavage. Preliminary results indicate MNU-mediated toxicity presented as decreased bodyweights and the presence of a palpable axillary mass in one animal. One early death was recorded due to bladder distention likely resulting from blockage of the urethral canal in a female animal treated with urethane. Interestingly, papilloma formation was observed at the site of tail tattoo in five male animals and 4 female animals treated with MNU, and in one female animal treated with urethane. The presence of papillomas at the site of tail tattoo in treatment groups implicates a possible synergy between sytemic carcinogen administration and the tissue repair and proliferation following damage from tattooing. These results, although preliminary, indicate the necessity for validation studies and the creation of an historical database demonstrating the responses of this test system to various classes of carcinogens. Further investigation of the interaction between carcinogens and skin wounding or other pseudo-promotional events may prove valuable.

411 EVALUATION OF ALTERNATE DOSE METRICS FOR BENZO[A]PYRENE INHALATION CANCER POTENCY USING A NASAL-PBPK HAMSTER MODEL.

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The current OEHHA inhalation cancer potency of 1.1E-3 $(\mu g/m^3)^{-1}$ is based on an applied dose metric and upper respiratory tract (URT) tumors in hamsters (Thyssen et al. 1981). A 13 compartment nasal-physiologically-based pharmacokinetic model was used to evaluate the ability of various dose metrics and dose averaging to provide an improved fit by the observed URT tumor incidence (Frederick et al., 1998). The model employed physiological and biochemical parameters scaled from a 380g rat to a 120 g hamster. Nasal biochemical values were estimated from dog tracheal epithelium (Gerde et al., 1997). Rates of hydrolysis and conjugation of the BAP oxidative metabolite were estimated from mean rodent values for butadiene monoxide (Kohn and Melnick, 1993). Two model forms were used: 1) a single species BaP model with metabolized dose (AMET) metrics in liver, lung and nasal tissue; and 2) a twin species BaP/BaP metabolite model with concentration based metrics for the BaP metabolite in the nasopharynx compartment. In both models daily exposures in mg/m3 were regressed against the best fitting model-derived internal metrics to estimate external equivalents. For the single species model the best fitting metric for URT tumors was liver AMET with daily average dose (χ^2 = 2.02; P = 0.36; quantal probit regression). This metric gave a human scaled potency of 2.5E-3 $(\mu g/m^3)^{-1}$. For the twin species model the best metric was the lifetime time weighted average (LTWA) area under the tissue concentration x time curve (NP-AUC) for the BaP metabolite in the nasopharynx compartment (χ^2 = 2.28; P = 0.32; quantal probit regression) with a human potency estimate of 1.8E-3 $(\mu g/m^3)^{-1}$. In both cases potency estimates were based on 0.1/ LED10 values (Benchmark Dose Software v.1.3.1) and provided superior fits by the URT data when compared to the applied dose LTWA metric (χ^2 = 7.3; P = 0.063). Further improvements in this dose response assessment will require additional chemical-specific and hamster-specific data.

412 TOXIC AND CARCINOGENIC EFFECTS IN THE LUNGS OF RATS AND MICE EXPOSED TO VANADIUM PENTOXIDE BY WHOLE-BODY INHALATION.

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Vanadium pentoxide (V_2O_5), commercially the most important vanadium compound, presents a hazard during the cleaning of oil-fired boilers and furnaces, the handling of catalysts, and during the refining, processing, or burning of vanadium-rich mineral ores or fossil fuels. The toxicity and carcinogenicity of V_2O_5 in male and female F344/N rats and B6C3F$_1$ mice was determined in a series of whole-body inhalation studies (NTP, 2001). In the 90-day study, rats and mice were exposed to particulate aerosols of V_2O_5 at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3. As expected, the lung was the primary site of toxicity in both species. This was indicated by concentration-related increases in absolute and relative lung weights, and increased incidences of inflammation and alveolar and bronchiolar epithelium hyperplasia/metaplasia. Fibrosis was only observed in rats. Based on the results from the 90-day study, exposure concentrations of 0, 0.5, 1, and 2 mg/m^3(rats) and 0, 1, 2, and 4 mg/m^3 (mice) were selected. In the 2-year study, significant concentration-related increases in inflammation, fibrosis, alveolar and bronchiolar epithelium hyperplasia/metaplasia, and squamous metaplasia were observed in male and female rats. Although not significant, chemical-related increases in alveolar/bronchiolar neoplasms occurred in male rats exposed to 0.5 and 2 mg/m^3, with the incidences in some groups exceeding the NTP historical control ranges. A marginal increase in alveolar/bronchiolar neoplasms was observed in female rats exposed to 0.5 mg/m^3. In mice, inflammation and alveolar and bronchiolar epithelium hyperplasia were observed in almost every exposed animal, while fibrosis also was observed, the incidences were less than that in rats. Alveolar/bronchiolar neoplasms were significantly increased in all exposed mice, providing clear evidence of carcinogenicity of V_2O_5 in mice.

413 EXPRESSION OF MUTANT HUMAN KI-ras INDUCES LUNG TUMORS IN BITRANSGENIC MICE.

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Ki-*ras* mutations have been implicated as an early event in the development of human and mouse lung adenocarcinomas. We developed a bitransgenic mouse model to regulate mutant human Ki-*ras* in a lung-specific and DOX-inducible manner. Monotransgenic mice containing the mutant Ki-*ras*CYS^{12} transgene linked to a tetracycline-inducible promoter are crossed to monotransgenic mice that constitutively express the reverse tetracycline transactivator from either the lung-specific SP-C or CCSP promoters. Induction of the Ki-*ras* transgene was detected by RT-PCR in bitransgenic mice following 1 week of DOX exposure. Bitransgenic mice exhibited hyperplastic lung foci after only 12 days of DOX treatment. By 3 months of treatment, extensive epithelial hyperplasia of the alveolar region of the lung tissue could be seen, as well as multiple macroscopic tumors, the majority of which were less than 1 mm in size. The tumor incidences following 3 months of DOX treatment were 80% and 100% for SP-C/Ki-*ras* and CCSP/Ki-*ras* mice, with tumor multiplicities of 2.0±2.0 and 7.2±5.8 (n=5), respectively. Histopathology analysis of one of these lesions identified it as a well differentiated adenoma, as the lesion contained normal sized nuclei and was encapsulated with no signs of invasion into surrounding tissue. These analyses are continuing on other tissue samples. Lung morphology of untreated bitransgenic mice and DOX-treated single transgenic mice was normal. These results indicate that induction of mutant Ki-*ras* in the lung is sufficient to induce the formation of lung tumors, providing strong evidence that mutation of Ki-*ras* is a critical, early event in lung tumor pathogenesis. This mouse model is being utilized to further define the role of mutant Ki-*ras* in lung tumorigenesis and should be an important new model for the testing and development of chemopreventive and chemotherapeutic agents for lung cancer. (Supported by NCI grant CA91909)

414 TUMORIGENICITY IN A/J AND RASH2 MICE FOLLOWING MAINSTREAM TOBACCO SMOKE INHALATION.

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Towards developing a rodent inhalation model for evaluating lung tumorigenicity, A/J strain and rasH2 transgenic mice were provided mainstream tobacco smoke (MTS) from Kentucky 1R4F reference cigarettes according to whole-body and nose-only exposure regimens. The whole-body regimen included a 5-month dosing period, with animals exposed to humidified air or 0.2 mg WTPM/L for 6 hrs/d (5 d/wk), and a 4-month recovery period. The nose-only regimen included 7-month dosing, with animals exposed to 0.4, 0.125 or 0.04 mg WTPM/L for 3 hrs/d (5 d/wk), and a similar recovery period. Gross examination of lungs was used to evaluate tumorigenic responses. Following whole-body exposure of A/J mice to MTS, both tumor incidence and multiplicity were decreased relative to sham. Inclusion of the recovery period led to increases for both indices, though no statistical differences were evident. Whole-body exposed rasH2 mice demonstrated increased tumor incidence and multiplicity in response to MTS dosing. Increases were enhanced during recovery, with MTS-exposed mice exhibiting a statistical increase in tumor incidence. Both strains demonstrated minimal body weight reductions during whole-body exposure relative to sham. Nose-only exposure of A/J mice to MTS resulted in reductions for tumor incidence and multiplicity relative to sham. These indices were increased during recovery, providing an apparent dose-related trend for MTS exposure concentration; statistical increases were limited to tumor multiplicity at 0.4 mg WTPM/L. Nose-only exposed rasH2 mice provided a markedly different response, with tumor incidence and multiplicity increased to the greatest extent at 0.04 mg WTPM/L; similar results were observed following recovery. The dissimilar responses exhibited by the two strains are possibly explained by their divergent profiles of body weight reduction during nose-only MTS exposure. A/J mice exhibited significant reductions (up to 20%) in an exposure-dependent manner, while body weight loss for rasH2 mice was unaffected by MTS concentration.

415 A 30-WEEK DERMAL TUMOR PROMOTION ASSAY USING SENCAR MICE FOR THE COMPARATIVE EVALUATION OF CIGARETTE SMOKE CONDENSATES.

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Mouse dermal initiation/promotion assays have been used for several decades to evaluate the potential carcinogenicity of cigarette smoke condensates (CSC). However, these studies have used highly variable methodoligies that differ in methods of CSC collection, duration of treatment, mouse strain, number of mice and endpoints measured. R.J. Reynolds uses a standardized protocol which utilzes female, SENCAR mice (40/group), which were treated with a single dose (75 ug) of dimethylbenz(a)anthracene (DMBA) as an initiator, followed one week later by treatment (three times/week) with 10, 20 or 40 mg "tar"/application of CSC for 29 weeks. End-points include survival, body weights, clinical observations, organ weights, dermal tumor development data and histopathology. As part of product stewardship efforts, CSC from Kentucky 1R4F reference cigarettes was included in four dermal tumor promotion studies conducted between 1994 and 2000. The combined results for the 1R4F cigarettes from the four studies are reported. The number of mice developing masses and the total number of masses produced were highly dose-dependent and reproducible. The average numbers of tumor bearing mice were 3.5, 15.5 and 32 and the average total numbers of tumors were 5.3, 79 and 208 for the low, mid and high dose groups, respectively. The combined results of these studies demostrate this comparative 30-week dermal tumor promotion assay provides a highly reproducible and quanitiative study design that can be used to assess the promotional activity of CSC.

416 COMPARATIVE 30-WEEK DERMAL TUMOR PROMOTION STUDY USING SENCAR MICE: COMPARISON OF CIGARETTE SMOKE CONDENSATE FROM A REFERENCE CIGARETTE CONTAINING DIRECT-FIRE FLUE-CURED TOBACCO AND A TEST CIGARETTE CONTAINING HEAT-EXCHANGE FLUE-CURED TOBACCO.

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A 30-Week dermal tumor promotion study was conducted to evaluate the relative tumor-promoting potential of cigarette smoke condensate (CSC) collected from a test cigarette containing flue-cured tobacco cured by heat-exchange to that of a reference cigarette containing flue-cured tobacco cured by direct-firing. Heat-exchange tobacco contains significantly lower concentrations of tobacco-specific nitrosamines compared to direct-fire cured tobacco. Mainstream CSCs were collected by cold trap from smoke generators using the Federal Trade Commission puffing regimen. Female SENCAR mice were "initiated" with a single 75-ug application of DMBA to the shaved dorsal skin. The CSCs 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 or 50 mice. In addition to the appropriate sentinel and vehicle controls, non-initiated mice received high-dose treatments of CSC from the reference and test cigarettes to demonstrate the main effect of CSC is promotion. End-points included body weights, clinical observations, organ weights, dermal tumor development data and histopathology. The number of dermal tumors and the number of tumor-bearing animals for both the reference and test CSCs were greater than the "DMBA-initiated" control group and increased with increasing dose. When corresponding doses of reference and test CSC were compared, there were no statistically significant differences noted with the exception of the mid-dose test CSC where the total number of microscopically-confirmed tumors was less than the reference CSC. Evaluation of these data in total indicates no toxicologically significant differences between the heat-exchange tobacco CSC and the direct-fire tobacco CSC.

417 TOXICOLOGICAL EVALUATION OF HONEY AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO.

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A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity that results from burning tobacco. The foundation of this evaluation strategy is comparative testing, typically including chemical and biological components. In the manufacture of cigarettes, honey is used as a casing ingredient to impart both aroma and taste. As part of ongoing stewardship efforts, cigarettes produced with honey (5% wet weight) as an alternative to invert sugar in tobacco casing material were subjected to extensive evaluation. Principal components of this evaluation included a determination of selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-Week dermal tumor promotion evaluation of mainstream cigarette smoke condensate in SENCAR mice, and a 13-Week inhalation study of mainstream cigarette smoke in Sprague-Dawley rats. Comparative analytical evaluations demonstrated that the substitution of honey for invert sugar as a casing material in cigarettes had no significant impact on mainstream smoke chemistry. In addition, *in vitro* and *in vivo* studies demonstrated that cigarettes containing tobacco cased with honey had comparable biological activity as cigarettes that contained invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufacture of cigarettes is acceptable.

418 DETERMINATION OF THE SKIN CANCER POTENTIAL OF BENZO(*A*)PYRENE (BaP) AND A PETROLEUM REFINERY STREAM IN K6/ODC MICE.

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ExxonMobil and the petroleum industry have historically utilized the mouse dermal carcinogenesis bioassay to assess the carcinogenicity of petroleum-derived materials. This assay, albeit an accurate predictor of carcinogenic potential, is expensive and takes two years to complete. Scientists have been searching for a faster, less costly but effective means to identify carcinogenic compounds. The K6/ODC transgenic mouse shows promise as a replacement for the conventional two-year rodent dermal bioassay. The skin of these mice is genetically tumor promoted probably as a result of the constitutive overexpression of ornithine decarboxylase (ODC).All that is needed for skin tumor development in this model is an initiating event which can be accomplished by low, even single doses of genotoxic carcinogens. In this study, six groups of K6/ODC mice (15 mice/group) were treated as follows: Groups 1, 2 and 3 received 25, 100 or 400 micrograms (µg) BaP in acetone, respectively, as a single dose applied topically to the dorsal area of the back; Group 4 received 750 µg of catalytically cracked clarified oil (CCO; a high-boiling carcinogenic petroleum material) in acetone as a single topical dose; Group 5 received 25 µg BaP in acetone applied topically once a week for the duration of the study (6- months).Groups 6 and 7 received 37.5 microliters acetone as a single dose or once a week for the duration of the study, respectively. Development of skin tumors was monitored weekly. After 20 weeks, 9/15 animals in Group 1, 15/15 animals in Groups 2 and 3 and 5/15 animals in Group 4 had developed papillomas and/or carcinomas. Of note, in Group 5 (25 µg BaP, 1 x week) 11/15 animals had developed tumors by week 14. Thus, repeated application of low dose BaP significantly decreased tumor latency. Tumors were not observed in either control group. These results clearly demonstrate that the K6/ODC mouse is very sensitive to the topical application of genotoxic carcinogens.

419 EVALUATION OF 2, 4–D PHOTOCARCINOGENIC ACTIVITY IN K6/ODC TRANSGENIC MICE.

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As a result of overexpression of the enzyme ornithine decarboxylase (ODC) in skin, the K6/ODC transgenic mouse appears to be extremely sensitive to tumor induction with both genotoxic chemicals and UV radiation. 2, 4–dichlorophenoxyacetic acid (2, 4–D), a phenoxy acetic acid herbicide has been used world-wide in agriculture and pest management with the accompanying potential for both incidental and accidental human exposure. It has been found to induce proliferation of peroxisomes in the liver of rodents and is a possible carcinogen. In this study, the carcinogenic effect of 2, 4–D on UV irradiation-induced skin tumors was evaluated by using K6/ODC mice. 2, 4–D (one of 2 doses) was applied topically 2 times per week on days when no UV radiation was given for a period of 22 weeks. Lomefloxacin was used as a positive control for enhancement of UV carcinogenesis, and unirradiated, chemical treated and irradiated and unirradiated vehicle controls were also used. No signs of phototoxicity were seen. Skin tumors were observed in all the irradiated groups. Tumor incidence was significantly increased in lomefloxacin plus UV irradiation, but not in 2, 4–D plus UV compared to vehicle plus UV exposed animals. There were no tumors in unirradiated control groups. The results suggest that 2, 4–D is not a carcinogen or enhancer of UV induced photocarcinogenesis in this model. Because of the positive result of lomefloxacin in this study, the K6/ODC mouse may provide a highly sensitive test system for detecting enhancers of photocarcinogenesis.

420 DERMAL EXPOSURE OF MALE AND FEMALE HEMIZYGOUS Tg.AC AND FVB/N MICE FOR 52 WEEKS TO N-NITROSODIETHYLAMINE (DEN).

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The Tg.AC (v-Ha-ras) transgenic mouse model has been proposed as a short term *in vivo* alternative or adjunct to the chronic bioassay to identify and characterize the mechanisms of action of carcinogens. To test the ability of Tg.AC mice to detect a

multi-organ genotoxic carcinogen and suspected human carcinogen, groups of 15-25 male and female hemizygous Tg.AC and FVB/N mice, the background strain, were exposed to 0, 50, 100, or 200 μg DEN/200 μl acetone topically twice weekly for up to 52 weeks. As a positive control, TPA (2.50 μg) was applied to the shaved dorsal surface of mice two times a week for up to 38 weeks in male and female Tg.AC and 52 weeks in male and female FVB/N mice. Although not dose-dependent, there was an increase in mortality of Tg.AC male mice exposed to DEN as compared to controls. Group mean body weight gain of DEN-exposed mice was decreased in comparison to the solvent control mice, for both strains (Tg.AC and FVB/N) and sexes. At necropsy, there was a very low incidence of papillomas in the Tg.AC male and female mice exposed to DEN. Papillomas were not induced by DEN in a dose-response manner, and only in one case was more than a single papilloma observed at the test site. In TPA-exposed male and female Tg.AC and FVB/N mice, the incidence of skin papillomas was 100%, 100%, 0%, and 0%, respectively. There was a high occurrence of lung masses in DEN-exposed Tg.AC and FVB/N male and female mice and an increased incidence of stomach tumors in DEN-exposed male and female Tg.AC and female FVB/N mice. Collectively, it can be concluded that dermal administration of DEN for up to 52 weeks of exposure did not induce a significant skin papilloma response in the Tg.AC model or the background FVB/N strain. DEN exposure, irrespective of genotype, induced lung tumors in male and female exposed mice. This work was sponsored by NIEHS (N01-ES-95442).

421 COMPARISON OF THE Tg.AC AND SENCAR DERMAL CARCINOGENESIS TEST SYSTEMS IN RESPONSE TO TPA PROMOTION FOLLOWING DMBA INITIATION OR VEHICLE TREATMENT.

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The Tg.AC mouse carries an activated v-Ha-ras oncogene linked to the promoter of the zeta-globin gene. These transgenic mice do not require chemical initiation and respond in a dose responsive manner to dermal applications of various tumor promoters including TPA, with development of squamous cell papillomas. The SENCAR mouse is a selectively bred animal for "sensitivity to carcinogenesis". SENCAR mice respond with similar paillomas to TPA promotion following a prerequisite chemical initiator treatment. The purpose of this study was to compare the Tg.AC and SENCAR test system responses to TPA so as to determine the more effective and responsive model for use in dermal carcinogenicity testing. Two groups of Tg.AC animals received a single dermal initiation treatment with either DMBA (50 μg) or vehicle. This was followed by 12 weeks of dermal TPA treatments (1.25 μg per dose per three times per week) beginning one week after initiator treatment. One group of female SENCAR animals received a single dermal initiation treatment with DMBA (50 μg), followed by 11 weeks of dermal TPA treatments (1.0 μg per dose per three times per week) beginning one week after initiatior treatment. Tumor incidence was recorded weekly and followed ILSI guidelines. At study termination, tumor multiplicity was highest in SENCAR animals followed by initiated male Tg.AC animals, non-initiated male Tg.AC animals, initiated female Tg.AC animals, and non-initiated Tg.AC females in descending order. Interestingly, latency of tumor formation based on the incidence of animals with papillomas was found to be shortest in SENCAR animals. Initiated Tg.AC animals (both sexes) also demonstrated decreased latency compared to the non-initiated animals. These results indicate that the SENCAR test system is the more effective and more responsive model at a lower cumulative dose of promoter. Additionally, in Tg.AC animals, development of skin papillomas resulting from TPA promotion is potentiated by a single pre-treatment with a mutagenic carcinogen.

422 VEHICULAR EFFECTS OF DIMETHYL SULFOXIDE (DMSO) ON DERMAL CARCINOGENESIS IN TETRADECONYL PHORBOL ACETATE (TPA)-TREATED HEMIZYGOUS Tg.AC TRANSGENIC MICE.

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Hydrophobic test material must often be solubilized in DMSO to facilitate administration, particularly at high concentrations approaching typical maximum tolerable doses. The purpose of this study was to examine the vehicular effects of DMSO on dermal carcinogenesis in TPA-treated Tg.AC transgenic mice. The Tg.AC mouse carries an activated v-Ha-ras oncogene linked to the promoter of the zeta-globin gene. These mice respond in a dose responsive manner to dermal applications of various tumor promoters including TPA, with development of squamous cell papillomas. Four groups of five animals per sex were treated three times per week for 12 weeks with incremental doses of TPA delivered in DMSO (2.5, 5.0, 10 and 20 μg TPA respectively for each 100 μl dose). Two additional groups of 5 animals per sex, a negative or vehicle control (DMSO alone) and a positive control (TPA 1.25 μg per dose delivered in acetone), were also included in the study. Tumor incidence was recorded weekly and followed ILSI guidelines. At study termination, statistical analyses revealed significant increases in tumor incidence in male animals treated with 5.0, 10, and 20 μg TPA per dose and female animals treated with 10 and 20 μg TPA per dose compared to vehicle control animals. A positive linear trend was observed in animals carrying site-of-application skin tumors, indicating a definitive dose response. However, when compared to the positive control group it was determined that treatments in the range of 10 to 20 μg TPA per dose in DMSO would be required to obtain similar incidences and tumor multiplicity as groups treated with 1.25 μg TPA in acetone. In conclusion, at low doses of TPA (< 10 μg per dose) the vehicular effects of DMSO result in decreased incidences and multiplicities, and increased latency periods for the appearance of papillomas. At present the mechanism of these effects are unclear, however they can be overcome at high doses of promoter.

423 THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 (MRP1/ABCC1) PROTECTS THE TESTICULAR TUBULES AGAINST METHOXYCHLOR DAMAGE.

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The multidrug resistance-associated protein-1 (MRP1/ABCC1) is a 190kDa membrane-bound glycoprotein that mediates cellular efflux of a variety of xenobiotics, typically as glucuronide, sulfate, or glutathione conjugates. We examined the ability of MRP1 to transport methoxychlor, a commonly used pesticide with estrogenic and antiandrogenic metabolites, which can also be conjugated with glutathione. NIH 3T3 cells stably expressing MRP1 were 2.5-fold more resistant to the toxicity of methoxychlor, and eliminated methoxychlor twice as rapidly than the mock-transfected counterparts. We then examined whether mrp1 expression could alter the toxicity of methoxychlor *in vivo* using male FVB/mrp1 knockout mice (FVB/mrp1-/-) over the length of a spermatogenic cycle. Methoxychlor treatment reduced CYP-mediated testicular testosterone metabolism in both the FVB and the FVB/mrp1-/- mice, although it did not significantly affect testicular morphology in the FVB mice. However, methoxychlor treatment greatly reduced the number of developing spermatocytes and reduced the integrity of the germinal epithelium in the FVB/mrp1-/- mice. These results suggest that the elimination of methoxychlor by MRP1 is important in protecting the testicular tubules from damage.

424 CHILDREN'S EXPOSURE TO DEET AND OTHER TOPICALLY-APPLIED INSECTICIDES.

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Use of topical repellents on children to control mosquitoes, ticks, and biting flies is common and, for achieving good control of these nuisance and disease-carrying arthropods, sometimes necessary. Anecdotal reports suggest that repellents may be applied more frequently and for a longer duration than recommended, but no studies characterizing the actual usage patterns and exposure of children have been reported. In summer 2002, a cross-sectional survey on the use patterns of repellents and possible associated effects was conducted at Maryland campgrounds. Information requested included products used, number of applications, duration of exposure, areas of the body treated, postapplication practices, and acute signs or symptoms. Information on parents' decision-making process was also compiled. The study yielded 301 respondents using repellents on their children. Deet was the most commonly used active ingredient (83.4%); aerosols were the most common formulation (42.5%). Most parents (65.4%) applied the repellent to their child only once within a day. Over a third of respondents (38.8%) treated their children's clothing as well as their skin. Forty one (13.6%) treated their child's hands, and about a third (34.9%) treated the child's face. Of these, 11.4% sprayed the product directly onto the child's face. Over half of the children did not remove the repellent by washing, swimming or other means before going to bed. More than a third of parents failed to read or follow label directions. Differences in patterns of use between mother's application and father's application were noted. Relatively few respondents reported increasing (or planning to increase) use of repellents due to concerns about West Nile Virus or Lyme disease. Only two cases of adverse reactions were reported. Minimization of children's exposure to pesticides is always desirable. While this study does not support a finding of acute adverse health effects from practices in common use, it does provide documentation of practices leading to undesirable exposure. Educational outreach to change parents' usage patterns is called for.

425 HUMAN EXPOSURE TO FIPRONIL FROM DOGS TREATED WITH FRONTLINE.

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The objective of this investigation was to determine fipronil residues in gloves worn while handling dogs after Frontline application. Veterinarians and animal care technicians handle large numbers of dogs every day, and there are currently no reports regarding the exposure to Frontline that may occur in these professionals. Frontline contains the active insecticidal ingredient fipronil (9.8%), which is known to provide fast, effective and convenient treatment and control of fleas and ticks in dogs for at least 30 days. Fipronil exerts its toxicity, including convulsions and seizures, through a well-established mechanism, i.e., non-competitive inhibition of GABA receptors. Frontline (1.34 ml) was applied topically on adult household dogs and gloves worn for 5 min during handling were collected (at the intervals of 24 hr, and 1, 2, 3, 4, and 5 weeks post-Frontline application) as the source samples for fipronil residues determination. At no time did the dogs show any clinical signs of fipronil toxicity. Glove extracts were analyzed for quantitation and confirmation of transferable residues of fipronil using GC/MS. The highest concentration of fipronil (589.33 + 205.70 ppm) was detected after 24 hr of Frontline application. Transferable residue of fipronil was of slightly lesser magnitude after one week, and followed a further descending trend during the 2nd, 3rd, and 4th weeks. Fipronil residue was undetectable in the gloves collected at the 5-week interval. Glove extracts from the control dogs did not reveal any residue of fipronil. These results suggest that in spite of the sequestration of fipronil in the lipids of skin and hair follicles, gloves were contaminated with a significant residue. Thus, repeated exposure to such contamination may result in potential health risks to veterinary personnel.

426 DEVELOPMENT AND APPLICATION OF A MULTI-COMPARTMENT KINETIC MODEL FOR PREDICTING THE FATE OF PARATHION AND ITS METABOLITES IN HUMANS.

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A multi-compartment kinetic model was developed to predict the time evolution of parathion and its metabolites, p-nitrophenol and alkylphosphates, in the human body and excreta, under a variety of exposure routes and scenarios. The biological determinants of the kinetics were established from published data on the *in vivo* time profiles of parathion and its metabolites in blood and urine of human volunteers exposed by intravenous, oral and dermal routes. In the model, body and excreta compartments were used to represent the amounts of each of the following: parathion, p-nitrophenol and alkylphosphates. The dynamics of inter-compartment exchanges was described mathematically by a differential equation system that ensures conservation of mass, in moles, at all times. The model parameters were determined by adjusting statistically the explicit solution of the differential equations to the experimental human data. Extensive use was made of the different time scales involved in the biological processes to simplify the differential equations and enable no more than two model parameters to be estimated per fit. Simulations provided a close approximation to kinetic data available in the published literature. When simulating a dermal exposure to parathion, the main route-of-entry for workers, the absorption half-life was set to 8 hr. The model predicted an overall urinary elimination half-life of parathion as p-nitrophenol of 19 hr and as alkylphosphates of 31 hr. The fraction of absorbed dose accumulated in urine as p-nitrophenol and alkylphosphates were determined experimentally to be 37% and 46% respectively. With this, the model can estimate the absorbed dose of parathion from measurements of amounts of metabolites accumulated in urine over a given period of time. In particular, the model was used to determine the absorbed dose of parathion from measurements of p-nitrophenol in urine of workers exposed to the pesticide in fruit orchards.

427 A TOXICOKINETIC MODEL FOR THE RISK ASSESSMENT OF WORKER EXPOSURE TO TRICLOPYR THROUGH MEASUREMENTS IN URINE SAMPLES.

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A toxicokinetic model was developed to establish links between the absorbed dose, the time varying body burdens and the excretion levels of trichlopyr in humans. The biological determinants of the kinetics were established from published data on the *in vivo* time profiles of trichlopyr in the blood and urine of human volunteers. In the model, compartments represent the amounts of trichlopyr in the body and in excreta. The dynamic of inter-compartment exchanges was described mathematically by a differential equation system that ensures conservation of mass at all times. Through back calculations, the model was used to evaluate the absorbed dose of trichlopyr in eight workers starting from measurements of the amounts of trichlopyr accumulated in urine over a 20-hr period following the onset of an 8-hr work shift. The workers exhibited excretion values of trichlopyr in their 20-hr urine samples ranging from 4 to 50 μmol. This corresponds, according to model predictions, to an absorbed dose between 0.8 to 10.5 times the reference dose (RfD) established by the US Environmental Protection Agency (EPA). The workers, who were exposed no more than a few consecutive months to trichlopyr, did not however show any apparent adverse effects, as documented by questionnaires. This raises questions concerning the adequacy of using the EPA RfD to estimate the health risk from short-term exposure to trichlopyr in workers. The EPA RfD is very conservative; it is based on the results of an animal study and an uncertainty factor of 100 was applied to account for inter-individual and species variations in toxic response.

428 CHARACTERIZATION OF ATRAZINE METABOLISM BY HUMAN GLUTATHIONE S-TRANSFERASES (GSTs).

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Atrazine is one of the most widely used herbicides in the United States, and has been detected occasionally at low levels in drinking water sources. Although atrazine exhibits relatively low toxicity to mammals, it has tested positive in some genotoxicity assays and has been implicated as a potent endocrine disrupter in frogs. The metabolism of atrazine in humans has not been fully characterized. Rodent studies suggest Phase I-dominated metabolism with minor Phase II-mediated metabolism likely effected by pi class GST. However, in human urine, mercapturates of atrazine are major metabolites, yet the specific GST form(s) responsible for GSH conjugation have not been identified. Using recombinant alpha, mu, pi and theta class human GSTs, we have demonstrated that only hGSTP1-1 displayed significant activity toward atrazine, with a specific activity of 7.1 nmol/min/mg protein. We have also confirmed that mouse GST pi protein is likely responsible for the glutathione-dependent metabolism of atrazine in mouse liver; the recombinant mGSTP1-1 had a specific activity of 7.3 nmol/min/mg protein. As expected, human liver- which expresses very little hGST pi protein- displayed little activity toward atrazine. Whether genetic variants of GSTP1 exhibit important catalytic differences in GST-mediated conjugation of atrazine has not been determined. However, from the crystal structure it is clear that the Ile105Val amino acid change in the hGSTP1*B genetic variant lies in the hydrophobic substrate pocket whereas the hGSTP1*D variation (Ala114Val) lies outside that area. Molecular docking studies suggest that both the wild type and the hGSTP1*B variant can accommodate atrazine in the pocket. Homology modeling and substrate docking of the atrazine-GST conjugate in the binding site are consistent with a thiolate nucleophilic attack and stabilization for the Cl- leaving group, and suggest that the hGSTP1B variant may have altered catalytic activity toward atrazine, relative to the common form. (Supported by P30ES07033).

429 COMPELLING EVIDENCE THAT FOMESAFEN CAUSES MOUSE LIVER TUMORS BY A MECHANISM OF PPARα ACTIVATION THAT IS NOT RELEVANT TO HUMANS.

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Recent advances in the understanding of how peroxisome proliferator-activated receptor α (PPARα) ligands cause rodent liver tumors have led to growing consensus on key data elements in the mode of action (ILSI, 1998). Fomesafen is a diphenylether herbicide that has produced liver tumors in mice, but not rats, at dietary levels of 100 and 1000 ppm. In shorter term studies, treatment of mice with fomesafen at 100 and 1000 ppm produced changes classically associated with PPARα activation in rodents: increased liver weight and hepatic peroxisomal volume. In addition, concordant histopathological changes (centrilobular hypertrophy and eosinophilia) and decreased plasma triglyceride levels were observed. Assessment of hepatocyte replication by BrdU labelling indicated increased cell replication at 100 ppm and 1000 ppm within 7 days, and this trend continued after 28 and 56 days. Peroxisomal β-oxidation (as measured by palmitoyl-CoA oxidation) in males and females was increased after 7 days at 100 ppm (4-5 fold) and 1000 ppm (6-8 fold). After 28 days, palmitoyl-CoA oxidation was increased at 10 ppm (3-4 fold) as well as at 100 ppm (4-9 fold) and 1000 ppm (8-9 fold). Finally, hepatocytes isolated from control mice, PPARα null mice, and humans were used

for *in vitro* investigations of the response to PPs. In wild type mouse hepatocytes, fomesafen (250mM) increased the rate of peroxisomal β-oxidation, increased DNA synthesis and decreased apoptosis. In contrast, PPARα null mice hepatocytes and human hepatocytes were refractory to the effects of fomesafen. These data demonstrate that activation of PPARα in the mouse mediates the multiple hepatic effects of fomesafen, but human hepatocytes are not responsive to the key events mediated by PPARα in mice. The combined evidence indicates that fomesafen belongs to the PP class of rodent specific hepatocarcinogens that induce tumors in mice *via* a mode of action that is not relevant to man.

430 EFFECTS OF CHLORPYRIFOS AND METHYL PARATHION ON HEPATIC CYTOCHROME P450 IN NEONATAL RATS.

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The adult pattern of hepatic cytochrome P450 is imprinted neonatally. Organophosphorus compounds might affect expression of cytochrome P450 in liver directly, or indirectly by changing cholinergic activity or hormone secretion. Also, their desulfuration can result in inactivation of cytochrome P450. These actions might cause changes in the levels and pattern of cytochrome P450 isozymes in neonates that could persist into adulthood. Studies were performed to examine the effects of chlorpyrifos (2 or 5 mg/kg) and methyl parathion (5 mg/kg) on hepatic P450s of neonatal rats. Pups of each sex were treated dermally once daily from postnatal day 3 to postnatal day 15. The rate* of NADPH-dependent conversion of methyl parathion to methyl paraoxon (*nmol/min/mg prot) in microsomes from livers of control pups decreased over 20 minutes from 0.91±0.07 to 0.34±0.22; an effect consistent with desulfuration-dependent inactivation of P450. Neither rate was affected by methyl parathion treatment (0.74±0.06, 0.46±0.16). In contrast, chlorpyrifos treatment inhibited the initial rate at which methyl parathion was converted to methyl paraoxon by 45% (0.51±0.04) and the final rate by 90% (0.05±0.03); the latter effect suggesting that chlorpyrifos is a more efficient suicide substrate than is methyl parathion. In general, rates of p-nitrophenol production from methyl parathion paralleled those of methyl paraoxon production. Both chlorpyrifos (0.14±0.01 versus 0.09±0.01 nmol/mg prot) and methyl parathion (0.12±0.01) increased total hepatic microsomal cytochrome P450 content. The level of CYP2E1 was also increased in liver microsomes of treated pups. In contrast, the level of CYP2C11 (an isozyme implicated in oxidative desulfuration) was reduced by treatment with chlorpyrifos, but increased by treatment with methyl parathion. These data indicate that the effects of organophosphorus compounds on hepatic P450s reflects a combination of inhibition and induction. The mechanisms and specific isozymes involved remain to be fully identified. (Grant R06/CCR419466 from the CDC).

431 EXPOSURE TO A COMMERCIAL HERBICIDE MIXTURE CAUSES A DECREASE IN LITTER SIZE IN MICE.

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We investigated the developmental toxicity in mice of a commercial formulation of herbicide containing a mixture of 2, 4-dichlorophenoxyacetic acid, mecoprop, dicamba and inert ingredients. Pregnant mice were exposed to one of four different doses of the herbicide mixture diluted in their drinking water, either during preimplantation and organogenesis or only during organogenesis. Litter size, birth weight and crown-rump length were determined at birth. Dams were sacrificed by carbon dioxide asfixiation and the number of implantation sites was determined by staining with ammonium sulfide. The data, although apparently influenced by season, showed an inverted or u-shaped dose response pattern for reduced litter size, with the lowest dose - which corresponds to the reference dose for 2, 4-D - producing the greatest decrease in the number of embryos implanted and number of pups being born. Fetotoxicity, as evidenced by a decrease in weight and crown-rump length of the newborn pups was not significantly different in herbicide-treated litters.

432 EFFECT OF PYRETHRINS ON RAT HEPATIC XENOBIOTIC METABOLISING ENZYME ACTIVITIES.

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High doses of Pyrethrins (PY) have been shown to produce liver and thyroid tumors in rats. The aim of this study was to examine the effect of PY on some markers of hepatic xenobiotic metabolism and to compare PY with the well known rat liver and thyroid tumor promoter sodium Phenobarbital (NaPB). Female Sprague-Dawley rats were fed diets containing 0 (control), 100, 3000 and 8000 ppm PY for 7, 14 and 42 days and for 42 days followed by 42 days of reversal. In addition, male rats were fed 0 and 8000 ppm PY diets for the same time periods and male and female rats were also fed diets containing 1200-1558 ppm NaPB for 7 and 14 days. Liver microsomes were prepared and assayed for protein and total cytochrome P450 (CYP) content, CYP-dependent enzymes and thyroxine UDPglucuronosyltransferase (UDPGT). The treatment of male and female rats with NaPB for 7 and 14 days and 8000 ppm PY for 7, 14 and 42 days produced significant increases in microsomal CYP content and a marked induction of CYP2B-dependent 7-pentoxyresorufin O-depentylase and testosterone 16beta-hydroxylase activities. Significant increases were also observed in CYP3A-dependent testosterone 6beta-hydroxylase and thyroxine UDPGT activities. Overall, the effects of PY were qualitatively similar to those of NaPB. However, on a mmol/kg/day intake basis, NaPB was a significantly more potent inducer of CYP2B and CYP3A forms, being 9.1 and 3.4 times more potent than PY in male and female rats, respectively. In female rats PY exhibited a threshold of 3000 ppm, where similar but less marked effects to those seen at 8000 ppm were observed . In both sexes the hepatic effects of PY were reversible on cessation of treatment. These results suggest that PY induces hepatic CYP-dependent enzymes and thyroxine UDPGT in the rat, much like other non-genotoxic agents that produce liver and thyroid tumors in this species *via* a threshold-based mechanism. (Supported by the Pyrethrin Joint Venture).

433 MECHANISTIC TOXICITY STUDY IN RATS WITH PYRETHRINS: PATHOLOGIC EFFECTS.

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The purpose of this study was to investigate how Pyrethrins (PY) produced liver and thyroid gland tumours in rats. Rats were given 100, 3000 or 8000 p.p.m. PY in the diet for 7, 14 or 42 Days. A recovery group was included. Phenobarbital treated groups were included for comparison. Animals were given bromodeoxyuridine (BrdU) prior to necropsy. After completion of treatment animals were killed. Blood samples were analysed for thyroid hormone levels (reported separately). Liver and thyroid glands were weighed and examined histologically and the BrdU labelling index was assessed. Frozen liver samples were subjected to microsomal enzyme analysis (reported separately). There were no effects seen in animals receiving 100 p.p.m. PY. However, in groups receiving 3000 or 8000 p.p.m. PY histological examination revealed liver cell hypertrophy in 60-100% of animals at each time point. This correlated with increased liver weights of approximately 30-60% above control values. In the animals killed after 7 or 14 days there were BrdU labelling indices of 3-10 fold the control values. Histological examination of thyroid glands showed follicular cell hypertrophy in 40-100% of animals at each time point, correlating with approximately 20-50% increases in organ weight. BrdU labellng indices were 3-6 fold greater than controls in animals killed after 14 days. Phenobarbital treated animals gave similar results. These results are consistent with the induction of liver mixed function oxidases with increased catabolism of thyroid hormones causing a secondary stimulation of thyroid follicular cells. Results of serum hormone and liver enzyme analyses confirmed this. In conclusion, Pyrethrins, like Phenobarbital, act through a dose related proliferative response in the liver and a secondary proliferative stimulation of thyroid follicular cells which is specific to rats. These effects are reversible and only appear above a dose threshold.

434 MECHANISTIC TOXICITY STUDY IN RATS WITH PYRETHRINS: EFFECTS ON THYROID HORMONES.

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The purpose of this study was to investigate how Pyrethrins (PY) produced liver and thyroid gland tumours in rats. Rats were given 100, 3000 or 8000 p.p.m. PY in the diet for 7, 14 or 42 Days. A recovery group was included. Phenobarbital treated groups were included for comparison. Other details of the experimental method are reported separately. After completion of treatment animals were killed and blood samples were taken and analysed for thyroid hormone levels (tri-iodothyronine (T3), thyroxine, reverse tri-iodothyronine (rT3) and thyroid stimulating hormone (TSH)). In male animals receiving 8000 p.p.m. PY there were statistically significant reductions in serum thyroxine levels from 3.69 to 2.55 at Day 7, 2.91 to 2.24 at Day 14 and 3.96 to 3.11 at Day 42 (ng/ml). T3 levels were reduced at Days 7

and 14. In the same groups TSH levels were significantly elevated from 4.46 to 7.69 at Day 14 and 2.68 to 6.95 at Day 42 (ng/ml). In females, there were significant increases in serum TSH which appeared earlier than in males. In females receiving 8000 p.p.m. PY there were increases in TSH from 1.87 to 4.77 at Day 7, 1.97 to 7.88 at Day 14 and 2.07 to 7.82 at Day 42 (ng/ml). Animals treated at 3000 p.p.m. had smaller increases. There were no effects seen in animals receiving 100 p.p.m. Phenobarbital treated animals gave similar results. These results are consistent with increased catabolism of thyroid hormones in liver causing a secondary stimulation of thyroid follicular cells *via* the pituitary gland. Liver enzyme analyses (reported separately) confirmed this. In conclusion, Pyrethrins, like Phenobarbital, act through a dose related metabolic response in the liver which is specific to rats and a secondary proliferative stimulation of thyroid follicular cells. These effects are reversible and only appear above a dose threshold.

435 EFFECTS OF PYRETHROID COMPOUNDS ON ALKALINE PHOSPHATASE ACTIVITY IN ESTROGEN RECEPTOR POSITIVE HUMAN BREAST CANCER CELLS.

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Pyrethroids are one of the most commonly used insecticides in worldwide, but it remains unclear whether pyrethroid compounds possess endocrine disrupting activity or not. T47D cells, an estrogen receptor positive human breast cancer cell line, is known to induce alkaline phosphatase (AlkP) only in response to progestins. Because the action of estrogen may be changed by the action of progestins, it is important to examine the potential to produce progestin-mediated effects for determining endocrine disrupting activity of chemicals. In this study we investigated the progestagenic/ antiprogestagenic effects of pyrethroid compounds using AlkP activity assay and expression of progesterone receptor in T47D cells. After a 48 hr exposure period, progesterone significantly increased AlkP activity in a dose-dependent manner, and maximum activity was observed at the level of 10-8M. However pyrethroid compounds (bioallethrin, cypermethrin, deltamethrin, fenvalerate, permethrin, sumithrin, and tetramethrin) showed no increase in AlkP activity at any concentration. Among seven pyrethroid compounds fenvalerate and permethrin significantly decreased the progesterone-induced AlkP activity, but only at a relatively high concentration (10-5M). The present study suggests that some pyrethroid compounds (fenvalerate and permethrin) may have weak endocrine disrupting effects *via* progesterone related mechanism. Supproted by NITR/Korea FDA Grant ED2001-19

436 IDENTIFYING TRIAZINE HERBICIDES ON EPA DRINKING WATER CONTAMINANT CANDIDATE LIST (CCL) FOR COMMON MECHANISM OF TOXICITY AND CUMULATIVE RISK ASSESSMENT.

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The USEPA's Office of Research and Development (ORD) and Office of Water (OW) have identified a subset of triazine herbicides that may be considered for a potential common mechanism of toxicity, based on a weight-of-evidence approach developed by the EPA. Chlorotriazine compounds selected exclusively from the CCL included: atrazine, simazine, propazine and cyanazine. Also, triazine degradants in drinking water — desethylatrazine (DEA), desisopropylatrazine (DIA) and diaminochlorotriazine (DACT) — were included. Animal studies indicate that these compounds induce mammary gland tumors in female rats, and cause a disruption of hormonal homeostasis (i.e., alteration of the estrus cycle, attenuation of the LH and PRL surge, delayed puberty in both males and females, etc). Available data indicate that these compounds interfere with control of the hypothalamic-pituitary axis (HPA), possibly by interacting within the hypothalamus. The association with LH surge is indicative of a common mechanism of toxicity. Hence, the incidence of mammary tumors is postulated to be related with the HPA. Evidence to-date indicates that no specific molecular entity has been identified as either the proximate toxicant or its receptor, although it strongly suggests that all four parent chlorotriazines act *via* the same mechanism. The proposed grouping for cumulative risk assessment, based on a common mode of action (disruption of the HPA axis), includes atrazine, simazine, propazine, cyanazine, DEA, DIA, and DACT. However, in performing cumulative risk assessment, it is important to consider the occurrence, use, and existing regulations of each of the parent compounds. ** The views expressed in this paper are those of the authors, and do not necessarily reflect the views and policies of the USEPA.

437 EFFECT OF CHLORPYRIFOS ON THE EXPRESSION AND FUNCTION OF AN EFFLUX MEMBRANE TRANSPORTER IN INTESTINAL CELLS.

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Chlorpyrifos (CPF) and its metabolite, chlorpyrifos-oxon (CPO), have been reported to affect the expression of P-glycoprotein (P-gp) in rat intestine. Changes to the expression of P-gp, a membrane efflux transporter, may result in changes to the oral bioavailability of xenobiotics such as pesticides and drugs. To determine the effect of CPF on the expression of *mdr1* (the gene coding for P-gp), Caco-2 cells were treated with CPF (30 μM) over a 24 h period. RNA from the cells was isolated at 0, 4, 8 and 24 h and reverse transcribed. The cDNA was subjected to a competitive PCR (compPCR) reaction to quantify the *mdr1* copy number in treated and control cells. The competitor fragment for the compPCR assay was designed with a sequence that annealed with the same primers as the target *mdr1* sequence but resulted in a smaller size product (350 bp vs 507 bp). The gene copy number for a particular treatment was determined by running several compPCR reactions with a range of competitor concentrations. CPF treated cells exhibited a significant increase in *mdr1* copy number with a maximum effect at 8 h (100% increase) compared to control cells (copy numbers: CPF_{8h} = 122, 446±13; $Control_{8h}$ = 61, 374±352). CPO treated cells did not exhibit a significant change in *mdr1* expression. Based on the expression results, functional studies were performed with Caco-2 cells treated with CPF for 8 h. The efflux ratio (ER) of verapamil was calculated from the efflux (basolateral to apical) and influx (apical to basolateral) permeabilities ($ER=P_{efflux}/P_{influx}$). Cells treated with CPF for 8 h exhibited an increase in ER, 27% and 13%, at the two verapamil concentrations tested, 0.01 μM and 100 μM, respectively. The effect of CPF on *mdr1* expression and efflux transporter function suggests that exposure to CPF may modulate the oral bioavailability of xenobiotics, particularly those administered with a concurrent exposure to CPF. This effect could potentially lead to pesticide-drug interactions that result in drug toxicity.

438 EFFECT OF CYP SUBSTRATES ON THE METABOLISM OF CHLORPYRIFOS IN HUMAN LIVER MICROSOMES.

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Metabolism of chlorpyrifos (CPF) to its active and inactive metabolites occurs through the catalytic activity of the cytochrome P450 (CYP) enzyme superfamily. Several CYP isoforms may be responsible for CPF metabolism including CYP3A4, the predominant CYP isoform in the human liver. Concurrent exposure to CYP substrates or inhibitors could affect the balance between CPF activation and detoxification. More importantly, CPF exposure may dramatically affect the disposition of drugs that are also substrates for CYP3A4 and potentially other CYP isoforms. The aim of this study was to examine the inhibitory effect of CYP substrates on the metabolism of CPF in human liver microsomes. Ketoconazole and doxycycline, two common antibiotics that are known CYP substrates with a demonstrated high affinity for CYP3A4, were investigated. Human liver microsomes were incubated with radiolabeled CPF and either ketoconazole or doxycycline at varying concentrations for 5 minutes at 37°C to determine K_i. The formation of chlorpyrifos-oxon (CPO), the active metabolite, was determined by HPLC utilizing radioactivity detection. Based on the enzyme kinetics of the inhibition studies, the formation of CPO was suppressed in the presence of ketoconazole and doxycycline with a decrease in Vmax of 43% and 20%, respectively, and calculated K_i values of 0.094 μM and 0.173 μM for ketoconazole and doxycycline, respectively. The pattern of inhibition suggests a mixed competitive-noncompetitive mechanism is occurring in the metabolism of CPF in the presence of either inhibitory substrate. The results suggest that concurrent exposure to CPF and other CYP substrates may decrease the formation of CPO, the toxic metabolite of CPF. Results from these studies also establish that ketoconazole and doxycycline inhibit CPF metabolism and infer that CPF may also interfere with metabolism of other CYP substrates. Therefore, these studies provide a framework for ongoing studies in our lab on pesticide-drug interactions and their potential for toxicity.

439 ESTROGENIC ACTIVITIES OF PYRETHROID COMPOUNDS IN MCF-7 BUS CELLS.

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Pyrethroids are extensively used as insecticide in agriculture and home. Several studies have reported that pyrethroids are relatively safe to humans and wildlife. However, some studies have suggested that pyrethroids possess estrogen-like activity. Thus, the purpose of this study was to investigate the effects of pyrethroid compounds on cell proliferation, and expression of ERs and pS2 using estrogen receptor positive human breast cancer cell line (MCF-7 BUS cells). Seven pyrethroids

(bioallethrin, cypermethrin, deltamethrin, fenvalerate, permethrin, sumithrin, and tetramethrin) were tested with 17b-estradiol as a positive control. Among the pyrethroid compounds tested, only sumithrin increased the MCF-7 BUS cell proliferation in a dose-dependent manner, maximum induction of cell proliferation was observed at dose of 10-5M. In ER expression, 17b-estradiol (10-10M) decreased the level of cytosolic ERa and ERb protein expression compared with the vehicle control, and sumithrin significantly decreased the expression of ERa and ERb protein at high concentrations, 10-7 ~ 10-5M, in a dose-dependent manner. Similarly to 17b-estradiol, sumithrin dose-dependently increased pS2 mRNA expression. The other six test compounds used in the present study did not show any estrogenic effect at all concentrations (from 10-11 to 10-5M). These findings suggest that sumithrin could be considered to induce weak estrogenic activity *via* ER related pathways. Supported by NITR/Korea FDA Grant ED 2001-19.

440 INTESTINAL METABOLISM OF ORGANOPHOSPHATE INSECTICIDES: POTENTIAL FIRST-PASS METABOLISM.

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Chlorpyrifos (CPF) and diazinon (DZN) are organophosphate (OP) insecticides, and their toxicity is mediated through CYP450 metabolism to CPF-oxon and DZN-oxon, respectively. Detoxification of CPF and DZN is also mediated by CYP450 and A-esterase (A-EST) metabolism of CPF- and DZN-oxon, resulting in the formation of trichloropyridinol (TCP) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP), respectively. This study evaluated the potential role that intestinal metabolism (CYP450 and A-EST) may play in the first-pass clearance of OPs. Microsomes prepared from whole liver or isolated intestinal enterocytes demonstrated similar CYP450 and A-EST metabolic profiles. CYP450 content, as measured by reduced CO spectra, was ~10-fold lower in enterocyte than hepatocyte microsomes. For enterocyte CYP450 metabolism, the overall metabolic efficiency for the conversion to their active oxon metabolites was ~5-fold greater for CPF than DZN. When metabolism was compared per nmol P450 (nmol/min/nmol P450), the Vmax was ~3 and ~2 times higher in enterocytes than liver microsomes for CPF-oxon and TCP, respectively. The affinity (Km) for the metabolism of CPF to CPF-oxon was the same in liver and enterocyte microsomes, however the Km for TCP production was lower in enterocytes. Due to the smaller volume of intestine, the lower amount of CYP450, and the higher Km for TCP in the enterocyte microsomes, the catalytic efficiency was lower in intestine than liver for CPF-oxon, DZN-oxon and TCP. Enterocytes also demonstrated A-EST metabolism of CPF- and DZN-oxon. Although the Km for the substrates were comparable in hepatic and enterocyte microsomes, the Vmax was significantly faster, 69- to 255-fold in liver. These results suggest that intestinal metabolism may impact first-pass metabolism of OPs following low-dose oral exposures that would be expected from residues on foods. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608).

441 HPLC ANALYSIS OF VINCLOZOLIN AND ITS METABOLITES IN SERUM.

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The fungicide vinclozolin (V) is used predominantly for treatment of grains, fruits, ornamental plants and turfgrass. V administered to rats is hydrolyzed to 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3'5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline (M3). V, M1 and M2 have antiandrogenic properties both in vivo and in vitro by interacting with the androgen receptor. However, data on V and its metabolites in biological samples is limited. The aims of this study were to optimize a method for the analysis of V and its metabolites by HPLC and to evaluate the stability of V in rat serum. A gradient program with a mobile phase consisting of 60-75% methanol:acetonitrile (70:30) and 0.05 M phosphate buffer (PB) pH 3.3 at 1 ml/min, C-18 column and wavelength of 212 nm were used. The method was validated using spiked serum samples (pH 1.0) in a concentration range of 2-10 µg/ml. All analytes were extracted with acetonitrile (pH 2.5) from 100 µl aliquots. Retention times for M3, M1, M2 and V were 10.3, 12.8, 15.4 and 18.2 min, respectively. Detection limits for analytes ranged from 8.3 to 36.2 ng/ml. The recoveries were from 60 to 105% and the coefficient of variation was lower than 10% for all analytes. The relative concentration of V in PB pH 7.4 and serum (37°C) decreased over 4 h. V was more stable in PB than in serum. M3, M2 and M1 were observed in both media and at 48 h M3 had the highest relative concentration (50-60%). High serum levels of V, M1 and M3 in rats orally exposed to V (100 mg/kg) during 24 h after dosing were observed. These results suggest that the metabolism of V is both enzymatic and non-enzymatic. A better understanding of the biotransformation and pharmacokinetics of V will clarify the relationship between toxicity and tissue dose of V and its metabolites. (Funded in part by cooperative agreement CR 828790 with the NRC. This abstract does not represent USEPA policy).

442 CHLOROPYRIFOS OXON AND CARBARYL INHIBITION OF TRANS-PERMETHRIN HYDROLYSIS IN HUMAN LIVER FRACTIONS.

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Permethrin is a pyrethroid insecticide used in the Gulf War with other deployment related chemicals such as chlorpyrifos, pyridostigmine bromide and N, N-diethyl-m-toluamide. The hydrolysis of permethrin by esterases is a major detoxification process. Chemical interactions among deployment related chemicals have been suggested as a potential cause of Gulf War Related Illnesses. In this study the inhibitory effects of chlorpyrifos on trans-permethrin hydrolysis in humans is investigated using human liver fractions. Chlorpyrifos is an organophosphorus insectide and chlorpyrifos oxon (CFO), an active metabolite, is a potent inhibitor of t-PMT hydrolysis. The parent chlorpyrifos must be metabolically activated to the oxon by cytochrome P450 (CYP) to exert inhibitory effects. Pyridostigmine bromide and N, N-diethyl-m-toluamide did not inhibit trans-permethrin hydrolysis. Complete inhibition by CFO indicated that the esterases involved in trans-permethrin hydrolysis are B-esterases, which are inhibited by organophosphorus pesticides. Ki values for CFO inhibition of trans-permethrin hydrolysis are 20 nM in the cytosolic fraction and 100 nM in the microsomal fraction. The pattern of inhibition kinetics was non-competitive irreversible reflecting the known mechanism of organophosphorus pesticides, covalent modification of the active site of esterases. Carbaryl, another anticholinesterase agent with a similar inhibition mechanism, also showed the same inhibition kinetics but with higher Ki values, which was expected from the fact that carbaryl is reversible and a less persistent esterase inhibitor compared to organophosphorus compounds. Different from CFO, carbaryl does not completely inhibit trans-permethrin hydrolysis even at high concentrations. This indicates that two or more esterases are involved in trans-permethrin hydrolysis in human liver fractions, all sensitive to organophosphate but variably sensitive to carbaryl.

443 DEFINING MULTIGENE DOSE-RESPONSE RELATIONSHIPS BY MICROARRAY ANALYSIS.

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Microarray studies often focus on increasing replicates to maximize statistical power. As a result, conducting complete dose response microarray studies can be cost prohibitive. However, in drug safety assessment, margin of safety (MOS) calculations are based on the difference between efficacy and toxicity. If genes are to serve as biomarkers to refine MOS calculations, then understanding their dose response relationships is critical. The purpose of this investigation was to develop methods to generate ED50 and maximum response (Rmax) values from microarray data. Peroxisome proliferator-activated receptor (PPAR) agonists are ideal for this type of investigation since their effects are transcriptionally-regulated. We determined PPAR-induced global liver gene expression profiles in rats exposed to either Fenofibrate (Feno), a weak, but specific PPARα agonist, or another potent PPAR agonist (PPARag). Eight doses were chosen spanning the ED50s for efficacy parameters (lowered triglyceride and/or glucose) with gene expression data collected on pooled liver RNA samples from control and treated rats. Overall, Feno induced more gene changes than PPARag. Both agonists induced fatty acid (FA) and drug metabolism-related gene expression. Comparing ED50 values for FA genes affected by Feno and PPARag yielded gene-based potency information. Consistent with the affinities for PPARα, Feno ED50s were right-shifted compared to PPARag. However, ED50 values for genes induced by either Feno or PPARag vary between genes suggesting gene selective responses. FA gene changes and gene-based potencies correlate with induction of *in vitro* and *in vivo* liver peroxisomal β-oxidation by Feno and PPARag. Feno, while less potent, produced higher Rmax values for FA gene expression compared to PPARag. Based on these studies, a dose response-based microarray data analysis design is proposed that is both cost effective and maximizes the value of microarrays by yielding ED50 and Rmax values that allow for compound comparisons.

444 GENOMIC AND PROTEOMIC PROFILING IN A PARKINSONIAN MODEL OF NEURODEGENERATION.

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The pathogenic mechanisms underlying Parkinson's disease (PD) remain enigmatic. In an effort to identify early molecular events associated with PD, we profiled genomic and proteomic changes in the MPTP mouse model of PD. cDNA and antibody microarray analysis revealed time-dependent (1h-48h) changes in striatal gene expression following dopaminergic neurotoxicity and associated reactive gliosis. A medley of genes exhibiting altered expression is tabulated. Other genes expressed included cytokines & chemokines, growth factors, transcription factors, protein kinases and genes related to stress, cell cycle and apoptosis. Further,

by proteomic profiling using ProteinChip®-SELDI-TOF® we identified smaller peptides & proteins associated with MPTP-neurotoxicity. These included neuropeptides (substance P, neurokinin A, brain natriuretic peptide), neuronal protein 15.6, PEA-15, MRF-1, cytokines & chemokines (IL-9, IL-17, MCP-1, MCP-5, MIP-2, MIP-3), growth factors (BMP, CT-1, LIF), transcription factors (ATF-3, BTF-3, CREB-bp, IEF-3, NTF-2) and stress proteins (MT-1C, MT-1H, MT-3). These findings helped identify early effectors/mediators and have provided a foundation for (1) further characterization of candidate genes involved in the neurodegenerative process and (2) identifying potential targets for therapeutic intervention.

GENE	cDNA ARRAY Fold change (1h-24h)	ANTIBODY ARRAY Fold change (48h)	GENE	cDNA ARRAY Fold change (1h-24h)	ANTIBODY ARRAY Fold change (48h)
Max n-2	-	2.4	Cyclin E	1.9 to 2.1	1.7
DAC-1	1.7 to 2.1	-	E2F-1	2.0	1.5
DP-5	1.9		Ets-1	2.5	1.8
Gfap	2.2 to 2.7	2.0	c-fos	1.7 to 2.1	1.5
Neurofil	2.2 to 2.9		Mdm2	2.0 to 3.0	1.8
Neurogenin3		2.6	Nfat-1	1.6 to 2.5	2.0
NPAR	1.7 to 2.3	-	p53	1.8 to 2.1	1.8
p35	2.0 to 14.0		p73	1.6 to 2.0	1.7
Synaptotag		2.2	Pten	1.9 to 2.3	1.6
Vimentin	1.7 to 2.2		Rb-3	1.7 to 2.6	1.5
			Zip-37	2.7 to 3.7	2.0

445 DIFFERENTIAL TRANSCRIPTION FACTOR ACTIVATION AND GENE EXPRESSION PROFILES IN HUMAN VASCULAR ENDOTHELIAL CELLS ON EXPOSURE TO RESIDUAL OIL FLY ASH (ROFA) AND VANADIUM.

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Endothelial cells, that line the vascular tree, serve as a selective thrombogenic barrier between blood components and tissues. Dysfunction of vascular permeability contributes to the pathogenesis of a wide range of diseases. Whether the vascular endothelial cells play any role in the observed cardiovascular effects of PM and/or its constituents is unclear. Acute injury to a cell on exposure to a toxicant can initiate a complex series of biological responses, which in turn link to transcriptional regulation of genes. Assessing the temporal and functional relationship of transcription factor activation and differential expression of genes could reveal novel interactions in the initiation and progression of acute injury. In this study primary cultures of human umbilical vein endothelial cells were exposed to saline, ROFA (1mg/ml) or vanadium (V) (1mM) for 20 minutes to investigate the immediate injury and or stress response. Differential activation of an array of 54 transcription factors was analyzed in the nuclear extracts of cells using Transcription Factor array (Panomics, Inc., Redwood City, CA) and the gene expression using human plastic microarray (Clontech, Palo Alto, CA). Analysis of Transcription factor regulation indicated exposure specific activation of AP-1, AP-2, EGR, E2F and p53 in ROFA and V exposed compared to saline exposed cells. Similarly the gene expression data revealed exposure-specific differential expression in ~1200 genes. Around 700 genes were induced 5-20 fold and ~500 genes were suppressed (5-10 fold) due to ROFA or V exposure. Hierarchical clustering analysis on the gene expression data generated three unique gene clusters representing exposure specific alteration in gene expression. Differential activation of transcription factors and regulation of down stream gene expression observed in this study suggests ROFA specific effects on endothelial cells in mediating PM toxicity. (This abstract does not reflect USEPA policy

446 COMPARATIVE STUDY OF DNA MICROARRAY DATA ANALYSIS: PRINCIPAL COMPONENT ANALYSIS VERSES FISHER LINEAR DISCRIMINANT ANALYSIS.

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DNA microarray technology has been extensively used in toxicology studies in recent years. Among many potential applications of this approach, it is DNA microarray technology has been extensively used in toxicology studies in recent years. Among many potential applications of this approach, it is expected that monitoring gene expression changes induced directly or indirectly by different classes of toxicants should ultimately allow molecular- and mechanistic-based classification of chemicals. To test the feasibility of classifying chemicals based on their effects on genes expression, DNA microarray data derived from exposure of primary cultures of rat hepatocytes to two model chemicals, namely cadmium and hydrazine, were analyzed using different statistical techniques. In an attempt to develop an algorithm that requires minimal data pre-processing, changes in expression levels of all 984 genes on the microarray were included in the analysis. Principal Component Analysis (PCA) was initially used to classify chemical and dose effects. It however failed to separate experimental groups (27.4% accuracy) with respect to chemical treatment or dosage due to the effects of animal variability and adaptation to *in vitro* culture environment on overall gene expression. In contrast, using Fisher Linear Discriminant Analysis (FLDA) we could successfully separate experimental groups on the basis of time, chemical and dosage with a high degree of accuracy (75%) using data from two cadmium experiments and 2 hydrazine experiments as training data and data from one experiment of either treatment as the test data. Our results demonstrated FLDA is a useful technique for DNA microarray data analysis. It requires no data filtering and thus minimizes biases that might be generated by subjective criteria during this process.

447 A DYNAMIC GENETIC NETWORK MEDIATES DOSE-DEPENDENT OXIDATIVE STRESS RESPONSES.

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Comparison of the yeast and human genomes confirms that the genetic pathways mediating the cellular responses to toxicant stress are remarkably conserved. We have exploited this conservation to develop a yeast functional genomics model for the cellular response to oxidative stress. Yeast cells exhibit a highly reproducible graded pattern of dose dependent cellular responses to oxidant exposure, similar to those reported for mammalian cells: adaptation, cell cycle checkpoint arrest, apoptosis, and necrosis. In microarray gene expression profiling of hydrogen peroxide stressed cells, we observe distinct transcriptional patterns that distinguish chronic from acute exposures. Using OmniViz data mining software, we have constructed a genetic network model for these data which is dynamic over time. Cells that survive acute oxidative stress have incurred extensive genetic damage to both their mitochondrial and nuclear genomes. We are using two functional profiling approaches to determine the cellular pathway and target dependencies of this genotoxicity. First, by selecting the subset of nuclear genes that are essential for mitochondrial function, we have applied a "target-based gene filter" to visualize aspects of our genetic network model that are specific to the mitochondrial target. Second, we have constructed a "phenotype macroarray" of yeast gene knockout strains and have used it to identify susceptibility genes for oxidative stress. We find that functional profiling identifies genes that are important for defense against oxidative stress but which are not identified by gene expression profiling. In addition, functional profiling with our phenotype macroarray distinguishes among oxidative stressors that act by similar mechanisms of toxicity but which may have different cellular targets (such as hydrogen peroxide, menadione, and tert-butylhydroperoxide). We conclude that our transcriptional and functional profiling methods provide overlapping but complementary approaches to whole genome analysis of the mechanisms of cellular toxicity.

448 EXAMINATION OF THE POTENTIAL AH RECEPTOR AGONISM OF PHA-X673 AND PHA-X680 USING DNA MICROARRAYS.

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Profound induction of hepatic cytochrome P450 in rodents can be predictive of rodent liver carcinogenicity and can identify potential drug-drug interactions. In particular, induction of cytochrome P450 1A1 (CYP 1A1) can be indicative of an interaction with the aryl hydrocarbon or dioxin receptor signaling cascade. Short-term toxicology studies are ideally positioned in the drug development testing funnel to identify potential development limiting toxicities, such as profound P450 induction. Presented herein are results from a 7-day rat toxicology study with two compounds, PHA-X673 and PHA-X680, two compounds known to induce CYP 1A1 *in vivo* similar to the known Ah-agonist, TCDD. Male Han Wistar rats were dosed with either PHA-X673 (100 or 600 mg/kg/day, BID, po) or PHA-X680 (600 mg/kg/day BID, sq). Two control groups for the different dose administration routes (po and sq) were included. Due to excessive toxicity, animals in the PHA-X680 group and the respective dosage route control group were sacrificed early on Day 4. All animals treated with PHA-X673 survived to necropsy on Day 8.

PHA-X673 at the highest dose induced CYP 1A1 mRNA levels over 10, 000 fold as determined by Taqman analysis and increased specific activity over 2, 000 fold. PHA-X680 also increased CYP 1A1 mRNA levels and activity (~200 fold for each). Microarray analysis (Affymetrix Rat ToxChip) of mRNA from livers from animals treated with PHA-X673 and PHA-X680 revealed several Ah-inducible genes were upregulated, including a 145 fold and 10 fold increase in CYP 1A1, respectively. Further, PHA-X673 and PHA-X680 both increased hepatocyte proliferation, yet had no apparent effect on apoptosis, as measured by PCNA and caspase-3 immunohistochemistry, respectively. These data indicate that PHA-X673 and PHA-X680 profoundly activate the Ah receptor and may represent novel tools for understanding Ah receptor mediated toxicity.

449 FROM "OMICS" TO INSIGHT: THE USE OF A NOVEL COMPUTATIONAL APPROACH TO STUDY GENE-GENE INTERACTIONS.

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The linear approaches used historically in toxicology to unravel gene networks underlying the toxic response can be overly simplistic. In the present studies, a novel computational method was used to identify gene sets that predict a specific target gene. Transcript levels were categorized into ternary expressions and randomly divided into training and test sets. The conditional probability that a target gene takes on one of three transcriptional states was estimated for all possible predictor gene set patterns. The goodness of the predictor sets was verified by cross validation against the test data set and averaged over all iterations. The predictability of the gene set for each target was quantified by the coefficient of determination ((T=target) average ($|T_{obs}-T_{mean}|$) - average ($|T_{obs-Tpred}|$)]/average ($|T_{obs}-T_{mean}|$). This measure, along with cross-validated errors, provided a good criterion for identification of the optimal predictor sets. To maximize possible gene combinations, 40 Affymetrix mouse arrays of murine embryonic kidney, fetal heart, and vascular smooth muscle subjected to different chemical treatments were used to form predictor/training data sets. Biologically relevant gene predictor sets were calculated for the specified target genes, including Ahr and Cyp1b1. For Ahr, the best gene predictor set included angiotensin II receptor type 2, meprin 1-alpha, alpha-internexin, Mac-2 antigen and pigment epithelial derived factor. For Cyp1b1 the best predictor set included glutathione-S-transferase pi2, cytochrome P450 3a16, platelet derived growth factor inducible protein, runt-related transcription factor, and HMG1-c. With this information in hand, unique clues of biological interrelationships are being identified. (Supported by NIH grants ES04849, ES09106, CA90301, ES07273, and ESO9804).

450 DIFFERENTIAL EXPRESSION OF BRAIN PROTEINS IN THE DEVELOPING MICE EXPOSED TO METHYLMERCURY AND PCB CONGENERS THROUGH PERINATAL TRANSFER: A PROTEOMICS APPROACH.

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To explore the effects of methylmercury (MeHg) and PCB congeners either alone or in combination on neurodevelopment, the expression patterns of proteins were analyzed in cerebellums of mice exposed to neurotoxicants *via* perinatal transfer. From gestational day (GD) 7 until postnatal day (PND) 21, each group of pregnant C57BL/6 mice were exposed to control, MeHg alone (40 ug/kg), PCB 153 alone (5 mg/kg), PCB 126 alone (5 ug/kg), the mixture of PCB 153 (5 mg/kg) + PCB 126 (5 ug/kg), the mixture of PCB 153 (5 mg/kg) + MeHg (40 ug/kg), and the mixture of all three chemicals. At PND 9, and PND 30, cerebellums of neonatal mice were collected and total proteins were extracted. Two-dimensional electrophoreses were performed and protein expression patterns were compared between control and chemical-treated groups. At PND 9, forty nine protein spots were differentially expressed in the groups exposed to single chemical and thirty two protein spots were differentially expressed in the groups exposed to chemical mixtures. At PND 30, forty five protein spots were differentially expressed in the groups exposed to single chemical and thirty four protein spots were differentially expressed in the groups exposed to chemical mixtures. Individual protein spots are being identified by liquid chromatography-tandem mass spectrometry. The elucidation and quantitation of expression changes of critical proteins during the neurodevelopmental process will be important in the understanding of the toxicologic interactions of MeHg and PCB congeners. (Supported by NIEHS R03 ES 10116 and ATSDR Cooperative Agreement U61/ATU 881475)

451 MICROARRAY ANALYSIS TO EXAMINE CHANGES IN EXPRESSION LEVEL OF GENES AS A FUNCTION OF TIME OF DAY IN FISCHER 344 RAT LIVER.

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Circadian rhythm appears to regulate various biological processes, including hormonal activity and various aspects of metabolism. This fundamental element of life seems essential to consider in toxicology studies since circadian variation may influence the endpoints measured. To determine the influence of circadian rhythm on gene expression, we used microarray analysis, a powerful tool that measures on a massive scale relative expression level of the biological relevant genes that are expressed at any condition. Oligonucleotide microarrays used consisted of 3959 genes (Clontech, Palo Alto, CA). Sixteen *ad libitum* fed 12-months-old male Fischer-344 rats were sacrificed at four different time of day (0600, 1100, 1700, and 2300 hr, n=4 at each time point, 12 hr light (0200-1400)/ dark cycle). Liver tissues were stored in RNALater at -20°C until processed for RNA isolation. Total RNA isolated using TRI reagent was reverse transcribed to cDNA for indirect labeling with fluorescent cyanine dyes, Cy3 or Cy5. Each individual test sample was compared to a reference sample that was prepared by pooling equal amounts of total RNA extracted from livers of all 16 animals. Differentially labeled test and reference cDNAs were hybridized on a microarray. Reverse labeling was also performed to reduce dye-specific biases in signal intensity. Hybridized arrays were scanned using GenePix 4000B scanner and analyzed by GenePix Pro 3.0 software. Preliminary results indicated that of 3959 genes evaluated, 1369 (~35%) were expressed. Among the expressed genes, 37 genes had rhythmic circadian expression showing evidence of significant effect ($p<0.05$) of time of day. Most of 37 genes were associated with signal transduction pathways, immune function, and lipid metabolism.

452 VARIATION IN GENE EXPRESSION PROFILES FOR 17-α ETHINYL ESTRADIOL IN THE FATHEAD MINNOW, *PIMEPHALES PROMELAS.*

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The fathead minnow is an excellent freshwater fish model for biological indicator development for the following reasons: 1) the species widespread distribution in North America and 2) a lengthy history of use in acute and chronic testing of contaminants, effluents and receiving waters by North American and European ecotoxicologists. The inherent, dynamic patterns of gene expression that are present in an individual animal, within a specific tissue, will vary not only within the individual fish at other time points, but also among different individuals within the same population, between life stages, exposure duration and concentration of stressor. We have developed a low-density cDNA microarray representative of differentially regulated genes in response to a 24 hour exposure of 5 ng/L 17-α ethinyl estradiol for male fathead minnow (*Pimephales promelas*) liver. To examine the differences in individual variation, life stage, and dose concentration, we screened the array with samples derived from the exposure of individuals to different combinations of these 3 variables. Our data suggest that gene expression profiling will provide a sensitive measure of environmental estrogen response in the presence of age and other inter-individual variations.

453 IDENTIFICATION OF TRANSCRIPTOME FINGERPRINTS FOR THE DNA DAMAGING AGENTS BLEOMYCIN AND HYDROGEN PEROXIDE IN L5178Y MOUSE LYMPHOMA CELLS.

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Genomic technologies offer the prospect of defining the cellular pathways leading to chemically induced toxicity including genotoxicity. It is generally believed that some aspects of genotoxicity is associated with changes in the transcription levels of certain genes, especially those involved in DNA repair and cell cycle control. Additionally, it is hypothesized that chemicals that share a common mode of genotoxic action should display similarities in gene expression changes. We have evaluated these hypotheses by analyzing the transcriptome profiles of mouse lymphoma (L518Y/TK+/-) cells treated with bleomycin and hydrogen peroxide, two mutagens that induce genotoxicity through the generation of reactive free radicals. The cells

were treated for 4 hours and mRNA was isolated at the end of the treatment or after a 20 hour recovery. Transcriptome analyses were performed using Clonetech Mouse 1.2K cDNA microarray (1176 genes) and hybridizing with 32-P labeled cDNA synthesized from the mRNA of the treated cells. In addition, the mutant frequencies induced by each chemical in the treated cells were also determined to confirm the adequacy of the experimental conditions to induce genotoxicity. Of the 1176 genes examined, each of the two mutagens altered the expression (1.5-fold or greater) of only 2 genes at the end of the 4 hour treatment. In cells allowed to recover for 20 h, bleomycin and hydrogen peroxide affected the expression of 8 and 5 genes respectively. Many of the affected genes were upregulated and are associated with apoptosis. Of these genes, 3 were in common between chemicals, granzyme A, integrin beta 7 and 45kDa calcium-binding protein precursor. The expression of none of the DNA repair genes present on the array was affected. These results show that bleomycin and hydrogen peroxide have both unique and commonly regulated genes that may serve as useful biomarkers of exposure to agents causing DNA damage by free radical mechanisms.

454 GENE ARRAY ANALYSIS OF THE VENTRAL PROSTATE IN RATS EXPOSED TO EITHER VINCLOZOLIN OR PROCYMIDONE.

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Vinclozolin (Vi) and procymidone (Pr) are antiandrogenic fungicides. While changes in gene expression have been described in the ventral prostate (VP) following vinclozolin exposure, similar studies have not been conducted with procymidone, a compound proposed to share a common mechanism of action (see VS Wilson et al., SOT 2003). Here we examine the gene expression profiles of animals exposed to both compounds. 100 day old male SD rats were surgically castrated and administered silastic implants either with or without testosterone. A subset of testosterone treated animals were then orally dosed using 200 mg/kg of either vinclozolin or procymidone in corn oil. Four treatment groups with six animals per group were utilized: castrated (C), testosterone (T), testosterone+Vi (V), and testosterone+Pr (P). Tissue from the VP was collected from 3 animals per group at 20 hrs and 4 days after the start of treatment for isolation of total RNA followed by analysis using Clontech Atlas 1.2 Toxicology arrays. In support of the hypothesis that Pr shares a common mechanism or mode of action with Vi, similar changes in gene expression were observed in the C, P and V groups at both the 20 hr and 4 day time points. While only 36 genes were affected at 20 hrs, 156 genes were altered 4 days after the start of treatment. This may in part reflect regression of the VP at the later time point. Of note was an up-regulation of the androgen receptor after 20 hrs of treatment along with an up-regulation of clusterin after 4 days. Both changes were predicted in the C, P and T groups prior to the conduct of the study. Of interest for future studies is the kallikrein family of serine proteases which appeared to be a more robust marker of androgenic activity than clusterin. The effects of Vi after 4 days of treatment were, in a number of cases, greater than those of procymidone, suggesting that vinclozolin may be a more potent antiandrogen than Pr. A replicate of this study is currently being conducted. This abstract does not necessarily reflect EPA policy.

455 TOXICOGENOMIC EFFECTS OF LOW-LEVEL AS (III) EXPOSURE TO HUMAN KIDNEY CELLS.

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In order to answer the question of whether exposure of human kidney cells to low-level arsenic would affect gene expression. HEK293 cells were incubated with 1, 10, and 25 μM arsenite [As (III)] for 6 or 24 hr. Total RNA from treated and control cells was isolated, reverse transcribed, and labeled with Cy3 or Cy5, and hybridized to a high-density gene array system representing 5K non-redundant human cDNA clones. Hybridizations were performed at least three times using independent total RNA preparations to ensure reproducibility. In this study, 17 genes were up-regulated, and 14 genes were down-regulated, based on a 2-fold increases/decreases and consistent occurrence. Because the microarray we used contain a relatively small fraction (one seventh) of the expressed genes in HEK293 cells, by extrapolation As (III) could affect as many as 200 genes in the whole genome of HEK293 cells. The expression of affected genes showing a dose-dependent (1 - 25 μM) trend which was apparently not time-dependent (6 hr versus 24 hr). The affected genes indicate that even this realistic, low-level arsenite exposure was recognized by the HEK 293 cells (e.g., metallothionein genes) and produced an oxidative stress (e.g., heme oxygenase gene). These affected genes were characterized as stress response genes, proto-oncogene, hormone, transcription factors, chemokine receptors, proteolytic enzymes, EST and unknown genes. These findings imply that arsenite induces complex cellular injury and the cellular adaptation to As (III) is associated with alterations in the expression of many genes (NIEHS 04940).

456 HYDRAZINE AFFETCS EXPRESSION OF LIPID TRANSPORT AND METABOLISM GENES IN C57BL/6J MOUSE LIVER.

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The antituberculosis drug isoniazid (INH) can cause hepatic necrosis or steatosis. Neither the pathogenesis of the hepatotoxicity nor the damaging products are well understood. Both acetylisoniazid and hydrazine (HD) have been implicated as hepatoxic metabolites of INH. A study was initiated to test the hypothesis that alterations in lipid transport and metabolism are initiated prior to hepatic cell death. Gene expression profiles were determined after oral or intraperitoneal (ip) administration of the putative hepatoxic metabolite HD. Adult male C57Bl/6J mice received 100 mg/kg orally or by ip injection while control mice were given saline. Twenty-four hours later livers and blood samples were obtained from some animals for evaluation of hepatic cytotoxicity. Livers were harvested from the remaining animals, RNA isolated and used to synthesize cDNAs labeled with Cy3 or Cy5. Labeled cDNAs from control and treated animals were hybridized to a 5300-gene chip then scanned to detect fluorescence intensities. The expression ratios for all 3 animals from each group were subjected to a limit-fold change restriction. Both routes of administration of HD resulted in changes in gene expression; however, the expression of a greater number of genes was changed following the oral dose (270 genes and ESTs) compared to the ip dose (92 genes and ESTs). These included genes involved in lipid metabolism/transport (Apoa4, Apoa5, Aplp1) and energy balance (Atp2a1, Abcg1, and Ckmm). There were no significant increases in serum ALT levels or in hepatic activity of caspase 3 and caspase 8. These markers indicate that HD induced alterations in hepatic gene expression at a dose that did not result in hepatic necrosis or in capspase-mediated apoptosis. This suggests that the effects on lipid metabolism and transport do not arise as a secondary response to cell and tissue death but are associated with HD initiated patterns of gene expression. (Supported by R01 ES10047 and P30 ES06694).

457 PROTEOMICS CHARACTERIZATION OF THE EFFECTS OF A KINASE INHIBITOR ON PROTEIN EXPRESSION IN RAT LIVER.

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Protein kinases are key regulatory enzymes of signal transduction pathways and form a target family of great relevance in finding new drugs for treatment of numerous diseases. Compound X is a kinase inhibitor that has been developed for the treatment of asthma. In a 14-day oral toxicity study in rats, compound X produced an increase in liver weight and a marginal increase in liver enzymes (≤3-fold) at the high dose of 200 mg/kg but not at a pharmacological dose of 10 mg/kg. A proteomics study was initiated to identify molecular mechanisms which could be indicative of potential toxicity in the liver. After protein extraction, 2D gel electrophoresis was performed using differential gel electrophoresis technology. Protein identification of the up- and down-regulated spots was performed by mass spectrometry. Results showed that compound X, at the low dose of 10 mg/kg, induced over-expression of 40 proteins whereas at 200 mg/kg it gave an up-regulation of 69 proteins and a down-regulation of 20 proteins. Data bio-analysis showed that a number of biochemical pathways were modulated such as lipid and fatty acid metabolism, amino acid and protein metabolism and citric acid cycle. Proteins involved in anti-oxidant defence and xenobiotic metabolism were found to be strongly affected by treatment, suggesting that toxicity might be linked to induction of oxidative stress by a reactive metabolite in liver. In addition, mass spectrometry results showed that a few proteins such as carboxylesterase 4, fumarylacetoacetase and 3-oxo-5 beta steroid 4-dehydrogenase were detected as several spots corresponding to different isoelectric points. Opposite effects of compound X on the expression ratios of these different protein forms were observed at the highest dose, suggesting that drug-induced post-translational modifications could be used as a toxicity-specific signature of compound X in the liver.

458 GENOMIC ANALYSIS OF ALACHLOR-INDUCED ONCOGENESIS IN RAT OLFACTORY MUCOSA.

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The herbicide alachlor induces olfactory mucosal tumorogenesis in a highly ordered temporal process in specific regions of the rat nasal cavity. We used GeneChip analysis to test the hypothesis that histological progression and oncogenic transfor-

mation are accompanied by gene expression changes that might yield clues as to the molecular pathogenic mechanisms for the formation of these tumors. Acute alachlor exposure was associated with up-regulation of matrix metalloproteinases (MMP-) 2 and 9, as well as tissue inhibitor of metalloproteinase-1, carboxypeptidase Z, and many other genes related to extracellular matrix homeostasis and collagen regulation. Heme oxygenase, an indicator of oxidative stress, was upregulated early and maintained elevated expression. Post-acutely, expression of ebnerin, related to the putative human tumor suppressor gene DMBT1 and the polarity reversal protein hensin, progressively increased in alachlor-treated olfactory mucosa. The induction of ebnerin was confined to the epithelial cells undergoing progressive morphological changes that preceded tumor formation. Neoplastic progression from adenomas to adenocarcinoma was highly correlated with upregulation of genes that play key roles in the wnt signaling pathway. Activated wnt signaling within the adenocarcinomas was confirmed by immunohistochemical localization of beta-catenin to adenocarcinoma cell nuclei, but not in earlier adenomas or foci of respiratory metaplasia. These observations suggest that alachlor-induced initiation and progression of olfactory mucosal carcinogenesis is based on early alterations in extracellular matrix components, induction of oxidative stress, upregulated ebnerin expression, and final transformation to the malignant state accompanied by wnt pathway activation.

459 GENOMIC AND IMMUNOFLUORESCENCE ANALYSIS OF INTERACTIVE GENE NETWORKS IN OXIDANT-INDUCED ATHEROGENESIS.

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Oxidative injury to cells within the vascular wall is a critical event in the pathogenesis of atherosclerosis. To study the cellular and molecular basis of this complex interaction, this laboratory has adopted an *in vivo* model of repeated cycles of oxidative injury by allylamine (AAM), a cardiovascular-specific toxicant that induces vascular lesions reminiscent of those seen in human atherosclerosis. AAM is metabolized within the vascular wall by a vascular specific enzyme, known as semicarbazide sensitive amine oxidase, to acrolein and hydrogen peroxide. These metabolites compromise redox homeostasis in vascular smooth muscle cells leading to oxidative stress, activation/ repression of redox-regulated genes, peroxidative injury, and cell death. To study these interactions, six week-old (175-180g) male Sprague-Dawley rats were gavaged daily with 35 or 70 mg/kg/day AAM or water as control (1ml/kg/day) for twenty days. The occurrence of aortic vascular lesions was documented by light microscopy. mRNA from thoracic aortas was isolated and hybridized against the rat genome Affymetrix microarray. Frozen sections of the same vessels were also processed for immunofluorescence analysis of selected proteins at sites of vascular injury. Tissues were visualized using a Bio-Rad RTS200MP confocal microscope equipped with 4, 6-diamidino-2-phenylindole, dihydrochloride (DAPI) long-pass (LP) and fluorescein-5-isothiocyanate (FITC) filter sets. Multiple gene and protein expression patterns were altered by AAM-induced oxidative injury. Key interactive gene networks involved in diverse cellular activities such as adhesion, extracellular matrix composition, cytoskeletal arrangement, growth, and signal transduction were altered by repeated cycles of chemical injury. (Supported by NIH grants HL 22863, HL 62539 and ES09106).

460 DETERMINATION OF E3 PROTEIN INTERACTIONS: CLUES TO CLAM TUMORIGENESIS?

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Gonadal tumors of the softshell clam (Mya arenaria) have been found in several eastern Maine populations. While the etiology of these germinomas is unknown, one hypothesis is that environmental contaminants are contributing to their formation. Sites with a high prevalence of gonadal cancer have been polluted by herbicides historically contaminated with dioxin. Using a differential display polymerase chain reaction, an E3 ubiquitin-protein ligase was initially identified in gill tissue from clams exposed to dioxin in the laboratory. Ubiquitin-protein ligases are responsible for ubiquitinating a target protein, thereby specifying it for degradation. Clam E3 function as a ubiquitin-protein ligase was confirmed by an *in vitro* ubiquitination assay. Clam E3 also has significant sequence similarity to E6-AP, a well-characterized ubiquitin-protein ligase that plays a role in the abnormal degradation of the tumor suppressor p53 in human cervical cancer. Increased clam E3 protein levels have been observed in female tumor-bearing gonads compared to normal gonadal tissue, suggesting a role of E3 in these tumors. Clam E3 also contains the key regions for p53 binding and degradation. The interaction of clam E3 with clam p53 was tested with GST pull-down assays. Results indicate that clam E3 interacts with clam p53, although the specificity of this interaction has not yet been confirmed. A yeast two-hybrid system is being employed to identify proteins that interact with clam E3, which may potentially include p53. Yeast vectors encoding N-terminal and C-terminal LexA-E3 bait fusion proteins have been constructed for screening clam gill and gonad cDNA libraries for potential interactors. Additionally, clam p53 will be constructed as a target protein for clam E3 in yeast to specifically determine if these two proteins interact. By discovering potential targets and interacting proteins of clam E3, we hope to further understand the mechanism of gonadal tumor formation in the softshell clam.

461 IDENTIFICATION OF RAT LIVER CYTOSOL PROTEIN TARGETS OF ACRYLONITRILE *IN VIVO* USING TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MASS SPECTROMETRY.

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Acrylonitrile (AN) is an organic compound produced in large quantities by the chemical industry. It is used as a monomer in the production of various polymers. Acrylonitrile is an acute toxin. The long-term goal of this research is to identify the mechanism responsible for the acute lethality of AN. Several mechanisms have been proposed to explain the toxicity of AN: Glutathione depletion with subsequent oxidative tissue damage, metabolism by P450 followed by release of cyanide (CN) and covalent binding to tissue proteins. Glutathione depletion doesn't seem to be the major factor involved in lethality because diethylmaleate, which is a potent glutathione depletor, is not particularly toxic. Cyanide, although toxic by itself, is not the only mechanism involved in the acute toxicity of AN, because preventing CN formation by P450 inhibitors does not prevent AN induced lethality. Covalent binding to tissue proteins could alter the function of vital proteins and might prove to be a major component in AN-induced toxicity. This study addresses this last mechanism. Rats were treated with 115 mg/kg (LD90) of C14 labeled AN. Liver cytosolic proteins were separated by two-dimensional polyacrylamide gel electrophoresis using immobilized gradients of pH 3-10. Radiolabeled spots were in-gel digested and identified by peptide mass fingerprinting using matrix-assisted laser desorbtion/ionization mass spectrometry. More than 10 radioactively labeled proteins have been identified to date and efforts are continuing to complete a database of rat liver protein targets of AN. This database will be a valuable resource for investigation of the biochemical basis of AN acute toxicity. Understanding the mechanism of toxicity of AN will help design better treatments for AN intoxication, as well as for compounds with a similar mechanism of toxicity. (The project described was supported by grant number ES06141 from the National Institute of Environmental Health Sciences, NIH).

462 IN SILICO IDENTIFICATION OF ESTROGEN RESPONSE ELEMENTS IN HUMAN AND MOUSE SEQUENCES.

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The biological actions of estrogen are mediated by estrogen receptor (ER) alpha and beta. Both ERs specifically bind to estrogen response elements (EREs) with high affinity. EREs are detected in the promoter and transcribed regions of many estrogen responsive genes. The perfect ERE (pERE) is a 13 bp (basepair) palindrome with two 5 bp arms and 3 bp spacer in between (GGTCAnnnTGACC). Direct binding of ER to the ERE can activate gene expression in response to estrogen. Half ERE sites can respond to ER signal through transcriptional modulators such as AP1 or Sp1. The purpose of this study is to identify EREs and other estrogen responsive regulons in silico within mouse and human genomic sequence data. Approximately 8, 000 mouse and 15, 000 human gene promoter sequences were extracted from the UCSC genome browser (http://genome.ucsc.edu), indexed by RefSeq ID. A JAVA program to search for pERE and one bp mismatch EREs in the dataset has been developed and has initially identified 341 mouse and 569 human genes that contain pEREs within their promoter or transcribed region. Twenty-five of these ERE-regulated genes were conserved between human and mouse, and published reports confirm estrogen regulation for at least 15 of the in silico identified genes. In a refined search of the 5000 bp upstream from the transcription start site, 59 mouse genes and 70 human genes were identified. Of these, 51 mouse genes and 47 human genes have products with known function including 9 genes related to JAK/STAT signal transduction pathway, 7 genes related to transcriptional regulation, 7 gene products located in nucleus and 12 gene products associated with the membrane, all of which are consistent with known anchors of estrogen. Many of the remaining genes currently do not have well defined functions, but their role in estrogen-induced responses is suggested. This work was supported by National Institute of Health Grant ES 011271.

463 RECIPROCAL REGULATION OF CYTOCHROME P450 3A1 AND P-GLYCOPROTEIN IN RAT LIVERS BY CARBON TETRACHLORIDE AND CHLOROFORM.

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P-Glycoprotein (MDR1) is expressed on the bile cannalicular membrane in the liver, where it plays a critical role in the biliary excretion of a wide array of cytotoxic xenobiotics. Cytochrome P450 3A (CYP3A) enzymes are the most abundant P450 enzymes in the liver, and they transform a wide array of xenobiotics including many substrates of MDR1. A number of studies have described similar regulation (e.g. coinduction) of MDR1 and members of the CYP3A family, suggesting a cooperative relationship between these key detoxification proteins. Here, we describe a reciprocal regulation of MDR1 and CYP3A1 in the rat liver in response to treatment with carbon tetrachloride (CCl4) and chloroform (CHCl3). Livers from Sprague-Dawley rats injected intraperitoneally with CCl4 (0.25 ml/kg and 1 ml/kg) and CHCl3 (0.25 ml/kg and 0.50 ml/kg) were sacrificed at 6h, 24h, and 72h after administration for microarray gene expression measurement. Statistically significant ($p < .01$) induction of MDR1 was observed at 6, 24, and 72 hours after administration of both doses of CCl4, with maximum induction observed at 24 hours for both the 0.25 ml/kg (1.9-fold) and 1 ml/kg (3.5-fold) doses. Statistically significant ($p < .05$) suppression of CYP3A1 was observed at 6 and 24 hours after administration of CCl4, with maximum suppression observed at 24 hours for both the 0.25 ml/kg (-2.3-fold) and 1 ml/kg (-5.4-fold) doses. Statistically significant, maximum induction of MDR1 and maximum suppression of CYP3A1 were also observed after administration of 0.5 ml/kg CHCl3 at 24 hours. There was a negative correlation between the expression of MDR1 and CYP3A1 in CCl4- (-0.667) and CHCl3-treated (-0.374) liver samples, but not in samples treated with dexamethasone, a known CYP3A1 and MDR1 inducer (0.267). In summary, our results show a similar reciprocal modulation of MDR1 and CYP3A1 by CCl4 and CHCl3.

464 CHEMICAL DISRUPTION OF GLOBAL GENE EXPRESSION DURING NEPHROGENESIS: ROLE OF ARYL HYDROCARBON RECEPTOR.

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This laboratory has recently shown that unregulated activation of aryl hydrocarbon receptor (Ahr), a member of the large basic helix-loop-helix (bHLH) and PAS homology domain family of transcription factors, participates in the regulation of mesenchymal-to-epithelial transition during nephrogenesis. This response involves modulation of alternative splicing and post-transcriptional control of the Wilms' tumor suppressor (wt1) gene. The present studies were conducted to test the hypothesis that Ahr mediates disruption of nephrogenesis by BaP. In these experiments, organ cultures of E11.5 metanephric blastema from Ahr wild type, heterozygous and null C57BL/6J mice were treated daily with 3 μM BaP for 4 days. Microarray analysis identified eight discrete gene classes altered by hydrocarbon challenge. These genes are involved in cellular differentiation, cell proliferation, apoptosis, cell cycle progression, stress response, transformation, extracellular remodeling and transcriptional control. While many of the genes identified are known to participate in nephrogenesis (GDNF, frizzled receptor, IGFR, syndecan, and laminin), others such as Sry, oncostatin M, pinin, GATA-3, CCAAT/enhancer-binding protein, Sox-18, Nrf-2, FBG-MuSV, GRO1 oncogene, cyclin G and fibulin were novel targets for developmental dysregulation. Genes of the Ahr gene battery, such as cytochrome P450s, diaphorase, and glutathione S-transferase were affected in an Ahr dependent manner. A significant degree of reciprocity in terms of Ahr dependence, was observed in the regulation of several novel target genes, most notably kidney androgen regulated protein and hnRNP-associated with lethal yellow. These data implicate unregulated Ahr signaling, either through P450-catalyzed bioactivation or transcriptional and post-transcriptional interference, in genomic alterations during disruption of nephrogenesis by BaP. (Supported by NIH grants ES04917, ES09106 and CA90301).

465 DIFFERENTIAL MODULATION OF HEPATIC CASPASE GENE EXPRESSION BY TAMOXIFEN AND ESTRADIOL IN SPRAGUE-DAWLEY RATS.

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As part of our program in the application of toxicogenomics to evaluate drug-induced hepatoxicity, we report here our results on the effects of two agents, the hormone estradiol and its hepatotoxic analog, the antibrest cancer drug tamoxifen. Sprague-Dawley rats were treated with each compound intraperitoneally (i.p.) for 24, 48 and 72 hours. The animals were sacrificed at 72 hours post-treatment, and the livers were harvested for total RNA extraction and for histopathology evaluation. We hybridized the total RNA from the livers to our Rat Comprehensive Toxicology (RCT) microarrays and measured the gene expression as relative to control. Gene expression results were analyzed using MatrixExpress(tm) software. We found that the caspase genes were modulated in response to the treatments with estradiol and tamoxifen. Estradiol administration apparently led to the suppresion of the expression of caspase 1, caspase 3 and caspase 7 genes, while tamoxifen administration led to the induction of the expression of caspase 3 gene, with no apparent effect on caspase 1 and 7 genes. Neither compounds had significant effects on caspase 6 gene expression. We will continue our research to evaluate the relationship between the differential effects of estradiol and tamoxifen on caspase gene expression and their hepatotoxic potential. Our results suggest that toxicogenomics may be used as a tool to identify the differences in biological effects the effects of structurally-similar drugs and thereby guide the selection of most appropriate drug candidates for further development.

466 USE OF A TOXICOGENOMIC APPROACH AS A POTENTIAL MEANS TO INVESTIGATE IDIOSYNCRATIC HEPATOTOXICITY OF QUINOLONES AND THIAZOLIDINEDIONE COMPOUNDS.

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Idiosyncratic drug toxicity, defined as toxicity that is dose-independent, host-dependent, and usually cannot be predicted during preclinical or early phases of clinical trials, is a particularly confounding complication of drug development. This type of toxicity is not limited to any one class of drugs, but has been seen in a wide spectrum of compound classes including quinolones and thiazolidinediones (TZDs). Clearly, an understanding of the mechanisms that lead to idiosyncratic hepatotoxicity would be extremely beneficial for the development of new compounds. The application of microarrays towards toxicology, termed toxicogenomics, has been used to identify toxic mechanisms. Several recent publications have utilized microarray analysis to identify discrete gene changes. In addition, research has shown that compounds associated with a particular mechanism of toxicity yield similar gene expression profiles. We are examining two classes of compounds: quinolone antibacterials and TZDs for diabetes. MTT studies were conducted to identify cytotoxic dosage range. Subsequently, several quinolone compounds were supplemented into human hepatocyte incubation medium. Due to known low aqueous solubility of quinolones, HPLC analysis was completed to verify compound concentration and to be cognizant of compound degradation. The total cellular RNA was harvested and subjected to microarray analysis using a human genome chip. Preliminary results reveal that clinafloxacin has a distinct global gene expression pattern from the other quinolones investigated. Furthermore, Mitoscan assay results suggested that troglitazone is a more potent mitochondrial damaging agent than the other TZDs studied. These experiments will provide more information regarding the use of human hepatocytes in toxicogenomics studies. Ultimately, we hope to identify marker genes that can be used to screen compounds for potential idiosyncratic toxicity.

467 MICROARRAY ANALYSIS OF ADIPOGENESIS: USING TCDD AS A TOOL TO DETERMINE GENE CHANGES CRITICAL FOR DIFFERENTIATION.

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The multipotential C3H10T1/2 cell line can be differentiated into adipocytes using a hormonal mixture (IDM/BRL) consisting of insulin, dexamethasone, isomethylbutylxanthine and a ligand for PPARγ. In the first 24 hours of differentiation, IDM/BRL generates an essential change in cell morphology and an increase in gene expression related to the commitment to adipogenesis. Between 24 and 48 hours of differentiation, several endpoints associated with cell cycle regulation are downregulated, including several cyclins and the phosphorylation of RB. Microarray analysis has been carried out on the IDM/BRL-induced changes present at the interface of these two phases, 24 hours after treatment. The analysis was carried out under conditions in which IDM/BRL was added to depleted media (unrenewed serum protocol), and not in the presence of fresh serum. The use of the unrenewed serum protocol has the benefit of eliminating multiple inessential gene changes caused by serum addition. In the initial analysis, 304 genes were identified that changed significantly in three independent experiments. Of these genes, 130 decreased in expression and 174 increased in expression. Typical markers of the commitment phase of adipogenesis were identified, including C/EBPβ, C/EBPδ, SREBP1 and SCD1. Interestingly, 10 genes associated with the cytoskeleton were downregulated by IDM/BRL treatment. Other genes involved in cell signaling, extra cellular matrix and cellular redox status were also identified. The environmental contaminant TCDD has previously been shown to inhibit adipocyte differenti-

ation through a mechanism that is dependent upon active ERK. TCDD therefore has additionally been used as a tool to inhibit gene changes required for adipocyte differentiation. Gene changes associated with IDM/BRL that are reversed by the combination of TCDD and ERK identifies mechanisms that are critical for differentiation.

468 INVESTIGATION OF DNA REPAIR AND CELL CYCLE ARREST FOLLOWING AFLATOXIN B_1 TREATMENT IN YEAST EXPRESSING HUMAN CYTOCHROME P450 1A2.

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Aflatoxin B_1 (AFB) is a human hepatotoxin and hepatocarcinogen produced by the mold *Aspergillus flavus.* In humans, AFB is bioactivated by hepatic cytochromes P450 (CYP) 1A2 and 3A4 to a genotoxic epoxide that forms N^7-guanine DNA adducts. Relatively little is known about the cellular response to these bulky DNA adducts. Characterization of global gene expression patterns will lend insights into the molecular responses to DNA damage that may result in gene alterations that either protect cells from further damage and mutagenesis, or result in necrosis or apoptosis. Previously, we characterized transcriptional responses to AFB genotoxicity using yeast cDNA microarrays, using dose and time points that are minimally cytotoxic but substantially genotoxic, in a human CYP 1A2-expressing diploid strain of yeast. In the current study, flow cytometric analysis demonstrated a dose and time-dependent S phase delay under these same treatment conditions. Additional microarray studies were analyzed by cluster analysis. It was found that *RAD51* and ribonucleotide reductase genes (*RNR1*, *RNR2*, *RNR3*, *RNR4*) were co-upregulated in a dose and time-dependent manner, as well as *RAD53* and several other DNA repair genes. Haploid mutants defective in DNA repair genes and cell cycle checkpoint were transformed with human CYP 1A2 to investigate the role of recombinational repair and *MEC1* pathway after AFB treatment using a colony forming assay and flow cytometry. Both *rad51* and *mec1* exhibited decreased cell survival rates after AFB treatment, relative to their wild type. The S phase delay induced by AFB was abolished in *mec1-1* and *mec1-1/rad53* strains, but not in *rad51*. These results indicate that, in a yeast model, *RAD51* recombinational repair plays an important role in repair of AFB-induced DNA damage, and *MEC1* is involved in mediating AFB-induced S phase delay. (Supported by grants R01ES05780, P30ES07033 and U19ES011387).

469 IMPACT OF THE PEROXISOME PROLIFERATOR CLOFIBRIC ACID ON CELLULAR STRUCTURE AND TRANSCRIPT PROFILE IN MOUSE LIVER UPON 1-WEEK IN FEED TREATMENT.

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The new field of toxicogenomics, with the aid of genome-wide arrays, promises to deliver a complete view of all the molecular processes in the cell. Such a complete view should enable us to simultaneously monitor the on-target effect of a drug as well as its toxicological side effects and other perturbations of the biological system. To deliver on this promise we need a robust experimental design capable of detecting small, but statistically significant, changes in the gene expression level combined with functional annotations to a level where the biological interpretations can easily be made. In this regard gene ontologies are emerging as a useful tool to unify and simplify biological interpretation. In the present study we investigated the treatment-related effects of Clofibric acid (CLO), a well-studied peroxisome proliferator and non-gentoxic liver carcinogen in rodents. CLO was mixed into the diet and administered to groups of five mice at doses of 0.25 or 0.5% for one week. The equally sized control group received only the diet. In the liver traditional toxicological end points such as organ weight, histomorphology and cellular ultrastructure were investigated in conjunction with a genome wide transcript profiling using DNA micro arrays. The results indicate that all known morphological and molecular changes in the cell such as peroxisome proliferation, oxidative stress, inflammatory response, changes in lipid and glucose metabolism, and apoptosis could be detected in a single micro-array experiment.

470 CARCINOGENICITY PREDICTION BY PROTEOME ANALYSIS: 2D-DIGE-BASED EXPRESSION MONITORING.

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Proteomics aims to chart the ebb and flow of tens of thousands of proteins at once to produce snapshots of cell status. These protein expression patterns potentially represent signatures of organ responses to specific chemicals and may offer insights into the processes accompanying tumorigenesis. We ran proteomic analyses on liver homogenates of control and treated animals using the quantitative fluorescent 2D-DIGE method (Amersham Biosciences, UK Limited). The 10 chemical compounds tested included genotoxic and non-genotoxic carcinogens as well as non-carcinogens. Each gel contained differentially labeled homogenates from treated and control animals. For example, 2D-DIGE of one carcinogen treated liver (250mg/kg/day, 28days) indicated significant ($p<0.001$) quantitative changes in 108 liver proteins compared to controls. To enable comparison among different chemical treatments, all gel images (>300 gel images) were matched to one master gel using Decyder software. Based upon such matching, we were able to apply data mining with bioinformatics tools such as cluster analysis of the various protein expression profiles. All four tested non-carcinogens were successfully grouped into the same cluster. This study is part of an on-going systematic large scale project supported by METI (Ministry of Economy, Trade and Industry, Japan) utilizing emerging technologies such as toxicogenomics and toxicoproteomics to developing quick and reliable alternatives to traditional long-term rodent protocols for carcinogen risk assessment.

471 THE EFFECTS OF POOLING RNA SAMPLES IN A DNA MICRO ARRAY TOXICOGENOMICS STUDY WITH CLOFIBRIC ACID IN MICE.

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It is a common practice to pool RNA samples for DNA micro array analysis with the obvious benefit of reducing the size, cost and complexity of an experiment and also simplifying the data analysis. However, it is important to understand what information is lost in the pooling process so that the best balance between pooling and not pooling in an experimental design can be achieved. In this experiment Clofibric acid was mixed into the diet and administered to groups of five mice at doses of 0.25 or 0.5% for one week. The equally sized control group received only the diet. Liver RNA from individual animals was analyzed on MGU74A GeneChip expression probe arrays. In parallel we pooled equivalent amounts of RNA from all livers of each experimental group and analyzed them along with the replicate samples to answer the following question: what information is lost during pooling and can we still reach the same biological conclusions? A key observation made from this data set was that, while pooled and replicate data may appear to agree on the array wide scale, they do not yield the same results as robust statistical methods of gene selection cannot be applied to unreplicated pools. This is due to the loss of our ability to measure the consistency and reliability of gene expression changes in the pooled data. A second observation was that global measures of similarity might not be sensitive enough to detect outliers, such as small subsets of transcripts that are affected by a technical cause. In both cases, replicate data was more informative that pooled data without replication. Pooling samples for analysis on micro arrays may have a benefit by reducing variance in large studies, but the pool must be replicated to provide robust results. That is given a large number of replicate biological samples, creating several pools of equal size is better than creating a single pool. In this way we get the benefits of reduced variance from pooling without loosing the ability to measure variance.

472 BACKGROUND AND CHEMICALLY INDUCED VARIATION IN CDNA MICROARRAY PROFILES: EVALUATION OF THE INFLUENCE OF RAT STRAIN AND DIET.

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DNA microarray technology is being employed in a number of screening, hazard identification and mechanistic studies. Several potential sources of variation in transcriptome levels of test animals were investigated using membrane-based, 1176 gene cDNA microarrays. The influence of rat strain (male CD vs CDF), and diet (ad libitum, diet restriction, and phytoestrogen-free) on background and benzo(a)pyrene (B[a]P)-induced gene expression profile in liver was examined. Results were compared to functional assays of several Phase I and II enzymes. In untreated controls of both strains, the numbers of expressed genes were significantly higher in the restricted and phytoestrogen-free groups than ad libitum groups. In CD rats, B[a]P-treatment resulted in a higher numbers of up- and down- regulated genes in estrogen free group rats relative to either other diet group. In particular, relatively few genes were down-regulated in the ad libitum group. In CDF rats, B[a]P treatment resulted in more up-regulated genes in restricted and ad libitum groups than phytoestrogen-free group rats and little difference between diets for down-regulated genes. CYP1A1 and CYP1A2 were up-regulated by B[a]P treatment in both strains while the metallothionein gene was up-regulated only in CD rats under all dietary conditions. Gene expression was reflected in induction of

specific Phase I enzymes in liver of rats treated with B[a]P. Results demonstrate the need to take strain and diet into consideration when interpreting cDNA microarray data.

473 SIMULTANEOUS MULTIPLEXED TOXICOGENOMIC AND TOXICOPROTEOMIC PROFILING.

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Toxicogenomic and toxicoproteomic biomarkers are becoming critical to efficient lead development, since multi-analyte assay panels revealing patterns of gene and protein expression can predict toxic effects. In addition, researchers require multiplex analyte assays that analyze hundreds of mRNAs or proteins from thousands of samples. Traditional methods such as microarrays, quantitative PCR and ELISA assays do not meet these demands. Here we describe the eTagTM Assay System as an ideal method for the investigation of both gene and protein expression profiles used for the characterization and classification of chemical toxicants. The eTag Assay System is ideal for high throughput sample analysis using completely solution-phase assays that are easy to automate and that work with limited biological samples. This - solution phase system does simultaneous quantitative detection of specific mRNAs and proteins direct from cell lysate in a homogeneous and isothermal manner. In both the eTag gene expression and protein assays, eTag reporters are released from target recognition complexes in direct proportion to the amount of specific mRNAs or proteins in the sample. These released eTag reporters are then separated and quantified using capillary electrophoresis. eTag reporters are low molecular weight fluorescent labels with unique and well-defined electrophoretic mobilities and can be clearly resolved using standard capillary electrophoresis instruments. The eTag Assay System provides the multiplexed analyte results that have low background, high precision, small bio-sample requirements, wide dynamic range, and complete separation of signals generated for each individual mRNA and protein. Custom configured analyte panels can be easily assembled from existing core multiplex panels in a modular fashion. In addition to assay validation, analysis of genes and proteins involved in toxicological profiling will be presented. This new system meets the unique demands of toxicogenomic and pharmacogenomic screening in lead discovery.

474 DATA INTEGRATION IN THE NEW ERA OF TOXICOGENOMICS.

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The application of new technologies such as genomics, proteomics and metabonomics to toxicity research has created the need to store, integrate, mine, and visualize enormous amounts of data. “Panomic” analysis of tissues and fluids from even a single investigative study can easily generate over a million data points. Integration of these data is a challenge because the data are generally stored in separate databases and are of distinct formats. To bring these disparate data into a common resource, we developed an Oracle-based information management system capable of capturing the data derived from different technical platforms and the tools and an interface to make that data easily accessible to investigators. We have used this system to integrate the genomic, proteomic, and metabonomic data from an individual animal with conventional clinic chemistry, hematology, and histopathology data from the same animal. We believe such a data integration approach greatly increases the value of toxicogenomic studies by enabling us to use statistical approaches to identify previously unknown relationships among diverse data types.

475 THE ACUTE EFFECT OF ETHANOL ON CNS AND ITS DEPENDENCE ON ALDH2 POLYMORPHISM MEASURED BY THE SIMPLE LIGHT REACTION TIME USING ELECTROMYOGRAM.

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The acute effect of ethanol on CNS was measured by the simple light reaction time using electromyogram (LRT-EMG) and its interaction with ALDH2 polymorphism was analyzed. Our institutional ethical committee approved this study project. The subjects were 10 healthy males. Six of them were randomly allocated to exposure group and remaining was allocated to control group. Each subject of exposure group took ethanol (0.4/kg) orally and each subject of control group took water orally. LRT-EMG tests were performed after -0, 20, 60, 90, 120, and 180 min, and blood was corrected after -0, 15, 60, 120, and 180 min. The LRT-EMG measurement includes pressing a switch when red LED flashes and keeps it pressing until the time S1 when the trigger shoots and blue LED flashes. Subjects are noticed to release the button as quickly as possible when they recognized the appearance of blue LED light. At that occasion the EMG of muscle extensor indices of the dominant hand side and the voltage level that show on and off level of the switch were monitored. Reaction time was defined by the time difference between the button release and S1. Conduction time was defined by the time difference between the appearance of EMG and S1. Movement time was defined by the difference between Conduction time and Reaction time. We also measured ethanol and acetaldehyde concentrations of blood and ALDH2 gene type. The concentrations of ethanol and acetaldehyde in blood steeply increased at 15 min after the intake and atypical ALDH2 cases did not return to the control level even after 180 min. Among the atypical ALDH2 exposure group movement time decreased at 60 minutes after the intake but there were no change among the typical ALDH2 group. Acetaldehyde might enhance the speed of finger movement. Conduction time elongated among control group, whereas Conduction time did not change among exposure group. This elongation might due to the decline of the concentration induced by tiredness and boring occurred among control group and not among exposure group.

476 HEPATOTOXICITY BY ACUTE ETHANOL INTAKE IN *ALDH2* GENE TARGETING MOUSE.

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The genetic polymorphism of aldehyde dehydregenase-2 (ALDH2), which is the important enzyme in ethanol metabolism, has been characterized as *ALDH2*2* in contrast to the wild type, *ALDH2*1*. To investigate the *in vivo* significance of *ALDH2* polymorphism, mice lacking *Aldh2* were created for the use of gene targeting in embryonic stem cells (K. Kitagawa et al. 2000). The ethanol-induced cytochrome -CYP2E1- generates reactive oxygen species, activates many toxicologically important substrates, and may be the important pathway by which ethanol causes oxidative stress. There is concurrently modification of glutathione, which is the major redox buffer and which may be modulated by TNF-alpha secretion in the liver receiving ethanol. In this study, mRNA in the liver tissue of the *Aldh2* gene targeting mouse, which received 5 g/kg body weight ethanol p.o., was extracted for real time PCR assay for Cyp2e1 and cytokines. The amount of total glutathione in the liver tissue was measured at the same time. There turned out to be a significant difference in the Cyp2e1 mRNA expression by *Aldh2* genotypes.

477 INFLUENCE OF METABOLIC GENOTYPES ON CHROMOSOME ABERRATIONS IN BUTADIENE EXPOSED WORKERS.

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1, 3- Butadiene (BD) is believed to exert its genotoxic effects after metabolic activation to epoxides. Inherited variations in the activity of BD activating and detoxifying enzymes may be responsible for individual differences in susceptibility to BD exposure. In the present study the influence of metabolic genotypes on chromosomal aberrations (CA) in lymphocytes of 10 clerks (controls) and 30 workers exposed to low levels of BD (4-201 μ g/m3) was investigated. The study was approved by the Italian local health authority and informed consent was obtained from all participants. The total number of CA, gaps excluded, was nonsignificantly (p=0.15) higher in the workers (mean % aberrant cells =1.4) compared with controls (0.9%). The urinary BD metabolite N-acetyl-S-(3, 4-hydroxybutyl)-L-cysteine (0.48-4.55 mg/g creatinine) and hemoglobin N-(2, 3, 4-trihydroxybutyl)-valine adducts (17.7-61.3 pmol/g globin) were significantly correlated to CA in workers (r=0.46 and r=0.39, respectively) as well as in the total group (r=0.38 and r=0.35, respectively). Stratification according to the glutathione transferase (GST) M1 genotype showed that the correlations were only evident in the subgroup lacking GSTM1 (r=0.61 vs – 0.14 for the urinary metabolite and r=0.51 vs 0.02 for the hemoglobin adduct). Individuals lacking GSTT1 had significantly higher CA compared to those possessing this gene. Furthermore, polymorphisms in GSTP1 and alcohol dehydrogenase 1C also affected the CA frequency. In conclusion, genetic polymorphisms in genes relevant for the metabolism of BD appear to influence the frequencies of CA in human lymphocytes.

478 EXPLORATION OF ALTERNATIVE SPLICING OF MONOAMINE OXIDASE-B TRANSCRIPTS BY THE INTRON 13 POLYMORPHISM.

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The role of monoamine oxidase B (MAO-B) in Parkinson's disease (PD) has been an area of interest for many years due to its role in dopamine catabolism and bioactivation of MPTP. A common polymorphism in intron 13 of the MAOB gene, an A->G substitution located 36 bases upstream of exon 14, was discovered and subsequently shown to be associated with PD, with a relative risk of ~2 for carriers of the G allele. No direct phenotypic effect of the polymorphism has yet been elucidated. The goal of the current project is to investigate whether the intron 13 polymorphism is associated with alternative splicing of MAOB transcripts. cDNA from astrocytes of intron 13 genotype A (normal human astrocytes) and genotype G (human astrocytoma cell line 132 1N1) was obtained by reverse transcription of total RNA using gene-specific primers. cDNA fragments from the 3' region of exon 13 to the 5' region of exon 14 were then amplified by PCR and cloned. Sequences of 20 clones obtained from each genotype were identical to the sequence of the predicted normal transcript. We conclude that MAO-B intron 13 genotype does not directly affect splicing and thus a genetic explanation for the observed difference in Parkinson's disease risk due to intron 13 genotype remains to be discovered. This research was supported in part by National Institute for Environmental Health Sciences Grants ES-04696, 10750, 07032, 07033.

479 GENETIC SUSCEPTIBILITY TO ASBESTOS-RELATED DISEASES.

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The relationship between genetic susceptibility and environmental exposure in the development of disease has been hypothesized for many years. It has also been hypothesized that inherited differences in DNA sequence contribute to phenotypic variation, influencing an individual's risk of disease and response to the environment. A promising approach to dissect this association is to systematically explore the common gene variants that may be associated with disease. Although asbestos-related diseases (ARD) are among the most well studied occupational diseases, relatively little is known about the host factors that may affect individual susceptibility. Candidate genes implicated in susceptibility or resistance to ARD include those involved in inflammation and subsequent fibrosis. We are interested in the role of single nucleotide polymorphisms (SNPs) in these genes in the association between asbestos exposure and the development of ARD. Since 93% of genes contain a SNP, and 98% of genes are within 5 kilobases of a SNP, this approach provides a powerful tool for genetic analysis. DNA was isolated from blood samples donated by Libby MT and Missoula MT residents (as exposed and control populations, respectively). PCR was used to determine the distribution of the constitutional deletion of glutathione S-transferase M1 gene (GSTM1). PCR followed by restriction enzyme digestion (PCR-RE) was used for SNP analysis of the GSTP1 gene. Constitutional deletion of GSTM1 resulted in an odds ratio of 1.58 (95% CI 0.676-3.144), but without enough power to achieve significance. SNPs in the GSTP1 gene did not appear to contribute to the development of ARD, with the caveat that more samples are needed for conclusive analysis. PCR-RE was used to examine a SNP in the interleukin 1 beta (IL1B) gene, and was demonstrated to contribute to ARD susceptibility ($p<0.05$). Further studies will be needed as asbestos-response genes are identified in other ongoing investigations. This work was supported by R21 ES11676-01 (EAP).

480 GLUTAMATE CYSTEINE LIGASE CATALYTIC SUBUNIT TRINUCLEOTIDE REPEAT POLYMORPHISM AND TYPE I DIABETES.

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Glutathione (GSH) protects against reactive oxygen species (ROS)-mediated cell injury. Glutamate cysteine ligase (GCL), the rate limiting enzyme in the synthesis of GSH consists of catalytic (GCLC) and modifier (GCLM) subunits. T1DM is an autoimmune disease involving auto-reactive T-cells and ROS which cause pancreatic beta-cell destruction. We hypothesized, that individuals with low endogenous levels of antioxidant enzymes, such as GCLC, may be susceptible to T1DM. A polymorphic GAG trinucleotide repeat (TNR) exists in the 5' region of the GCLC gene. The specific aim was to determine the frequency of this polymorphism and associations with age-at-onset and islet autoantibodies in a group of 100 new onset 0 - 14 year old T1DM and in 100 healthy controls from Sweden. A fluorescent dNTP microsatellite analysis was performed whereby the GCLC TNR region was amplified by 2-step PCR utilizing genomic DNA. Statistical analysis was performed utilizing PRIZM. 3.0. Results show American Caucasians and the Swedish population have similar GCLC TNR allele frequencies, no significant difference in GCLC TNR allele frequency between T1DM and controls, a younger age-at-onset among females positive for the GCLC TNR 7 ($p = 0.027$). Autoantibodies against glutamic acid decarboxylase (GAD65 Ab), but not against IA-2 or insulin, showed higher levels among females positive for either the GCLC TNR 7 or TNR 9 allele ($p = 0.0014$). Since GCLC TNR 7 has been reported to be associated with lower GSH levels, we speculate that the GCLC TNR polymorphisms may contribute to gender associated differences in T1DM risk.

481 MUTATION SCANNING OF THE COMPLETE FLAVIN-CONTAINING MONOOXYGENASE GENE FAMILY IN AFRICAN-AMERICANS.

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The flavin-containing monooxygenases (FMO) catalyze the monooxygenation of numerous important N-, P- and S-containing drugs, pesticides and environmental toxicants. Most of the xenobiotics are transformed into more soluble compounds readily excreted or further metabolized by phase II enzymes, but in some cases FMOs can also catalyze the formation of reactive metabolites. Six FMO genes (FMO1-6) have been identified so far, but the major alleles of FMO2 and FMO6 encode non-functional proteins due to a nonsense mutation and splice-site abnormalities, respectively. Except for FMO3, little is known about the degree of variability found in human FMOs. The complete FMO family was scanned in 50 individuals of African-American descent using Detection of Virtually All Mutations-SSCP (DOVAM-S). In brief, the coding regions and splice sections were amplified using PCR and pooled using a PE Biosystems robotic device. The pooled products were run under 5 different nondenaturing electrophoretic conditions. A total of 49 sequence variants were identified, of which 29 were Variants Affecting Protein Structure or Expression (VAPSE), some of which are expected to be detrimental for the activity of the protein. The rest were either silent or located in intronic regions not expected to affect splicing of the mRNA. In FMO6 a nonsense substitution, Q105X was found in 66% of the alleles. A deletion, 337G, was found in 27% of the FMO2 alleles. Substitutions of highly conserved amino acid residues were also found in the remaining FMOs: FMO1 I303T and R502X, FMO4 E339Q and FMO5 P457L. In summary, this study demonstrates a high degree of sequence variability in the FMO family and it also suggests that only 3-4 of the FMOs are functional in most individuals. Some of the substitutions identified in this study might be useful markers in future association studies looking at sensitivity to toxicants and common disease

482 HIGH ACETALDEHYDE LEVELS AFTER ETHANOL GAVAGES IN ALDEHYDE DEHYDROGENASE 2 (*ALDH2*) GENE TARGETING MICE.

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Acetaldehyde, an intermediate metabolite of ethanol, is detoxicated by mainly aldehyde dehydrogenase 2 (ALDH2). A diminished enzyme activity of the mutant ALDH2 allele (*ALDH2*2*) due to single nucleotide mutation is associated with high sensitivity and a low tolerance to ethanol in humans. *ALDH2*2* is more commonly found in Japanese than in Caucasoid. We genearated Aldh2 gene tageting C57BL/6 (*Aldh2* -/-) mice. Their activity of acetaldehyde dehydroxylation was null in the mitchondrial fraction of the liver (K Kitagawa et al. FEBS Lett 2000).
After single oral administration of 5.0 g/Kg BW ethanol, we measured ethanol and acetaldehyde consentrations in the blood, brain and liver. Ethanol levels in blood, brain and liver of the *Aldh2* -/- were almost same as those of the *Aldh2* +/+ mice. The *Aldh2* -/- mice showed about ten times higher acetaldehyde levels in blood, brain and liver than wild-type C57BL/6 (*Aldh2* +/+) mice. On the other hand, in a free-choice ethanol and water drinking test, acetaldehyde concentrations in brain and liver of *Aldh2* -/- mice were almost equal to those of wild-type (*Aldh2*+/+) mice although the *Aldh2* -/- mice drank one third less ethanol than the *Aldh2* +/+ mice (T Isse et al. Pharmacogenetics *in press*). The primary genetic factor controlling the alcohol preference of the mouse C57BL/6 must be the *Aldh2* gene as it has been observed in the ethnic difference of alcohol sensitivity in humans. The *Aldh2* -/- mouse may be used for identification of genetic factor(s) involved in individual and ethnic difference of other toxicology, drug sensitivity, and pathophysiology studies.

483 DIFFERENTIAL RESPONSE OF HUMAN CELL LINES TO ARSENIC: EVALUATION OF CANDIDATE GENES.

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The epidemic-like public health problem caused by arsenic exposure in certain areas worldwide has made the understanding of arsenic toxicity and susceptibility a high priority. This project seeks to identify genes that modify arsenic toxicity *in vitro*, and to evaluate if genetic variation or gene expression profiles associate with arsenic susceptibility. A set of 24 lymphoblastoid cell lines was tested (4-6 times in duplicate) for acute toxicity and inducible tolerance to sodium arsenite. Following acute exposure to 1-50 mM sodium arsenite, cell lines were evaluated for survival using the MTS assay. There was little evidence of differential acute toxicity among these cell lines; the average EC50 for all cell lines was 13 mM (range 9.2-15.1 mM). To evaluate inducible tolerance to arsenic, cells were exposed to a nontoxic dose (1 mM) of arsenic for 72 hrs and subjected to a subsequent acute treatment with toxic doses of arsenic. Cell lines with and without adaptive tolerance to arsenic were identified. Growth rate was not associated with tolerance. Genes involved in arsenic transport or metabolism were evaluated for a role in arsenic tolerance. GSTO1 and Cyt19 are involved in arsenic reduction and methylation respectively. DNA sequencing of ~10 kb in 24 cell lines identified 2 SNPs in coding regions, 2 in promoters and 8 in noncoding intron sequence. SNP genotypes for ASNA1, an energy-dependent arsenic efflux pump, and GSTP1, involved in arsenic efflux and metabolism, were obtained from existing data sources (dbSNP). Preliminary analysis suggests that polymorphisms in ASNA1 and GSTP1 are not associated with differences in arsenic toxicity. Further analysis of the relationship between arsenic sensitivity, SNPs, and gene expression profiles (microarrays) is ongoing. Identification of human cell lines with reproducible adaptive tolerance to sodium arsenite provides a model system for discovery of arsenic susceptibility genes. Analysis of sequence variation and expression profiles may reveal molecular determinants of arsenic susceptibility.

484 GENOTYPING WITH TAQMAMA.

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TaqMAMA combines strengths of the fluorogenic 5-nuclease assay (TaqMan) with allelic discrimination of the mismatch amplification mutation assay (MAMA). Here we employ TaqMAMA to screen for known human genetic polymorphisms in two sets of experiments. First, we developed TaqMAMA genotyping assays for 11 human genetic polymorphisms. Genotypes were assigned correctly for all individuals at each of the 11 polymorphic sites. The signal/noise ratios for discrimination between alleles was ~10-fold to ~1, 000, 000-fold. In all cases data interpretation was rapid and could be automated. In addition, we show that SybrGreen can be used in place of more expensive fluorescent probes that are commonly used in TaqMan. Our second set of experiments was plasmid-based. The goal of these experiments was to explore the relationship between MAMA primer design and strength of allelic discrimination. Plasmids were created that contained all 16 possible nucleotide combinations in a two-nucleotide location. The amplification efficiency was then determined for each of 16 MAMA primers acting on these 16 plasmids to determine the TaqMan efficiency of perfect match and every possible mismatch. The data demonstrate that all types of genetic polymorphisms can be detected using TaqMAMA, and provide a guide for both MAMA primer design and DNA strand selection for the design of future genotyping assays using TaqMAMA. Collectively, the experiments show that TaqMAMA genotyping assays can be developed and deployed rapidly, with high probability of success, and be conducted at a cost and throughput for screening assays in toxicogenetics.

485 NEW POLYMORPHISMS IN THE HUMAN PARAOXONASE (PON1) GENE.

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Paraoxonase (PON1) is a high density lipoprotein (HDL)-associated enzyme involved in the metabolism of organophosphorus (OP) insecticides, nerve agents and oxidized lipids. Single nucleotide polymorphisms (SNPs) have been described in the 5' regulatory region (5), the coding region (L55M; Q192R) and in the 3' UTR (4), as well as a microsatellite in an intron [(CA)n]. The Q192R polymorphism significantly affects the catalytic efficiency for metabolism of some substrates while the 5' C-108T polymorphism affects levels of PON1. Analysis of the known 5' regulatory region and coding SNPs shows significant levels of recombination between PON1 regions. Resequencing the complete PON1 genomic region (~30 kb) in 47 individuals identified 192 SNPs in the region, including 1 novel cSNP, 8 new 5' and 12 new 3' UTR SNPs. Analysis of the linkage disequilibrium (LD) patterns between common variants demonstrates that a significant fraction of Caucasian haplotypes represent recombinants between Q192R and the promoter region. Thus, PON1 activity in a given individual likely represents a combination of intrinsic enzyme activity and differential expression resulting from cis acting regulatory elements, in addition to genetic background contributions. A rare nonsense SNP was identified at codon 194. Analysis of samples with discordant assignment by 2-D enzyme determination of PON1 status and genotyping revealed an individual whose functional PON1 status was explained by this SNP. (ES09883, ES07033l, ES09601/EPA-R826886, HL67406, HL66682)

486 CONTRIBUTION OF PARAOXONASE (PON1) LEVELS AND Q192R GENOTYPE TO ORGANOPHOSPHATE DETOXICATION: EVIDENCE FROM HUMANS AND "HUMANIZED" TRANSGENIC MICE.

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Biological monitoring studies demonstrate widespread exposure of the population to organophosphorous (OP) pesticides. Differences in detoxication efficacy of specific OPs among individuals are influenced by differences in catalytic efficiency or abundance of the HDL-associated enzyme, paraoxonase (PON1). PON1 hydrolyzes the highly toxic oxon derivatives of chlorpyrifos and diazinon. PON1 activity is determined in part by a coding region polymorphism (Q192R) that affects its catalytic efficiency, and in part by differences in plasma PON1 abundance. Infants represent a particularly susceptible population for OP exposure due to low abundance of PON1. Developmental onset of PON1 expression is variable, and PON1 abundance varies widely (> 13-fold) among adults. We describe a transgenic mouse model for investigating the PON1Q192R polymorphism. Mice were produced that expressed human transgenes encoding either the hPON1192Q or hPON1192R isoforms in place of mouse PON1, using human flanking sequences to drive expression faithfully in the liver. Mice expressing hPON1192R had similar PON1 levels as mice expressing hPON1192Q, but were significantly more resistant to dermal toxicity of chlorpyrifos oxon, and to a lesser extent chlorpyrifos, consistent with a higher catalytic efficiency of chlorpyrifos oxon hydrolysis for the hPON1192R isoform. This mouse model is providing critical information on the relative importance of the two isoforms for protecting against OP-related toxicity. Since the presence of the hPON1192Q allele is widespread in Northern Europeans, and to varying extents in other ethnic groups, these findings have importance for sensitivity to specific OP compounds in the general population. (ES09883, ES07033, ES09601/EPA-R826886, HL3300568, AHA 11133-GI1, T32 AG00057).

487 THE MODIFICATION OF CYSTIC FIBROSIS LUNG DISEASE BY A POLYMORPHISM IN A GLUTAMATE CYSTEINE LIGASE GENE.

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GSH modifies the clinical course of several pulmonary diseases. A polymorphism in a gene that regulates GSH synthesis might influence cystic fibrosis (CF) lung disease. We tested for an association between severe CF lung disease and a GAG repeat polymorphism in the glutamate cysteine ligase catalytic subunit gene (GCLC). In a cross sectional study, subjects were recruited from the CF clinic at the University of Washington. Relevant data were collected on each subject. Linear regression was used to test the relationship between CF lung disease and GCLC genotype. Gene-gene interaction between the GCLC polymorphism and CFTR genotype was measured using multiplicative interaction terms. Mechanistic studies were done to test the rate of GSH repletion after depletion, GCLC mRNA stability and rate of protein synthesis in cells with different GCLC polymorphism. 101 CF subjects participated in the study (51% male, mean (SD) age=26±8 yrs, mean FEV1=59±25% predicted). There was no association between GCLC genotypes and CF lung disease after adjusting for age, sex, P.aeruginosa infection and CFTR genotype (Coefficient (SEM) = 1.38 (1.86), p=0.46). However, after adjusting for gene-gene interaction between the GCLC polymorphism and the presence of a severe CFTR mutation, there was a significant association between GCLC genotypes and CF lung function (Coefficient (SEM) = 11.7 (4.5), p=0.01). There was a significant gene-gene interaction between GCLC and CFTR genotypes (Coefficient (SEM) = -8.4 (4.0), p=0.04). Also, the 9 GAG repeat was associated with faster GSH repletion, more stable mRNA and higher rate of protein synthesis in cultured lung fibroblasts. There is an association between the GCLC polymorphism and CF

lung disease and this association is significantly influenced by CFTR genotype. Mechanistic studies implicate a potential role for this GCLC polymorphism in regulating GCLC gene expression and protein synthesis. Supported by NIH grants P42ES04696, P30ES07033 and T32HL07287.

488 CLASSIFICATION OF SKIN SENSITISATION POTENCY USING THE LOCAL LYMPH NODE ASSAY.

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It has long been appreciated that chemicals that possess the potential to cause skin sensitization (allergic contact dermatitis) vary widely in their relative potency. However, until recently predictive test methods have not been well suited to measurement of this parameter. The local lymph node assay (LLNA) has been shown to provide a clinically relevant indication of the relative potency of skin sensitizers *via* the estimated concentration required to produce a 3-fold stimulation of proliferation in draining lymph nodes (EC3: the threshold for identification of a chemical as a sensitizer). In the present investigations we have examined the utility of a simple grading scheme for skin sensitizers based on order of magnitude differences in EC3 value. 50 chemicals have been classified as extreme (EC3 < 0.1%), strong (EC3 < 1.0%), moderate (EC3 < 10%), weak (EC3 ≤ 100%) or non-sensitizing (ie threshold for classification as a skin sensitizer not reached). It is suggested that this simple grading scheme provides a useful foundation on which to develop regulatory risk assessment/management strategies.

489 CURRENT REGULATORY STATUS OF THE LOCAL LYMPH NODE ASSAY.

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Although the ideal alternative to the guinea pig as a model for the predictive identification of skin sensitization potential would be an *in vitro* method, such is not yet available. Where complete replacement is not yet possible, refined and reduced alternatives represent the best option, particularly where they also provide a superior quality of information compared with the earlier protocols. This is the situation with regard to the murine local lymph node assay (LLNA), a method based on the fact that skin sensitizers stimulate T cell division in lymph nodes draining the site of application on the skin. The LLNA has been formally validated in the USA *via* the Interagency Coordinating Committee on the Validation of Alternative Methods, as well as *via* the European Centre for the Validation of Alternative Methods. The hazard identification protocol for the LLNA has now (April 2002) been accepted by the Organization for Economic Cooperation and Development as OECD Guideline 429. Given the OECD agreements concerning mutual acceptance of data, this means that the reduction and refinement opportunities offered by the LLNA can be fully realised. For these reasons, the UK competent authority, the Health and Safety Executive, has taken the step of identifying the LLNA as the preferred method for hazard identification. This regulatory acceptance of an objective, quantitative method for the identification and classification of skin sensitizers opens up the prospect of further categorisation of sensitizers according to potency, so permitting improvements to risk assessment and risk management to be made.

490 THE MOUSE LOCAL LYMPH NODE ASSAY: COMPARISON OF CELLULAR PROLIFERATION RESPONSE (CPR) USING VARIOUS VEHICLES.

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The mouse local lymph node assay (LLNA) is a refined *in vivo* alternative to traditional guinea pig tests for assessment of potential to cause skin sensitisation. A new OECD guideline (OECD 429) has recently been finalised which recognises the LLNA to be of equal merit to the traditional tests. The OECD 429 test guideline provides a list of vehicles ranked in order of preference; acetone:olive oil (4:1)(AOO), Dimethylformamide (DMF), propylene glycol (PPG) and Dimethyl sulfoxide (DMSO). Historically the aqueous vehicles acetone (AC) and absolute alcohol (AA) have also been common choices for such studies. The objective of this review was to determine whether the choice of vehicle affects the quantified background cellular proliferation response (CPR). In addition, as AOO is the preferred vehicle, assessment was made as to the consistency of the quantified CPR over a 7-year period. The protocols followed a standard design using pooled lymph node values. All studies used 6 to 8 week old female CBA/Ca strain mice obtained from Harlan UK Ltd., The 173 studies were performed between 1996 and 2002. The vehicles investigated were AOO, DMF, PPG, DMSO, AC and AA. The CPR was quantitatively measured as disintegrations per minute (DPM) after incorporation of ^{3}H-methyl thymidine. Comparison of the various vehicles revealed the mean CPR values for DMSO were generally higher (approximately 50%) than those of the other vehicles, whilst for the remaining vehicles CPR values were generally similar. Data for AOO treated animals over time indicated that between 1996 and 2000 the background CPR generally remained constant. However, data for 2001 were consistently lower than previous years. This is considered to be due to housing the studies in a full barrier maintained facility. In conclusion, the analyses show that the OECD test guideline preferred vehicles AOO, DMF and PPG produce similar background CPR. In addition, the responses in AOO are generally consistent over time but in the last year there has been a decline in the background CPR.

491 AN EUROPEAN INTER-LABORATORY VALIDATION OF ALTERNATIVE ENDPOINTS OF THE MURINE LLNA.

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The new OECD guideline 429 (Skin Sensitisation: Local Lymph Node Assay) is based upon a protocol, which utilises the incorporation of radioactivity into DNA as a measure for cell proliferation *in vivo*. The guideline also enables the use of alternative endpoints in order to assess draining LN cell proliferation. Here we describe the 1st round of an inter-laboratory validation of alternative endpoints in the LLNA conducted in 4 industrial laboratories, 3 CRO labs and 1 governmental agency laboratory. The validation study was managed and supervised by the Swiss drug agency SwissMedic. Statistical analysis was performed at the university of Bern, Department of Statistics. Ear-draining lymph node (LN) weight and cell count were used to assess proliferation instead of radioactive labelling of lymph node cells. In addition, the acute inflammatory skin reaction was measured by ear swelling and weight of circular biopsies of the ears to identify skin irritating properties of the test items. Hexyl cinnamic aldehyde and 3 blinded test items were applied to female, 8 to 10 weeks old NMRI and BALB/c mice. Results were sent to the independent study manager for statistically evaluation. The results of this first round showed that the alternative endpoints of the LLNA are sensitive and robust parameters. The use of ear weights added an important parameter assessing the skin irritation potential, which supports the differentiation of pure irritative from contact allergenic potential. The results highlighted that many parameter do have an impact on the strength of the responses. Therefore, such parameters have to be taken into consideration for the categorisation of compounds due to there relative sensitising potencies.

492 INTEGRATION OF PRIMARY IRRITATION WITH THE LLNA.

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The LLNA is a stand alone model to assess contact sensitization potential of chemicals, and is preferred by many governmental agencies unless technical difficulties with a test material requires the use of a guinea pig test. Historically, non-specific proliferation due to irritation has confounded LLNA interpretation. We have begun to test the hypothesis that chemicals which elicit a 10% increase in ear thickness (minimal irritating concentration-MIC_{10}) will produce a LLNA stimulation index near 3.0 due to the physiology surrounding primary irritation. Initial studies evaluated three concentrations of the isocyanates, MDI and IPDI, the anhydrides, TMA and PA, or DNCB applied to ears of mice for two days. Ear thickness was determined 24 hr later and following a third application, a LLNA was completed. MDI demonstrated a higher sensitization potential (EC_3=0.09%) than irritancy potential (MIC_{10}=0.28%). Conversely, 0.03% IPDI elicited a 3-fold proliferation but a 10% increase in ear thickness below 0.02% IPDI. In the case of TMA and PA, both demonstrated proliferation at chemical concentrations (EC_3=0.87% and 0.45%, respectively) below those required for primary irritation (MIC_{10}=4.6% and 0.95%, respectively). Likewise, 0.3%DNCB elicited 10% ear swelling while only 0.06%DNCB (EC_3) was needed to stimulate a 3-fold increase in LN proliferation. It appears that a specific irritating concentration and EC_3 values do not correlate to the same testing concentration for any given chemical and for 4/5 chemical tested, EC_3<MIC_{10}. As such, it does appear useful to minimize false positive proliferation

by testing chemicals at concentrations below which excessive irritation is elicited. An irritation screen over a range of soluble test material concentrations also serves to reduce unwarranted pain to laboratory animals. These studies suggest that simultaneous integration of ear swelling with lymph node proliferation can be beneficial when interpreting LLNA results. Additional studies evaluating chemical irritants (e.g., SLS) should be tested to confirm this testing scheme.

493 DEVELOPMENT OF THE LOCAL LYMPH NODE ASSAY IN NON-HUMAN PRIMATES.

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The selection of an appropriate non-rodent species for toxicology studies should be based on scientific considerations, which in the case of biopharmaceuticals should be to reproduce similar pharmacology to man. The local lymph node assay (LLNA) may be used as a model to monitor the pharmacology of immunomodulatory drugs, and because of the above need to evaluate biopharmaceuticals in non-rodent species, we have developed this model in the marmoset and cynomolgus monkey. Animals were given 3 daily topical applications of oxazalone to the dorsal surface of the ear and the draining lymph nodes dissected at necropsy on day 5. In some experiments, 7 oral administrations of cyclosporin A were given daily, commencing 2 days before the first dose of oxazolone. At necropsy the draining lymph nodes were removed and a single cell suspension of the lymph node was made using a cell strainer, then the cells were either resuspended in tissue culture medium for overnight culture with tritiated thymidine or processed for flow cytometry. *Ex vivo* proliferation of the draining lymph nodes demonstrated a dose-dependent increase in the number of proliferating cells in the marmoset, with stimulation indexes of 3, 7 and 12 being observed following the application of 1, 2.5 and 5% w/v oxazalone. Analysis of the cellular composition of the lymph nodes revealed that there was a dose dependent increase in the total number of lymph node cells, and in particular an increase in the proportion of B lymphocytes accompanied sensitization with oxazolone. In the cynomolgus monkey, the application of oxazolone was again associated with increased draining lymph node cellularity, B lymphocyte numbers and *ex vivo* proliferation, which could be inhibited in a dose dependent manor by cyclosporin A. In conclusion, these preliminary results demonstrate the potential of the LLNA to monitor immunomodulatory drugs intended to suppress the cellular immune response or influence cell migration in the non-human primate.

494 INTER-RELATIONSHIPS BETWEEN DIFFERENT CLASSES OF CHEMICAL ALLERGENS.

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Although sensitization of the respiratory tract induced by chemical allergens is not as common as skin sensitization and allergic contact dermatitis, it is nevertheless an important occupational health issue. Occupational asthma is associated with considerable morbidity and can be fatal. Several experimental approaches have been proposed for the identification and characterization of chemical respiratory allergens, but despite considerable progress none of these has yet been validated formally. In the absence of a widely accepted method for toxicological assessment of respiratory allergens it is appropriate to explore their relationship with skin sensitizing chemicals. In an analysis of a series of known chemical respiratory sensitizers we have found that without exception they elicit positive responses in one or more tests used for the prospective identification of skin sensitizing potential (guinea pig maximization test, the occluded patch test of Buehler and/or the local lymph node assay). We suggest that this observation could form the basis of an alternative paradigm for the initial characterization of sensitizing potential. Specifically, the proposal is that chemicals that fail to elicit positive responses in accepted methods for the identification of contact allergens are also regarded as lacking the potential to induce sensitization of the respiratory tract.

495 A HAZARD IDENTIFICATION MODEL FOR PROTEIN ALLERGENICITY: A MOUSE STRAIN COMPARISON.

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With the advent of biotechnology, the need for an animal model to identify allergic potential of proteins has increased. This laboratory's approach has involved i.p. administration of proteins to mice followed by evaluation of specific IgE (mouse Passive Cutaneous Anaphylaxis), total IgE, and specific IgG_1 and IgG_{2a} (ELISA $titer_{OD\ 0.5}$). BALB/c, CBA, B6C3F1, A/J and BDF1 mice were injected i.p. with 1 mg/ml ovalbumin (OVA) on days 1, 8, 15, 22, and 29. Tail bleeds were performed on day 21 followed by a terminal bleed on day 35. By day 21 (3 i.p. injections), A/J mice demonstrated increases in total IgE (10-fold) and OVA IgG_1 (titer=10, 993). Specific IgG_1 was not detected in any naive mice at dilutions of 1:10. While BDF1, B6C3F1, BALB/c and CBA mice exhibited little to no elevation in total IgE, B6C3F1 and BDF1 mice had appreciable increases in specific IgG_1 (titer=12, 150 and 35, 067). CBA mice had only 2/10 exhibit positive PCA reactions by day 21; sera from all other strains had at a least a 90% response rate at dilutions of 1:2. By day 35, A/J mice still had the only appreciable increase in total IgE. A/J mice also developed the highest OVA IgG_1 titer (>3x106), followed by BDF1 (>1.3x106) and B6C3F1 (920, 825). A/J mice had the largest IgG_{2a} titer (>59, 000) with CBA next at 6, 126. All strains demonstrated a 100% PCA response rate at 1:2 sera dilutions following 5 i.p. OVA injections with the following order of sensitivity: A/J>BDF1=B6C3F1>BALB/c>CBA. OVA specific IgG_1 demonstrated a better correlation with PCA reactions than did any other endpoint, including total IgE. A/J, BDF1 and B6C3F1 mice appeared most responsive to OVA exposures suggesting that evaluations of IgG_1 and PCA response by day 21, following only 3 i.p. injections, should be sufficient to evaluate allergenicity potential of proteins. Subsequent studies will investigate additional proteins including weak- and non-allergens to ensure that our model does not provide false positive results.

496 IMMUNOHISTOCHEMICAL ANALYSIS OF LOCAL LYMPH NODE ASSAY IN BALB/C MICE USING BROMDEOXYURIDINE.

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A murine local lymph node assay (LLNA) has been developed as an alternative test to guinea pig maximization test. The disadvantage of LLNA is the need for the use of radioactive material. In this study, we aimed to investigate immunohistochemical analysis of local lymph node assay in Balb/c mice using Bromodeoxyuridine (BrdU). Female Balb/c mice were treated by the topical application on the dorsum of both ears with strong allergens, 2, 4-dinitrochlorobenzene (DNCB), Toluene diisocyanate (TDI) and strong irritant, Sodium lauryl sulfate (SLS), once daily for three consecutives, respectively. The proliferation of cells in auricular lymph node, and ear were analyzed by labelling index (LI) of BrdU incorporation into cells. The cell number and weight of lymph node in the mice treated with allergens, DNCB and TDI were increased compared to vehicle control. There was an increase in the percentage of B220+ cell of mice treated with DNCB and TDI compared to vehicle control, but was not with SLS. Because we observed B cell increase in allergen-treated group, we measured stimulation index (SI) in cortex and medulla (C+M) of lymph node. The SI of C+M in lymph node of mice treated with DNCB and TDI were over three-fold increase compared to that of the control. However, the SI of C+M in lymph node of mice exposed to SLS was not significantly increased compared to vehicle control, although the lymph node weight of SLS was significantly increased. These results suggest that the measurement of SI in cortex and medulla with immunohistochemical profile of lymph node using BrdU could be one of the useful methods for screening for irritant and allergen.

497 DEVELOPMENT OF A NOVEL ANAPHYLAXIS MODEL IN MICE BY INTERMITTENT INTRAVENOUS INJECTIONS OF OVALBUMIN WITHOUT USE OF ADJUVANT.

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Several types of mouse anaphylaxis models have been used to investigate the immediate type hypersensitivity to foods, respiratory allergens or pharmaceuticals. In these models, mice were needed to be sensitized for a long period with the allergens *via* several routes, such as oral, intraperitoneal, subcutaneous and topical application, in combination with aluminum hydroxide gel or Freund's complete adjuvant. To the best of our knowledge, there has been no report in which the intravenous injection of an antigen without the adjuvant was used for sensitization. To develop a novel anaphylaxis model, 10-week female ICR (CD-1) mice were given by intermittent intravenous injections (3 times at 4-day intervals) of 0.5, 5, 15 or 50 mg/kg of ovalbumin (OA) alone. In addition, strain difference was examined using 10-week female BALB/c and C57BL/6. In ICR mice, anaphylactic signs were observed immediately after the third injection of OA at 0.5 mg/kg or higher. Elevations in serum histamine level and permeability of the subcutaneous vasculature were associated with the onset of the signs. Anti-OA IgE and IgG antibodies were detected by the passive cutaneous anaphylaxis reactions in sera collected from mice 4 days after the second injection. The anaphylactic reactions due to OA were also observed in BALB/c and C57BL/6 mice at 15 mg/kg or higher, but not 5 mg/kg, indicating less susceptibility in these two strains than in ICR. Bovine serum albumin (BSA) also induced anaphylactic reactions in ICR mice by the same procedures. However,

higher doses (5 and 50 mg/kg) were needed to induce the reactions, and neither anti-BSA IgG nor IgE antibodies were detected even in mice receiving 50 mg/kg. In conclusion, anaphylaxis reactions were shown to be induced in mice by 3 intermittent intravenous injections of OA and BSA, and especially the OA-induced anaphylaxis was characterized by the production of specific IgE antibody despite of the short sensitization period without the adjuvant.

498 A 4-WEEK IMMUNOTOXICITY STUDY IN RATS TO VALIDATE IMMUNOLOGICAL ENDPOINTS.

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Assessment of the immune system's capability to respond to antigens with the generation of specific antibodies, whilst under the influence of a test article, is required in toxicity tests according to the European guideline for repeated dose toxicity testing of medicinal products. The purpose of this study in rats was to validate methodology for the determination of Keyhole Limpet Haemocyanin (KLH)-specific antibodies under the influence of an immunologically active compound. Prograf, a commercially available immunosuppressant, was administered orally (gavage) to 5 rats/sex/group at dosages of 0.5 or 3 mg/kg/day, once daily for a period of 4 weeks. On days 14 and 22, KLH was administered subcutaneously, with an adjuvant (AluGel), to the two treated groups and a control (i.e. without Prograf treatment) approximately 1 hour following administration of Prograf. Terminal investigations included haematology parameters, titration of KLH-specific antibodies in serum (ELISA), macroscopic pathology, spleen and thymus weights, immunophenotyping of splenocytes (FACS analysis) and limited histopathology. At 3 mg/kg/day a minimal reduction of subcutaneous KLH-induced granuloma formation and a moderate to marked reduction of germinal centre development (axillary lymph node and spleen) were observed. Reduced CD4+ (T-cell) counts were found in the spleen of males, consistent with a suppressed production of KLH specific antibodies (IgG in both sexes, IgM in males only) and a higher incidence of atrophy in the periarteriolar lymphoid sheaths of males. Slight to moderate lymphopenia was present in both sexes at 3 mg/kg/day. These findings are consistent with the known pharmacological activity of Prograf. In conclusion, determination of antibody titres following immunisation of rats with KLH, with concurrent exposure to a test item, appears to be a valid method in the context of the immunotoxicity evaluation required by European regulation.

499 IMMUNOTOXICOLOGICAL ASSAYS: MEASURING AND REDUCING THE VARIABILITY.

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Immunotoxicity testing is being increasingly requested as part of the development of pharmaceuticals, pesticides, industrial chemicals and food additives. However, many of the functional assays are recognised as having high between- and within-study variability. For example, using historical data, the within-group coefficient of variation of the widely-used natural killer (NK) cell activity assay and the specific antibody formation in a plaque forming cell (PFC) assay, was found to be around 60%. Whilst between-animal variation is usually found to be the dominant source of variability in such assays, the multiple sample processing steps are also likely to contribute. Since little precise information is available on this, we therefore carried out a data-analysis investigation into the NK, PFC and other assays to formally separate out and measure the degree of variability from the various sources. Historical data were used and new validation studies were performed. It was found possible to improve the efficiency of certain of the assays by reducing the number of replicates or plates and by using randomized block designs to eliminate biases. In conclusion, the main method of reducing between-animal variability in non-invasive assays (blood rather than spleen samples) was shown to be the adjustment of data by pre-treatment values. Additional variability between studies and between sampling occasions was also found and is being looked at further.

500 COMPARATIVE STUDY OF THE IMMUNOTOXIC ACTIONS OF CYCLOSPORIN A, DEXAMETHASONE AND FUROSEMIDE IN THE RAT.

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In this study Sprague-Dawley rats were treated with cyclosporin A (at 10, 15 and 20 mg/kg), dexamethasone (15 mg/kg) and furosemide (70 mg/kg) by oral gavage for 28 days. Hematological investigations were performed on blood samples taken at terminal sacrifice. The antibody response to the T-cell dependent antigen Keyhole Limpet Hemocyanin (KLH) was evaluated by subcutaneous administration of KLH on Day 22, followed by ELISA determination of IgM levels on blood samples taken at terminal sacrifice. Lymphocyte subsets in peripheral blood were quantified by flow cytometry on samples taken at the end of the study. Organ weight analysis and histopathological examinations were performed on lymphoid organs. The methods used correspond to those proposed for the evaluation of the potential toxicity to the immune system in recent European testing guidelines for new drugs. The test materials were all well tolerated clinically, and treatments with all three resulted in reductions in bodyweight gain, thus providing evidence that the selected dose-levels resulted in some general toxicity. Treatment with cyclosporin A provoked a dose-related decrease in the IgM antibody response to KLH, but treatment with dexamethasone or furosemide did not affect this parameter. Treatment with cyclosporin A resulted in a reduction in the proportion of CD4 and CD8 T-cells in rats of both sexes. Treatment with dexamethasone or furosemide did not influence T-cell populations. None of the treatments adversely affected B-cell populations. The test methods clearly permitted identification of the immunosuppressive actions of cyclosporin A. The immunosuppressive action of dexamethasone was not detected by the KLH response or lymphocyte subset analysis at the dose-level selected for this study. The results with furosemide were consistent with the view that this drug does not have immunotoxic properties.

501 EVALUATION OF PRIMARY ANTIBODY RESPONSE TO KEYHOLE LIMPET HEMOCYANIN IN SPRAGUE-DAWLEY RATS.

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The Committee for Proprietary Medicinal Products (CPMP) and the Food and Drug Administration (FDA) recommend evaluating a primary T-dependent antibody response when assessing the immunotoxicity potential of new drug candidates. Although the immune response to sheep red blood cells (sRBC) in rodents is well documented, responses to other T-dependent antigens such as keyhole limpet hemocyanin (KLH) have not been fully characterized. The current study evaluated the primary IgM response to KLH in male and female Sprague-Dawley rats. Animals were given a single intravenous injection of KLH at 30, 100 or 300 μg per animal. Serum samples were collected prior to immunization, and on Days 3, 5, 7, 10, 14 and 21 post-immunization. IgM specific for KLH was measured by ELISA, using a detection antibody with high reactivity with rat IgM and no cross-reactivity with rat IgG. Anti-KLH IgM was undetectable in sera collected from animals prior to immunization. In contrast, robust dose-dependent antibody responses were seen in rats immunized with KLH. The peak IgM response in both male and female rats occurred between 5 and 7 days post-immunization. The timing of the IgM response to KLH in this study was consistent with reported responses to sRBC. However, the use of KLH as an antigen may provide a better standardized model compared to sRBC. Experiments evaluating the effects of known immunosuppressive agents such as cyclosporin A and cyclophosphamide on the primary antibody response to KLH are ongoing.

502 DEVELOPMENT AND VALIDATION OF AN ASSAY TO EVALUATE THE CANINE T-DEPENDENT ANTIBODY RESPONSE.

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New immunotoxicity testing guidelines have been adopted by the CPMP and are forthcoming from the FDA. The primary T-dependent antibody response (TDAR) is an assay recommended by both agencies. Immunotoxicity assays have been validated for mice and rats, however methods for the dog have not been reported. Our lab has developed a dog model for the assessment of a primary TDAR to keyhole limpet hemocyanin (KLH). Dogs were immunized with KLH using 3, 10 or 30 mg/dog by intramuscular injection. Serum samples were collected prior to immunization and on days 3, 5, 7, 10, 14 and 21 post-immunization. Serum samples were tested in a dog anti-KLH IgM ELISA developed in our lab. Immunization with KLH resulted in a robust antibody response at all doses of antigen. Peak IgM response occurred on day 7 post-KLH injection. The 10mg/dog dose elicited the highest response. After the peak response, the anti-KLH response flattened out or decreased slightly through the end of the study. The ELISA assay showed high specificity for IgM with little to no cross-reactivity with IgG. Intra-assay and inter-assay precision of endpoint titers (log 2) is less than 10%. Additional validation studies are planned to determine the sensitivity of the dog TDAR to a known immunosuppressive agent.

503 VALIDATION OF AN ELISA PROCEDURE WITH NITROPHENYL-CHICKEN GAMMA GLOBULIN (NP-CγG) AND KEYHOLE LIMPET HEMOCYANIN (KLH) AS ANTIGENS FOR TESTING HUMORAL IMMUNOTOXICITY.

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Limitations of the standard sheep red blood cell plaque assay for evaluating preclinical immunotoxicity have led to an interest in the feasibility of using alternative antigens, together with an ELISA format, for assessing humoral immunotoxicity.

The purpose of this study was to evaluate the predictive value of NP-CγG and KLH antigen challenge in determining the immunotoxicity of orally administred azathioprine (AZA), cyclophosphamide (CY), or cyclosporin A (CsA) at doses of 17, 10, or 25 mg/kg per day, respectively, for 30 days in F344 female rats. The effect of treatment on the immune response was assessed in a humoral model that consisted of the administration of nitrophenyl-chicken gamma globulin (NP-CγG) and keyhole limpet hemocyanin (KLH) antigens during immunosuppressive treatment and the measurement of resulting rat antigen-specific IgG and IgM, as well as total IgG, levels. Cellular assessment parameters included organ weights and cellularity, hematology, lymphocyte phenotype characteristics, spleen cell mitogen stimulation (T and B cell dependent), splenic natural killer (NK) cell cytotoxicity, and bone marrow cellularity and lymphocyte phenotype differential. Although decreases in several of the cellular assay parameters were induced by all three immunosuppressive agents, the only functional assays to demonstrate a statistically significant immunosuppressive effect were the antigen-specific serum IgG levels. The primary (Day 10; 15 days post immunization) and secondary (Day 25; 5 days post rechallenge) NP responses were significantly suppressed by >60%. The use of NP hapten provided consistent responses when analyzed with a sensitive, well developed, ELISA methodology. Absolute lymphocyte phenotyping and lymphocyte hematology were also predictive of T cell immunosuppression for all 3 compounds. The data suggest that these two parameters, NP-IgG humoral response and lymphocyte phenotyping, are sufficient for identifying immunosuppressive compounds.

504 CHARACTERIZATION OF KEYHOLE LIMPET HEMOCYANIN (KLH) AS AN ALTERNATIVE T-DEPENDENT ANTIGEN FOR ELISA IMMUNOTOXICOLOGICAL EVALUATIONS IN MICE.

J. A. Shea, V. L. Peachee and K. L. White. *Medical College of Virginia, Richmond, VA.*

Previous studies by the NTP/NIEHS identified the spleen plaque forming cell response to T-dependent antigen sRBC as one of the most sensitive functional assays for detecting immunomodulating compounds. Various regulatory agencies currently accept the sRBC ELISA for immunological evaluation. Problems with the sRBC ELISA is that depending on the sRBCs and animals, low affinity antibody can be produced following a single injection. Furthermore, lot to lot preparations of sRBC membranes can vary significantly. Since no standard antibody is available ELISA results are usually expressed as serum titers. The objective of this study was to optimize the KLH ELISA as an alternative T-dependent antigen and to evaluate how it compared to the plaque assay. Commercial KLH (Pierce) produced a uniform solution unlike preparations from other vendors. Mice were injected with 100 ug/animal iv and evaluated on days 3 - 7. Antibody response to KLH reached a plateau on days 4, 5, and 6, with day 5 having the least variability. The 100 ug/animal concentration was selected after dose response studies ranging from 30 ug/animal to 500 ug/animal demonstrated a plateau effect. The optimal ELISA conditions utilized Immunlon II plates coated with 0.5 ug/well of KLH in PBS incubated overnight. Purified mouse IgM mAB against KLH (PharMingen) was used as a standard. Daily treatment with cyclophosphamide ip, at doses from 5 mg/kg to 60 mg/kg resulted in dose dependent suppression on day 5. Animals treated daily with dexamethasone ip at 0.1 mg/kg to 5 mg/kg did not differ significantly from the vehicle controls. A similar lack of effect of dexamethasone was observed in the plaque assay when data were expressed as PFC/10^6 spleen cells. KLH as the T-dependent antigen in ELISA studies has several advantages over sRBC including; being commercially available, prepared under quality control conditions, produces a robust response with a single injection, and a KLH standard is available for quantitating serum antibody levels in mice. (Supported in part by NIEHS Contract ES55387).

505 VALIDATION METHOD FOR THE DETECTION OF ANTI KEYHOLE LIMPET HEMOCYANIN ANTIBODIES IN SPRAGUE-DAWLEY RAT SERUM BY ELISA.

N. Rouleau, J. Jean-Baptiste, G. Desilets, R. Riffon and L. LeSauteur. *Immunology Laboratories, CTBR, Senneville, QC, Canada.* Sponsor: D. Jones.

Immunotoxicity testing guidance have recommended the T-cell dependent antibody response assay; keyhole limpet hemocyanin used as an antigen and ELISA monitoring for antibody formation provides advantages over traditional methodology. Validation of a qualitative ELISA for the detection of KLH specific antibodies in sprague-dawley rat serum was performed. The ELISA method consisted of plates coated with KLH, adding the test serum, then the antibodies were detected using a peroxidase conjugated secondary antibody; a colorimetric substrate was used for color development. Parameters validated for the ELISA method included specificity, cut-off determination, intra- and inter-assay precision, and stability. High, medium, low and species (rat immunoglobulin) positive controls were included. Specificity, intra- and inter-assay precision met the acceptance criteria of mean A450 values above the cut-off value and the coefficient of variation ≦20%. A cut-off value was determined. Stability of samples at room temperature for approximately 6 hours, at 4°C for approximately 24 hours, and after 4 freeze-thaw cycles at −70°C, long-term storage stability of samples at approximately −70°C are ongoing. A similar method has been developed for monitoring IgM and both methodologies can be used for monitoring the T-cell dependent antibody response in a toxicology study.

506 VALIDATION METHOD FOR THE DETECTION OF ANTI-KEYHOLE LIMPET HEMOCYANIN ANTIBODIES IN CYNOMOLGUS MONKEY SERUM BY ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA).

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Immunotoxicity testing guidelines have recommended the T-cell dependent antibody response assay; keyhole limpet hemocyanin used as an antigen and enzyme linked immunosorbent assay (ELISA) monitoring for antibody formation provides advantages over more traditional methodologies. The ELISA method consisted of coating plates with KLH, and subsequently adding the test serum; the antibodies were then detected using a peroxidase conjugated secondary antibody, with a colorimetric substrate for color development. Validation of this qualitative ELISA for the detection of KLH specific antibodies in Cynomolgus monkey serum was performed. Parameters validated included specificity, cut-off determination, intra- and inter-assay precision, and stability. High, medium, low and species (monkey immunoglobulin) positive controls were used for the validation. Specificity, intra- and inter-assays previously met the acceptance criteria of mean A450 values over the determined cut-off value, coefficients of variation (CVs) < 20%. Stability of samples at room temperature for approximately 6 hours, at 4°C for approximately 24 hours, and after 4 freeze-thaw cycles at approximately −70°C met the acceptance criteria, long-term storage stability at approximately −70°C is ongoing. A similar method has been developed for monitoring IgM and both methodologies can be used for monitoring the T-cell dependent antibody response in support and toxicology studies

507 INTERLABORATORY STUDY OF THE PRIMARY ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN OUTBRED RODENTS FOLLOWING EXPOSURE TO DEXAMETHASONE.

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EPA guidelines provide a choice in evaluating immune system function in rats and mice using sheep red blood cells (sRBC), namely, an antibody-forming cell (AFC) assay or a sRBC-specific serum IgM ELISA. All four laboratories used the same source of sRBC to determine which of the two methods was more sensitive in detecting suppression of the antibody response by dexamethasone (DEX). CD rats and CD1 mice were injected iv with sRBC followed by ip injections of 0, 0.1, 0.3, 1, 3 or 5 mg/kg/day DEX. In rats, statistically significant reductions in antibody response as measured by AFC/106 splenocytes were first detected at 0.1 mg/kg/day (47% suppression) by one lab and at 1 mg/kg in 3 labs. In contrast, suppression measured by ELISA was first detected at 5 mg/kg (20-33% suppression). In mice, statistically significant reductions in the antibody response were detected at 1 mg/kg (52% suppression - AFC assay) by 1 lab and 3 mg/kg (55% suppression) by another lab. Suppression using the ELISA was detected at 3 mg/kg (7% inhibition). In conclusion, the PFC assay detected significant suppression at lower concentrations compared to the ELISA in both rats and mice. At concentrations of DEX where both assays showed significant suppression, greater suppression was observed using the PFC assay. These results in the mouse mirror those observed in an earlier collaboration using cyclophosphamide (CY), but differ from those in rats in which the rat ELISA detected significant suppression with CY at the same (2 labs) or lower (1 lab) concentration compared to the PFC assay. Additional compounds will need to be evaluated before concluding that one method is superior to the other in detecting suppression of the antibody response. (This abstract does not reflect EPA policy).

508 QUANTITATION OF ANTI-SHEEP RED BLOOD CELL (SRBC) MEMBRANE-SPECIFIC IGM: A NOVEL METHOD USING ABSORPTION OF SPECIFIC ANTIBODY.

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The primary humoral immune response against SRBC has been recommended in regulatory guidance documents to evaluate potential unexpected immunosuppression by pharmaceutical compounds or chemicals. Currently, specific IgM to SRBC

membrane antigens is most commonly assessed as a relative value based on the density of specific IgM-producing splenocytes in the hemolytic plaque assay, or serum titers. Both of these methods are labor-intensive andand present significant challenges for achieving and maintaining GLP compliance. The objectives of this study were to develop and validate a time- and cost- efficient ELISA to quantitate specific IgM against SRBC membrane antigens in rat serum that could be readily implemented as a GLP-compliant procedure. Serum was collected from rats immediately prior to, and 6 days following immunization with SRBC; total IgM was quantitated in these samples using a standard curve established with polyclonal goat IgG against rat IgM. In samples collected following immunization, total IgM was measured before and after absorption to fixed SRBC to determine the quantity of IgM specifically directed against SRBC membrane epitopes. To validate this assay, male rats were given cyclophosphamide at 0, 10 or 30 mg/kg/day beginning one day prior to immunization. An additional group of rats was immunized with 1 mg keyhole limpet hemocyanin (KLH) subcutaneously as a negative control group to assess non-specific binding of IgM to fixed SRBC. Results of this novel ELISA and those of the traditional titer method for determination of SRBC-specific IgM were compared. Additional parameters evaluated in these rats included routine hematology and histology of lymphoid tissues, and by flow cytometry, distribution of major lymphocyte subtypes in peripheral blood, spleen, thymus and mesenteric lymph nodes. Results demonstrated excellent correlation between the quantitative ELISA developed in this study and traditional titer method, and between histology and results of lymphocyte subtype distribution in peripheral lymphoid tissues.

509 AUTOMATED MEASUREMENT OF LEUKOCYTE MIGRATION IN NORMAL AND OXIDATIVE ENVIRONMENTS.

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We have developed a novel method (ECIS/taxis) for automated real time monitoring of leukocyte cell movement in response to chemotactic factors. In this report, we describe the migration of several primary and immortalized leukocytes in response to several chemoattractants. In the ECIS/taxis system, cells migrate in an under-agarose environment, and their positions are monitored using the electric cell substrate impedance sensor technology to measure the impedance change at a target electrode that is lithographed onto the substrate. Cell arrival is indicated by significant increase in the impedance at the target electrode. The intensity of migration of cells, in response to increasing doses of chemoattractant, was proportional to the increase in the absolute value of resistance measured by the target electrode. Modifications to the design of the target electrode have been shown to increase the sensitivity of the assay. The multiwell assay configuration allowed the detection of antagonistic effects of pharmaceutical drugs as well as the detection of the damaging effects of reactive oxygen species on the migrating cells. Finally, we have further used the system to evaluate the role of metallothionein as an antioxidant in the preservation of inflammatory phagocyte function following exposure to exogenous and endogenous oxidants. Higher levels of endogenous metallothionein correlated with a greater resistance to the effects of oxidant on the chemotactic response. These results suggest that ECIS/taxis measurements will expand the experimental and diagnostic capacity to evaluate normal and dysfunctional chemotactic cell movement, and that metallothionein induced as a consequence of toxicant exposure can have significant impact on the motility of cells in inflammatory environment. This work was supported in part by NIH Grants: EB00208 and ES07408

510 COMPARISON OF THE PHAGOCYTIC ACTIVITY IN RATS AND MONKEYS USING TWO COMMERCIAL KITS.

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Phagocytosis is an essential arm of non-specific defences against infections and is therefore an important part of immunotoxicity evaluation. In this study, phagocytosis was studied by flow cytometry in rats and in Cynomolgus and Rhesus monkeys, using 2 commercial human kits: the Phagotest© and the Bursttest© (Orpegen). The Phagotest© measures the percentage of phagocytes which have ingested bacteria and the number of bacteria in each cell, while the Bursttest© measures the percentage of phagocytes which have produced reactive oxygen radicals and the enzymatic activity per cell (oxydative burst). Overall, results in monkeys were similar to those obtained in man. Various interspecies differences were identified in this study. In the Phagotest©, the proportion of phagocytic cells was approximately 80% in monkeys, but only 60% in rats. Two stimuli are used in the Bursttest©, namely opsonized E. coli and phorbol myristate acetate (PMA). In monkeys, as in humans, PMA was more potent than E. coli, with 90% and 70% of positive phagocytic cells, respectively. In rats, only 60% of phagocytes were positive with either stimuli. In addition, these two kits can be used to calculate mean fluorescence intensity, which depends on the number of bacteria or enzymatic activity per cell. Results were not homogeneous due to the influences of sampling, number of bacteria and the technique. Although the Phagotest© and Bursttest© were both found to be suitable to evaluate phagocytosis in rats and monkeys, this study suggested that monkeys are preferable to rats for this investigation. This confirms our previous results using chemiluminescence.

511 INTERLABORATORY STANDARDIZATION OF FLOW CYTOMETRY DATA ACQUISITION PRACTICES.

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In today's global work environment, it is common for a research facility to have laboratories exist in multiple geographic locations. To harmonize flow cytometry practices in the toxicology groups across 3 major Pharmacia sites located in Skokie, IL, Kalamazoo, MI and Nerviano, Italy, a cross-site standardization study was conducted. The goals were to standardize instrument set-up and data acquisition protocols across sites to assure optimal alignment for light scattering and fluorescence resolution as well as appropriate compensation when using multiple fluorochromes. This standardization also harmonized quality control practices across sites using different model flow cytometers. Uniformly sized fluorospheres (Flow-Check™) were used to monitor photomultiplier tube (PMT) and system fluidic performance. Fluorescently labeled microparticles (CaliBRITE beads) were used to target fluorescence for each PMT to a specific channel and then set-up appropriate compensation. This protocol was followed each time sample data was acquired. To assess the adequacy of the harmonized set-up, CD-Chex®Plus, a commercially available normal human peripheral blood control preparation were immunophenotyped. All sites used the same labeling protocols and reagent lots. Both BD instruments (FACSCalibur, FACScan) showed very similar high voltage trends for the CaliBRITE beads as did the Coulter XLs. Compensation settings day to day for each instrument showed very little variability. In analyzing CD-Chex®Plus data, all three labs were within 5% of group mean values in quantitation of CD3+/CD45+, CD3+/CD4+, CD3+/CD8+ and CD45+/CD19+ cells. The values obtained for CD45+/CD16, CD56+ showed a little more variability, but were still considered acceptable. These acceptable immunophenotyping results confirm the success of the standardization protocol and demonstrate the ability to harmonize data collection across multiple geographic sites using five different flow cytometers and furthermore suggests that a common reference range may be developed across laboratories.

512 SEX DIFFERENCE IN THE RESPONSE OF MOUSE LIVER TO CAR-MEDIATED INDUCTION OF CYP2B10 AND HEPATOCYTE PROLIFERATION.

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The nuclear receptor Constitutive Androstane Receptor (CAR) binds DNA as a heterodimer with the retinoic-X receptor and activates gene transcription in a constitutive manner. Previously, *in vitro* studies have shown that the testosterone metabolites, androstanol, and androstenol, inhibit the constitutive transcriptional activity of CAR, suggesting that differences might exist in the response to CAR-mediated gene activation between different sexes. In this study, we have analyzed the response of female and male mice hepatocytes to two processes induced by the CAR ligand and non-genotoxic mouse liver carcinogen TCPOBOP, namely induction of CYP2B10 transcription and stimulation of hepatocyte proliferation. Results showed that levels of CYP2B10 mRNA were more abundant in the liver from female mice treated with TCPOBOP than those of males. Similarly, labeling index of female hepatocytes at 24, 30 and 36 hours after treatment was much higher than that found in males. The higher proliferative activity of female hepatocytes was associated with increased hepatic levels of cyclin D1, cyclin A, E2F and enhanced phosphorylation of p107. Administration of androstanol to males caused a decrease of labelling index and CyP2B10 mRNA levels. In conclusion, the results show that, at least two biological responses elicited by the CAR ligand TCPOBOP, namely, induction of microsomal detoxification and hepatocyte proliferation occur at higher levels in female than male mice, suggesting that CAR transactivating activity in males is partially counteracted by physiological higher levels of testosterone metabolites such as androstanol and androstenol.

513 ALTERATIONS IN AIRWAY INTRACELLULAR SIGNALING PATHWAYS FOLLOWING AIR POLLUTION PARTICLE (PM) EXPOSURE USING LASER CAPTURE MICRODISSECTION AND PROTEIN ARRAY TECHNOLOGIES.

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Understanding the mechanisms by which PM and associated constituents mediate adverse health effects would provide biological plausibility to epidemiological findings indicating an association between PM and increased rates of morbidity and mortality. Whole lung extracts have provided insight into mechanisms that mediate ambient air and PM-induced pulmonary injury. This approach, however, makes it difficult to elucidate detailed cellular and molecular pathology associated with PM-induced injury. Therefore, the majority of information regarding the means by which PM generates lung injury has been derived from *in vitro* studies where it is unclear to what extent these mechanisms can be extrapolated to the *in vivo* situation. Recent technological advances have allowed the direct study of susceptible cells or cell populations *in vivo*. In the present study we applied laser capture microdissection (LCM) and protein array technologies to examine the effect residual oil fly ash (ROFA) exposure had on airway intracellular signaling pathways associated with cell growth and differentiation. Sprague-Dawley rats were intratracheally instilled with 0.5 mg/rat ROFA in saline or saline alone. LCM was used to recover airway epithelial cells from the lungs of control and exposed rats. Protein array analysis examined alterations in cellular signaling pathways from protein extracted from LCM captured tissue. ROFA increased p-ERK:ERK at 6h and 24h ($P < 0.001$) post exposure when compared to saline or cage control animals. This study shows the utility of LCM and protein arrays in addressing environmental health issues and demonstrates *in vivo*, that lung injury due to PM exposure is associated with airway epithelial cell MAPK activation. *(This abstract does not reflect EPA policy.)*

514 INSULIN-LIKE GROWTH FACTOR 1 - ESTROGEN RECEPTOR α CROSSTALK IN MCF 7 BREAST CANCER CELLS.

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Polypeptide growth factors such as insulin-like growth factor I (IGF-I) induce proliferation of breast cancer cells through activation of growth factor signaling pathways and ligand-independent activation of estrogen receptor α (ERα). Treatment of MCF-7 cells with 10 nM IGF-I for 9 days resulted in a > 5-fold increase in cell number, whereas solvent (DMSO)-treated cells increased by approximately 2.5-fold. In contrast, MCF-7 cell proliferation was inhibited after treatment with 1 μM ICI 182, 780 (an antiestrogen), 15 or 5 μM PD98059 or LY294002 which inhibit mitogen-activated protein kinase kinase (MAPKK) and phosphatidyl inositol-3-kinase (PI3-K), respectively. Inhibition by these agents was cytostatic and did not induce cytotoxic responses at the concentrations used in this study. Cotreatment of cells with 10 nM IGF-I plus PD980059, LY294002 or ICI 182, 780 significantly decreased growth factor-induced cell proliferation only with the latter two inhibitors, whereas PD980059 was inactive as an inhibitor. PD980059 and LY294002 inhibited IGF-I-induced MAPK and Akt phosphorylation, respectively, whereas ICI 182, 780 did not inhibit MAPK or Akt phosphorylation. IGF-I also induced constructs containing the serum response element from the c-*fos* protooncogene promoter or multiple copies of the serum response factor element; however, in cotreatment studies, ICI 182, 780 did not inhibit these IGF-I-induced responses. These results suggest that both ERα and PI3-K are critical factors for mediating IGF-I-induced proliferation of MCF-7 cells and induction of c-*fos* is not critical for cell growth. Current studies are determining between PI3K-ERα interactions. (Supported by NIH ES09106 and ES09253)

515 ROLE OF DIOXIN-INDUCED MAP KINASE SIGNALING IN ARYL HYDROCARBON RECEPTOR PHOSPHORYLATION.

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Recent evidence has shown that treatment of mouse hepatoma Hepa-1 cells with 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces intracellular mitogen-activated protein (MAP) kinases that are required for maximal activation of the aryl hydrocarbon receptor (AHR). In this study, we investigated the role of TCDD in activation of signaling pathways leading to MAP kinase induction and AHR phosphorylation. In AHR-negative CV-1 cells, AHR-dependent reporter gene expression was potentiated by co-expression of AHR/ARNT with the upstream regulators of the MAP kinase pathways, MEKK1 and Raf1. This effect on AHR activity was suppressed by specific inhibitors of the ERK (extracellular signal regulated kinase) and the JNK (Jun N-terminal kinase), but not the p38 MAP kinase. ERK activation was completely abolished in a PLC-γ null cell line, while PLC-γ reconstitution of these cells restored ERK responsiveness to TCDD. These results suggest a role for PLC-γ, MEKK1 and Raf1 in TCDD-dependent activation of MAP kinases. *In vivo* ^{32}P-orthophosphate labeling of mouse hepatoma Hepa-1 cells showed that TCDD exposure led to rapid ERK activation followed by the transient induction of AHR phosphorylation. *In vitro*, ERK directly phosphorylated AHR isolated from untreated Hepa-1 cells. Hence, MAP kinases may act as central effectors of different pathways in TCDD signaling and their role on AHR activity is likely to be mediated through direct receptor phosphorylation. *(Supported by NIH ES06273, NIH ES10807, NIH P30-ES06096 and a grant from the Philip Morris External Research Program).*

516 REGULATION OF HIF TRANSACTIVATION AND CAP43 EXPRESSION BY NICKEL COMPOUNDS THROUGH PI-3K/AKT-DEPENDENT, P70S6K-INDEPENDENT PATHWAY.

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Nickel compounds induce a marked induction of hypoxia inducible factor 1 (HIF-1), and this is considered to be important in nickel-induced tumor promotion and progression. As yet, however, the signal transduction pathways leading to the induction of HIF-1 are not fully understood. We demonstrate in this study that exposing mouse epidermal C141 cells to either $NiCl_2$ or Ni_3S_2 results in the activation of the phosphatidylinositol 3-kinase (PI-3K) pathway, including PI-3K, Akt and p70 S6 kinase ($p70^{S6k}$). In addition, exposure to $NiCl_2$ and Ni_3S_2 leads to transactivation of HIF-1. HIF-1 transactivation in response to $NiCl_2$ and Ni_3S_2 can be inhibited by pre-treatment of the C141 cells with the PI-3K inhibitors wortmannin and LY294002, which also attenuates the activation of PI-3K, Akt and $p70^{S6k}$. Overexpression of a dominant negative mutant of PI-3K, Δp85, also led to inhibition of PI-3K, Akt, and $p70^{S6k}$, and impaired nickel-induced HIF-1 transactivation. In addition, overexpression of an Akt dominant negative mutant, Akt-T308A/S473A, was shown to block nickel-induced Akt phosphorylation and HIF-1 transactivation. In contrast, pre-treatment of cells with rapamycin, a $p70^{S6k}$ specific inhibitor, did not result in HIF-1 transactivation, but did attenuate $p70^{S6k}$ activity. HIF-1 activation has previously been shown to induce Cap43 gene expression and, consistent with this observation, we demonstrate here that inhibition of the PI-3K pathway using either wortmannin or LY294002, or by overexpression of Δp85 or Akt-T308A/S473A leads to a significant inhibition of nickel-induced Cap43 protein expression. Rapamycin, in contrast, did not inhibit Cap43 expression in a similar manner. In summary, our results demonstrate that nickel compounds induce both HIF-1 activation and Cap43 protein expression through a PI-3K/Akt dependent and $p70^{S6k}$-independent pathway. These results may help us understand the signal transduction pathways related to the carcinogenic effects of nickel,

517 INTERFERENCE OF POLYCHLORINATED BIPHENYLS WITH G-PROTEIN-COUPLED ATP-RECEPTOR SIGNAL TRANSDUCTION IN HUMAN MACROPHAGES.

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ATP stimulates different types of purine receptors to increase intracellular calcium concentrations ([Ca++]i) in monocyte derived human macrophages, the ion channel type P2X as well as the effector chain type P2Y receptors. Up to now it is poorly understood which type of receptors mediate the peak-like increase of [Ca++]i induced by ATP which is enhanced by the polychlorinated biphenyl congener PCB47 (Dehnhardt et al., 2000). The present investigation tries to characterize the contribution of P2Y receptors to this phenomenon. Human monocyte derived macrophage cultures treated with PCB47 were stimulated using the ATP as agonist to monitor single cell [Ca++]i using FURA-2 as fluorescence probe in an imaging system. cAMP levels were determined in macrophage cultures to monitor the adenylyl cyclase dependent branch of intracellular signal transduction. We also used the selective phospholipase C (PLC) inhibitor U73122 to block the PLC dependent branch of the P2Y receptor chain. Treatment of macrophage cultures using PCB47 in a concentration range between 10nmol and 100 μmol significantly reduced intracellular cAMP levels. Application of forskolin reduced this effect. In macrophages pretreated with U73122 (100 nmol to 10 μmol for 10 min) to inhibit PLC the peaklike intracellular [Ca++]i increase was drastically reduced in PCB 47 exposed as well as in unexposed controls. The benzoyl-ATP (bATP) stimulated [Ca++]i increase which is known to be induced only by P2X type of ATP receptors

was lower as compared to the [Ca++]i increase induced by ATP known to target both ion channel type and effector chain type of ATP receptors. These experiments show that beside P2X receptors P2Y receptors are targets for PCB47 interference in human macrophages. (partly supported by DFG, Germany)

518 DOWN REGULATION OF EGFR/MAPK SIGNALING IN THIOACETAMIDE TREATED DIABETIC RATS.

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Previously we have shown that hepatotoxicity of thioacetamide (TA) (300 mg/kg) was markedly potentiated in streptozotocin-induced diabetic (DB) rats [90% mortality in DB group vs 0% mortality in non-diabetic (NDB) group] due to combined effects of enhanced CYP2E1-mediated injury and compromised compensatory tissue repair. Diallyl sulfide, a specific inhibitor of CYP2E1, did not rescue diabetic rats from TA-induced mortality, underscoring the determining role of suppressed tissue repair in lack of survival. Even at the doses (30 in DB rats vs 300 mg/kg in NDB rats) that caused equal liver injury, tissue repair was compromised in diabetic rats, indicating that failing tissue repair is a function of diabetes. The objective of present study was to test our hypothesis that cellular mitogenic signaling is down regulated in diabetic rats. Our results indicate that TNF-aα and IL-6 mediated pro-mitogenic signaling in TA-treated DB rats is higher than TA-treated NDB rats over the time course of 0 to 48 h, indicating that the problem is not at the level of cytokine signaling. Western blot analysis of epidermal growth factor receptor (EGFR) revealed that EGFR is significantly down regulated in the DB rats and is further down regulated after TA treatment (300 mg/kg). Down stream events occurring through mitogen activated protein kinases (MAPKs), such as ERK1, ERK2 and p38 were also down regulated in the DB rats. After TA treatment (300 mg/kg), ERK1, ERK2 and p38 were further down regulated in DB rats. In contrast, in the DB rats treated with equitoxic dose of TA (30 mg/kg), EGFR, ERK1 and ERK2 were significantly up regulated as early as 6 h after TA treatment and either sustained or elevated thereafter as compared to the DB rats treated with 300 mg/kg of TA. These data indicate that down regulation of MAPK signal transduction pathway *via* EGFR may explain failed tissue repair in the unfavorable toxic outcome of TA treatment in DB rats. (Supported by Kitty DeGree Endowed Chair and LBRSF)

519 LACTOFERRIN MODULATES IMMUNE RESPONSE THROUGH THE ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASE IN RAW 264.7 CELLS.

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Lactoferrin, an iron binding glycoprotein produced by epithelial cells and neutrophils, is found in most biological fluids. To date, lactoferrin has been shown to have various biological functions: antitumor, anti-inflammatory, antifungal, antibacterial, protease, transcription factor, procoagulating, and immunomodulating activities. This protein may directly regulate the inflammatory responses. However, some of the proposed functions still remain unclear and controversial. In the present study, we investigated the mechanism of action of lactoferrin on macrophages. We treated bovine lactoferrin to RAW 264.7 cells, a murine macrophage cell line. Lactoferrin increased the production of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and effector molecules such as nitric oxide in a dose dependentmanner. As an upstream effectors in the inflammatory cytokine production, lactoferrin markedly induced activation of a transcription factor NF-κB and mitogen-activated protein kinases (MAPKs) such as p38, JNK, and ERK1/2. Taken together, we demonstrated here that lactoferrin directly activated macrophages by mediating NF-κB and MAPK signalings, resulting in increase of inflammatory cytokine production.

520 THE ROLE OF PHOSPHATIDYLCHOLINE PLC IN THE 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 determined 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. Inhibition of phosphatidylcholine specific phospholipase C (PLC), but not phosphatidylinositol specific PLC, prevented the inhibition of GJIC by 1-MeA. Apparently: the choline-specific PLC is a key upstream regulator of GJIC. In summary: 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.

521 HORMONAL REGULATION OF LACTATE DEHYDROGENASE-A IN BREAST CANCER CELLS IS DEPENDENT ON ACTIVATION OF PROTEIN KINASE C.

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Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis and LDH forms are upregulated in many types of tumors and cancer cell lines. LDH-A is increased in mammary gland carcinogenesis and in breast cancer cells, and treatment of estrogen receptor (ER)-positive MCF-7 cells with 17β-estradiol (E2) induces LDH activity. In this study, we show that E2 induces LDH-A gene expression in MCF-7 cells and the molecular mechanisms of this response have been further investigated using constructs containing the 1173 to +25 region of the LDH-A rat gene promoter (pLDH-A) which is highly homologous to the human and mouse gene promoters. Analysis studies of the LDH-A promoter showed that a series of promoter deletion constructs, pLDH-1 (-332 to +9), pLDH-2 (-249 to +9), pLDH-3 (192 to +9), pLDH-4 (-110 to +9), and pLDH-5 (-92 to +9), were E2-responsive, whereas pLDH-6 (37 to +9) was not inducible by E2. Subsequent mutation analysis of pLDH-7 (-58 to +9) demonstrated that a cAMP response element (CRE) was required and electrophoretic mobility shift assays indicated interactions of CREB and ATF-1 at this site. Although cAMP/protein kinase A activate constructs containing the CRE motif, hormonal activation of pLDH-7 is not blocked by PKA inhibitors. Subsequent studies with multiple inhibitors and a chimeric protein containing the yeast GAL4 DNA-binding domain fused to CREB demonstrated that hormonal activation of LDH-A was dependent on activation of protein kinase C through non-genomic pathways. (Supported by NIH ES09106 and E209253)

522 A POSSIBLE ROLE FOR ARSENIC AS A MEDIATOR FOR CARDIAC HYPERTROPHY.

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Previous reports have indicated that the MAP/ERK kinase, MEKK4, functions upstream of the p38 MAP kinase and that dominant-negative MEKK4 will disrupt arsenite-induced activation of the JNK MAP kinase. However, no agonists or upstream kinases have previously been reported that modulate its activity. The objective of this study was to identify and characterize proteins that regulate MEKK4 signal transduction, to determine a physiological role for MEKK4 and to see how arsenic could affect that system. Previous experiments indicated the calcium-regulated protein, annexin II, associates with MEKK4, indicating that calcium might regulate MEKK4 activity. Early experiments also indicated that MEKK4 could be phosphorylated on tyrosine, suggesting that a tyrosine kinase might regulate MEKK4 activity. A likely candidate for the upstream kinase is the calcium-regulated tyrosine kinase Pyk2. To investigate this, Pyk2 was immunoprecipitated from aortic smooth muscle cells treated with angiotensin II followed by an *in vitro* kinase assay to evaluate the kinase activity of Pyk2 on MEKK4. The ability of Pyk2 to phosphorylate MEKK4 on tyrosine was confirmed, but only in response to the angiotensin II treatment. As both angiotensin II and p38 have been reported to modulate COX II, we tested whether MEKK4 was involved in this pathway. HEK cells stably expressing the angiotensin receptor were transiently transfected with dominant-negative MEKK4 and a COX II-luciferase reporter construct. Dominant negative MEKK4 greatly reduced the luciferase activity, indicating MEKK4 modulates COX II production. Similarly we confirmed that arsenic could activate the COX II reporter and that dominant negative MEKK4 also inhibited

the effect. We hypothesize that Pyk2 phosphorylates MEKK4 on tyrosine in response to angiotensin II, subsequently regulating COX II production and that arsenic exposure is able to activate this signaling cascade. Ongoing studies are aimed at investigating the site of arsenic activity in this pathway and its possible role in cardiac hypertrophy. (NIH ES 12007 and ES 04940)

523 MECHANISMS FOR SELECTIVE ACTIVATION OF SRC FAMILY KINASES AND JNK BY LOW LEVELS OF CHROMIUM(VI).

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Inhaled hexavalent chromium (Cr(VI)) promotes pulmonary disease and lung cancer through poorly defined mechanisms. These mechanisms were studied in A549 lung epithelial cells to investigate the hypothesis that non-toxic Cr(VI) exposures selectively activate cell signaling that shifts the balance of gene transcription. These studies demonstrated that non-toxic doses of Cr(VI) increased reactive oxygen species and selectively activated JNK, relative to ERK or p38 MAP kinase. In contrast, only toxic, non-selective levels of exogenous oxidants stimulated JNK. However, JNK activation in response to chromium and exogenous H_2O_2 shared requirements for intracellular thiol oxidation, activation of Src family kinases, and $p130^{cas}$ (Cas). Cr(VI) did not mimic H_2O_2-mediated stimulation of JNK in cells containing only Src and did not activate Src or Yes in A549 cells. Instead, Fyn and Lck were activated in A549 cells indicating cell specific activation of Src family kinases in response to Cr(VI). Finally, Cr(VI) was demonstrated to directly activate purified Fyn *in vitro* and that the majority of this activation did not require oxidant generation. These data suggest that non-toxic levels of Cr(VI), which can shift patterns of gene transcription, are selective in their activation of cell signaling and that Cr(VI) can directly activate Src family kinases. *Supported by NIEHS grant ES10638.*

524 ROLE OF THE SERINE/THREONINE KINASE MEKK4 IN CYCLOOXYGENASE II REGULATION IN HUMAN KERATINOCYTES.

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We are interested in cellular arsenate and arsenite stress response mechanisms. The mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 4 (MEKK4) signaling pathway is implicated to respond to arsenate and arsenite stress. Using immortalized human keratinocytes, HaCaT cells, as a model system we are investigating the regulation of the serine/threonine protein kinase MEKK4. We show that extracellular calcium is essential for MEKK4 phosphorylation. We have evidence that ATP binding to a purinergic receptor leads *via* a G protein-coupled response to Ca2+ influx and to a localized rise of cytoplasmic Ca2+ concentration. Annexin II is a calcium-binding protein and preliminary evidence suggests that annexin II interacts with components of the MEKK4 pathway. Blocking the influx of extracellular Ca2+ results in lesser or basal state of MEKK4 activation. Stimulation of HaCaT cells with ATP, a purinergic receptor agonist, results in tyrosine phosphorylation of MEKK4. We have evidence that the MEKK4 pathway regulates cyclooxygenase II expression levels. To corroborate data obtained with immortalized keratinocytes we, in addition, employ normal human epidermal keratinocytes (NHEK). The inflammatory mediator interferon gamma has been reported to activate cyclooxygenase II expression. Indeed, we demonstrate an interferon gamma dependent tyrosine phosphorylation of MEKK4. Furthermore, the tyrosine kinase Pyk2 co-immunoprecipitates with MEKK4, suggesting that this tyrosine kinase may mediate the phosphorylation of MEKK4. Also present in this complex is the calcium-binding protein annexin II as identified by co-immunoprecipitation. This may indicate a calcium dependent regulation of tyrosine phosphorylation of the serine/threonine kinase MEKK4. Ongoing studies investigate the role of MEKK4 in arsenic signal transduction in keratinocytes.

525 *IN VIVO* DETECTIONS OF MULTIPLEX PHOSPHOPROTEINS AND CYTOKINES WITH BIO-PLEX PROTEIN ARRAY SYSTEM.

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Phosphoproteins and cytokines play pivotal roles in cell signaling events, mediating a wide range of physiological responses. These proteins are well defined targets for toxicology study. Bio-Plex Protein Array System based on Luminex technology has been proven a novel system to detect these protein markers in a multiplex format. We have presented *in vitro* data in last SOT meeting. At current study, we treated mice with synthesize substance A at concentrations of 1, 3, 10 and 30mg/dose and sacrificed the mice after 0, 0.5 and 17 hours. We collected the livers from those treated mice and prepared the tissue lysate samples. The samples were run with Bio-Plex multiplex phosphoproteins reagents and Bio-Plex Protein Array System. Five phosphoproteins were detected simultaneously from the liver tissue samples, including JNK, p38, Erk, IkBa and Akt. The results showed that phosphorylations of these kinases induced by the substance were dose-dependent and time dependent. The MAP kinase p38 was the most sensitive target and the phosphorylation was observed significantly at 3mg/dose of the compound. There was no significant phosphorylation detected within half an hour after dosing. However, after 17 hours, all the targets had more than 5-folds of phosphorylation observed compared with controls. The results clearly demonstrate that Bio-Plex Protein Array System and Phosphoprotein reagents are very reliable and efficient tools to detect phosphoproteins from tissues in multiplex format. We also have collected 9-plex cytokine data from rat tissue and 18-plex cytokine data from mouse tissues. These studies strongly support that Bio-Plex Protein Array System and multiplex assay reagents are very powerful tools for *in vivo* toxicology study, which can detect several phosphoproteins and many cytokine targets simultaneously from one tissue sample.

526 AH RECEPTOR-MEDIATED TRANSCRIPTIONAL SUPPRESSION OF INTERLEUKIN-6.

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The Ah receptor (AhR) mediates most of the toxic responses induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD exposure causes marked immune suppressive responses. The mechanism for the immune suppression by TCDD remains to be investigated. In an earlier study, we found mutually suppressive interactions between the AhR and a pleiotropic transcription factor nuclear factor kappa B (NF-kB). NF-kB is known to regulate a large group of immune and inflammatory responsive genes, including the interleukin 6 (IL-6). The IL-6 gene is known to be regulated directly by NF-kB and its promoter regions has been well characterized, making it a good system to analyze the effects of AhR/NF-kB interaction. In the present study, we found IL-6 mRNA transcripts can be induced in Hep1c1c7 (mouse hepatoma cell line) by either TNF-alpha (2.5 ng/ml, 30 min) or lipopolysaccharide (LPS, 1 ug/ml, 4 hrs) as determined by RT-PCR quantitation. TCDD treatment (10 nM) blocked the TNF and LPS-induced IL-6 gene expression. The suppressive effects of TCDD on the IL-6 gene expression are clearly dependent on the intact AhR complex, since TCDD treatment had no effect on the mutant cell lines Bpr or Tao, which either lack the AhR nuclear translocator (Bpr) or the AhR, respectively. Supported in part by NIEHS Grant ES 09859.

527 EFFECTS OF ESTROUS CYCLE AND SOY ON CYTOCHROME P450 1B1 (CYP1B1) PROTEIN EXPRESSION IN FEMALE RAT ADRENAL GLANDS.

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Previous work in our laboratory found that female NCTR Sprague-Dawley (SD) rats fed a soy- and alfalfa-free diet were more sensitive to 7, 12-dimethylbenz(a)anthracene (DMBA)-induced adrenal toxicity than expected based on extensive literature. Because CYP1B1 has been shown to be important in the bioactivation of DMBA to an adrenal toxin in rats, we are investigating the effects of diet and genetic background on CYP1B1 expression in rat adrenal glands. In this experiment, female SD rats were placed on standard NIH31 rat chow or soy- and alfalfa-free 5K96 diet from postnatal day (PND) 21 until sacrifice at PND50 ± 5. Stage of estrous cycle at sacrifice was predicted by daily vaginal cytology and confirmed by histological examination of the vagina. Serum was collected at necropsy and estradiol and progesterone levels measured. Immunohistochemical analysis confirmed that CYP1B1 was exclusively expressed in the zona fasciculata and zona reticularis in adrenal cortex, which are the regions vulnerable to DMBA-induced adrenal necrosis. Western blot analysis showed significantly higher CYP1B1 expression in diestrus than in proestrus in the adrenal microsomes of rats fed 5K96 but not in rats fed NIH-31. In both estrus and diestrus, CYP1B1 expression was higher, though not significantly so, in rats fed with 5K96 diet than rats fed with NIH-31 diet. *In vitro* adrenal microsome reactions showed that 4-OH estradiol formation from estradiol, a reaction associated with human CYP1B1, was slightly, but not significantly, higher in diestrus than in proestrus for both diet groups (diestrus: 0.037 ± 0.010 and 0.037 ± 0.003 pmole/μg/min (mean ± SD); proestrus: 0.029 ± 0.001 and 0.030 ± 0.013 pmole/μg/min for NIH31 and 5K96, respectively). DMBA metabolism is being investigated. Adrenal CYP1B1 appears to be regulated by modulation of hormones during the estrous cycle and soy may affect this regulation.

528 ORGAN SPECIFIC INDUCTION OF CYP1A1 AND 1B1 BY CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN ENGINEERED C57BL/6J MICE OF ARYLHYDROCARBON RECEPTOR GENE.

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Organ-specific induction of mRNAs of CYP1A1 and 1B1 by polycyclic aromatic hydrocarbons (PAHs) was investigated in wild and arylhydrocarbon receptor (AhR)-deficient C57BL/6J mice. CYP1A1 mRNA was detected in control mice at very low levels in liver, lung, heart, kidney, intestine, thymus, testis, uterus, ovary, and brain and was highly inducecd in these organs by benzo[a]pyrene in AhR(+/+) mice. In AhR(+/+) and AhR(-/-) mice, CYP1B1 mRNA was found to be constitutively expressed at significant levels in heart (the ratio of mRNAs of CYP1B1 to actin was about 0.7), kidney (about 0.7), intestine (about 0.3), testis (about 1.0), thymus (about 0.4), uterus (about 0.2), ovary (about 1.5), and brain (about 0.4), whereas it was low in liver and lung (<0.2 in these cases). CYP1B1 in the latter two organs was highly induced by PAHs in AhR(+/+) mice. The induction of CYP1B1 by PAHs was more extensive in organs where the constitutive expression of CYP1B1 was low. These results suggest that CYP1A1 and CYP1B1 are differentially regulated in their expression in extrahepatic organs of mice and could be induced by PAHs with different extents depending on the inducers used and the organs in AhR(+/+) mice. The findings of significant levels of constitutive expression of CYP1B1 in AhR(-/-) mice as well as AhR(+/+) mice in several organs including heart, kidney, thymus, testis, ovary, and brain in AhR(-/-) mice as well as AhR(+/+) mice are of importance in understanding the basis of toxicity and carcinogenesis by chemicals that are metabolized by CYP1B1. (Supported in part by Grants from the Ministry of Education, Science, and Culture of Japan and the Petroleum Energy Center.)

529 DEVELOPMENT OF AN *IN VITRO* MODEL SYSTEM TO EVALUATE SWITCH-LIKE BEHAVIORS OF HEPATOCYTES IN RESPONSE TO ENZYME INDUCERS.

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Receptor-mediated enzyme induction in the liver has been reported to demonstrate switch-like behaviors. As dose increases, more cells become fully induced rather than observing a proportionate induction in all hepatocytes. The inducing chemicals act *via* different receptor pathways, indicating that the switch-like response is a generic property of induction within these cells. We developed an *in vitro* model system using primary rat hepatocytes and a prototypical enzyme inducer to study this phenomenon. The induction of cytochrome P450 1A1 protein and mRNA was examined by treatment with PCB 126, an aryl hydrocarbon receptor agonist. Primary rat hepatocytes were exposed to concentrations of PCB 126 ranging from 10-5 to 10-11 M for various time points. Dose response curves in hepatocyte populations were established using western blots for total protein induction and real-time RT-PCR for mRNA. To analyze the switching response at the level of individual cells, the techniques of immunocytochemistry and in situ hybridization were used. The integrated responses of populations of hepatocytes were compared with responses of individual cells by visualization and quantification of single cell induction. Induction of individual cells and the dose response for increasing numbers of cells with increasing dose was qualitatively evident for both the protein and mRNA. Single cell quantification with a Bioquant imaging system permitted comparison of induction level on a cell-by-cell basis. Interpretations of distributions are based on methodologies evaluating a switch response in oocytes (Ferrell and Machleder, Science, 280, 895, 1998). Distributions of induced cells were compared with those generated from similar *in vivo* studies. Our *in vitro* model provides a system with which to characterize the switching response and to investigate the underlying molecular mechanisms (American Chemistry Council contract RSK0005).

530 REGIONAL INDUCTION OF CYP1A1 PROTEIN AND MESSAGE IN RAT LIVER DURING TREATMENT WITH MIXTURES OF PCB126 AND PCB153.

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Liver enzyme induction by various inducers is regional and has all-or-none, switch-like characteristics *in vivo* and *in vitro*. This has been studied in an *in vitro* switching model (CT French et al., this meeting). We have studied this switch *in vivo* by evaluating enzyme induction and liver tumor promotion due to a mixture of the co-planar PCB126 and the non co-planar PCB153 in Fisher rats using a modified Ito medium term initiation-promotion study. Two weeks after DEN initiation, rats were gavaged 3 times per week with PCBs at varying concentrations of the PCBs alone and as mixtures. In a second study, enzyme activities for CYP1A1 were estimated in liver microsomes from Sprague-Dawley rats treated with PCBs for 13 and 30 weeks. At moderate doses, there was a greater than additive increase in CYP1A1 activity in the 13/30 week dosing study and increased liver concentration of PCB153 in mixture treated rats in the Ito study. At moderate to high doses in the Ito study, there were less than additive increases in number and area of GST-P foci. Also in the Ito study, exposure to PCB126 caused regional induction of CYP1A1, starting in the centrilobular region at 0.1μg/kg/day and progressing outward until the entire liver was induced at 10μg/kg/day, then shifting to only the periportal region in rats treated with the highest dose mixture (10μg/kg/day PCB126; 10, 000μg/kg/day PCB153). The pattern of induction was the same for CYP1A1 mRNA as assessed qualitatively by in-situ hybridization and immunohistochemistry on sequential liver sections. Quantitatively, induction was assessed by measuring mRNA levels in individual cells. The unexpected regional interaction may correlate with the inhibitory interactions for GST-P positive foci and the increased concentration of PCB153 in liver of the high dose mixture-treated rats. (American Chemistry Council contract #1467, NIEHS grants SBRP ES05949, KO8 ES00314 and KO8 ES00380.)

531 ANALYSIS OF TCDD-MEDIATED INDUCTION OF *CYP1A1* IN IKKβ$^{+/+}$ AND IKKβ$^{+/-}$ MICE.

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Cytochrome P450 1A1 (CYP1A1) catalyzes the hydroxylation of a variety of xenobiotic compounds and is strongly induced by halogenated and polycyclic aromatic hydrocarbons through activation of the aryl hydrocarbon receptor (AhR). Several lines of evidence indicate a linkage between activation of the AhR / *Cyp1a1* signal transduction pathway and NF-κB activation, in both function and regulation. To further characterize this linkage, TCDD-mediated induction of *Cyp1a1* was investigated in IKKβ$^{+/+}$ and IKKβ$^{+/-}$ C57Bl/6 mice. IKK is a kinase composed of 2 catalytic subunits (α and β) and 1 regulatory subunit (γ). IKK phosphorylates IκB, which causes dissociation of IκB from the IκB - NF-κB complex and triggers degradation of IκB. Free NF-κB then translocates to the nucleus, binds to κB binding sites on DNA, and stimulates transcription of target genes. Embryonic fibroblasts from IKKβ$^{+/-}$ C57Bl/6 mice exhibit a 50% reduction in IKK activity and a 90% reduction in NF-κB DNA binding activity. IKKβ$^{+/+}$ and IKKβ$^{+/-}$ mice were treated for 18 hours with different doses of TCDD, ranging from 0.32 μg/kg to 125 μg/kg. Liver Cyp1a1 RNA and protein levels were quantitated. In IKKβ$^{+/+}$ mice, Cyp1a1 RNA and protein were detectable after treatment with 0.32 μg/kg TCDD. Cyp1a1 RNA and protein reached maximum levels after treatment with 1.6 μg/kg TCDD. At TCDD doses of 16 μg/kg, 45 μg/kg, and 125 μg/kg, Cyp1a1 RNA and protein levels observed were similar to those of mice treated with 1.6 μg/kg TCDD. At all TCDD doses, Cyp1a1 RNA and protein levels were not significantly different in IKKβ$^{+/-}$ mice when compared to IKKβ$^{+/+}$ mice. This suggests that TCDD-mediated induction of *Cyp1a1* is not affected by a significant loss of NF-κB activity *in vivo*. (Supported by USPHS grant ES10337.)

532 DEVELOPMENT OF A HUMAN *CYP1A1*-LUCIFERASE TRANSGENIC MOUSE MODEL.

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Regulation and expression of *CYP1A1* is mediated in part through activation of the Ah-receptor by environmental toxicants such as polycyclic and heterocyclic aromatic hydrocarbons, in addition to dietary compounds such as flavonoids. The human *CYP1A1* gene contains three Ah receptor enhancer sequences (XRE) that associate with the ligand activated Ah receptor resulting in enhanced transcription. Previously, the human *CYP1A1* promoter and 1600 bases of 5′-flanking sequence was subcloned upstream of the fire-fly luciferase reporter gene and stably transfected into HepG2 cells. This cell line, called TV101, has been used to identify Ah receptor ligands and to study the regulatory patterns of the *CYP1A1* gene. In these studies, the human *CYP1A1*-luciferase chimeric was microinjected into the pronucleus of fertilized mouse eggs and then transferred to pseudopregnant C57Bl/6 mice. From 53 offspring, the incorporation of the *CYP1A1*-luciferase gene was identified in four founders. Each founder was bred and transmission of the gene into the F1 population was examined for inducibility in each of the four litters. Upon treatment with a single dose of dioxin or 3-methylcholanthrene, induction of

hepatic fire-fly luciferase activity was observed in siblings from two of the breedings. Treatment of *CYP1A1*-luciferase transgenic mice with differing doses of TCDD ranging from 8 to 32 μg/kg demonstrated a concordance of liver induced luciferase activity with the accumulation of microsomal cyp1a proteins. Thus, these studies demonstrate the potential usefulness of a transgenic mouse model for examining the regulatory properties of the human *CYP1A1* gene. (Supported by Superfund Grant ES10337)

533 SMOKING INCREASES HUMAN NASAL EPITHELIAL CYTOCHROME P450 1A1 GENE TRANSCRIPTION.

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Respiratory tract carcinogenesis, mediated by polycyclic aromatic hydrocarbons (PAH) such as those associated with cigarette smoke, is in part due to induction of cytochrome P450 1A1 (CYP1A1) mRNA and protein expression with subsequent carcinogenic activation of PAH by CYP1A1. Respiratory epithelium is a key target tissue for these events. We therefore hypothesized that CYP1A1 mRNA expression level could be noninvasively monitored in human nasal epithelium, the most easily sampled respiratory epithelium, as a potentially pre-carcinogenic health effect of exposures to inhalants such as cigarette smoke. Subjects were healthy office and garage workers. Demographic, smoking, and occupational histories were obtained by questionnaire. Nasal epithelium was collected using Rhinoprobes(Tm) to obtain scrapings from subjects inferior turbinates. Tissue samples were stabilized using RNLater(Tm) to inhibit RNAses. RNA was extracted using Trizol(Tm). Isolated RNA was reverse transcribed, and abundance of mRNA encoding CYP1A1, IL-8, and G3PDH was evaluated by real time TaqMan(Tm)PCR. Cycle threshold (CT) was used as a measure of the mRNA level in each sample, standardized between samples by using the CT for G3PDH, and the following equation used to calculate a delta CT: CT (CYP1A1 or IL-8) minus CT (G3PDH) = delta CT. Data demonstrated a significant increase ($p < 0.001$) of CYP1A1 expression in current smokers (n=16) as compared to non-smokers (n=17) or former smokers (n=23) in our study group. No significant differences were seen in IL-8 expression levels between these groups nor were any associations apparent between occupational exposures and expression levels of CYP1A1 or IL-8. We conclude that CYP1A1 gene expression can be monitored noninvasively in human nasal epithelium, and that expression level can be modulated by exposures to agents such as cigarette smoke.

534 ROLES OF THE AH RECEPTOR IN OXYGEN-MEDIATED INDUCTION OF PULMONARY AND HEPATIC CYTOCHROME P4501A ENZYMES AND IN THE ATTENUATION OF HYPEROXIC LUNG INJURY.

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Administration of supplemental oxygen is frequently encountered in infants suffering from pulmonary insufficiency. However, hyperoxia causes lung damage in experimental animals, and may do so in humans. The mechanisms of oxygen-mediated lung injury are not completely understood. In the present investigation, we tested the hypotheses that: (i) hyperoxia would modulate pulmonary and hepatic cytochrome P450 (CYP)1A1/1A2 expression by Ah receptor (AHR)-dependent mechanisms; and (ii) AHR (-/-) mice would be more susceptible to hyperoxic lung injury than wild type (C57BL/6J) [(AHR (+/+)] mice. Two month-old adult male AHR (+/+) and AHR (-/-) mice were maintained in room air or exposed to hyperoxia (greater than 95% oxygen) for 24-72 h, and pulmonary and hepatic CYP1A1/1A2 expression was studied. Extent of lung injury was determined by measuring lung weight/body weight ratios and by histology. Hyperoxia caused significant increases in pulmonary and hepatic ethoxyresorufin O-deethylase (EROD) (CYP1A1) activities and pulmonary CYP1A1 mRNA levels in AHR (+/+), but not AHR (-/-) mice, suggesting that AHR-dependent mechanisms contributed to CYP1A1 induction. On the other hand, hyperoxia augmented hepatic CYP1A2 expression in AHR (+/+) as well as AHR (-/-) animals, suggesting that AHR-independent mechanisms contributed to this phenomenon. AHR (-/-) mice exposed to hyperoxia were more susceptible to hyperoxic lung injury, as indicated by significantly higher lung weight/body ratios, increased pulmonary edema, and enhanced neutrophil recruitment into the lungs than similarly exposed wild type animals. Taken together, our results support the hypothesis that there is a mechanistic link between the AHR, induction of CYP1A enzymes, and development of hyperoxic lung injury. (Supported in part by NIH grant ES09132 and by the American Lung Association.)

535 ALLEVIATION OF HYPEROXIC LUNG INJURY IN THE NEWBORN RAT BY RETINOIC ACID.

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Supplemental oxygen is frequently used in infants having pulmonary insufficiency, but prolonged hyperoxia contributes to the development of chronic lung disease in these infants. Hyperoxic exposure during the newborn period leads to acute lung injury and abnormal lung maturation in rats. Cytochrome P450 (CYP) enzymes have been implicated in lung injury induced by hyperoxia. In this investigation, we tested the hypothesis that neonatal exposure of rats to a combination of retinoic acid (RA) and hyperoxia would lead to decreased susceptibility of these animals to lung injury and improved lung maturation in adulthood, compared to those exposed to hyperoxia only, and that modulation of cytochrome P450 (CYP)1A and/or CYP4F4 expression contributes to the beneficial effects of RA. Newborn Fisher rats were maintained in room air or exposed to hyperoxia (≥95% O_2) for 7 days. Some animals were treated i.p. with RA (0.5 mg/kg) or vehicle (saline), once daily for 5 days. Animals were sacrificed 1 or 30 days after termination of hyperoxia, and lung injury was assessed by measuring lung weight/body weight (LW/BW) ratios and by histology. Pulmonary CYP1A1 and CYP4F4 expression was studied by RT-PCR. Exposure of animals to hyperoxia alone for 7 days showed higher LW/BW ratios at 1 day compared to those exposed to RA + hyperoxia. Hyperoxia significantly suppressed CYP1A1 mRNA expression at 1 day, and this effect was potentiated by RA treatment. On the other hand, while hyperoxia dramatically attenuated CYP4F4 expression, this effect was counteracted by pretreatment with RA. At the 30 day time point, the oxygen-exposed animals showed retarded alveolarization. In contrast, RA + hyperoxia-exposed animals showed improved alveolarization. The differential regulation of CYP1A and CYP4F4 expression by hyperoxia and hyperoxia + RA suggests that these enzymes may contribute to the beneficial effects of RA in alleviating lung damage induced by hyperoxia. (Supported in part by NIH grant KO8 HL04333 to XC)

536 EFFECTS OF LIGHT AND DARK BEERS ON HEPATIC CYTOCHROME P450 EXPRESSION IN MALE RATS RECEIVING ALCOHOLIC BEVERAGES AS PART OF TOTAL ENTERAL NUTRITION.

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Alcoholic beverages contain many congeners in addition to ethanol. Therefore, consumption of alcoholic beverages may have considerably different effects on expression of hepatic microsomal monooxygenases than the relatively selective induction of CYP2E1 observed following ethanol consumption. In the current study we compared the effects of two beers: larger (a light roasted beer) and stout (a dark roasted beer) infused intragastrically into groups of N = 10-13, 300 g male Sprague-Dawley rats for 21 d using a system of total enteral nutrition (TEN) with a group of rats infused an equivalent amount of ethanol isocalorically. At the end of the infusion period, rats were sacrificed and liver microsomes prepared. Cytochrome P450s CYP1A1/2, CYP2B1, CYP2E1, CYP3A and CYP4A expression was assessed by Western immunoblot analysis. In addition monooxygenase activities were assayed for the following substrates: ethoxy-, methoxy-, pentoxy- and benzyloxyresorufin, tesosterone, midazolam, erythromycin and p-nitrophenol. No effects of larger or stout were observed on relative expression of CYP2E1or CYP2B1 or activity towards p-nitrophenol or pentoxyresorufin. However, higher expression of CYP1A2, CYP3A and CYP4A were observed in stout-infused relative to larger and ethanol-infused rats ($p \leq 0.05$). In addition, although no differences were observed in alkoxyresorufin, midazolam or testosterone metabolism between groups, stout-infused rat had greater erythromycin N-demethylase activity (a CYP3A substrate) and higher lauric acid 12-hydroxylase activity (a CYP4A substrate) than other groups ($p \leq 0.05$). Therefore stout contains congeners which are inducers of cytochrome P450s other than CYP2E1. Supported in part by R01 AA08645 (TMB).

537 EFFECTS OF CHRONIC ETHANOL ON HEPATIC CYP2C11 IN MALE RATS: INTERACTIONS WITH THE JAK2-STAT5B PATHWAY.

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Chronic alcohol consumption results in altered expression of many genes. These effects are often secondary to alterations in hormonal actions. GH is secreted from the pituitary gland in a sexually dimorphic pulsatile pattern and this secretory pat-

tern is known to be important in the regulation of gene expression involving the JAK-STAT signal transduction pathway. Chronic alcohol treatment in male rats results in: 1) demasculinization of the GH pulse pattern; and 2) decreased expression of the male-predominant hepatic CYP2C11. Using the total enteral nutrition (TEN) model, Western and Northern analysis and electrophoretic mobility shift assays, we have evaluated the involvement of the JAK-STAT pathway in male rats (8-10/group) chronically infused intragastric diets with or without ethanol for 45 days. We have found that hepatic levels of CYP2C11 mRNA and protein, STAT 5b and phospho-STAT 5b levels were reduced ($P \leq 0.05$) in ethanol-infused rats as compared to rats not infused with ethanol. However, the levels of JAK2 were elevated ($P \leq 0.05$) in ethanol-treated rats. These results suggest that: 1) the JAK2 suppression in the classic GHR-JAK-STAT-CYP2C11 pathway is masked by ethanol-induced increases in other JAK2-dependent pathways; or 2) ethanol does not reduce CYP2C11 through the classical GH-GHR-JAK2-STAT 5b pathway. Supported by NIAAA08645 (TMB).

538 A CELL-BASED ASSAY FOR SCREENING INDUCERS OF CYTOCHROME P450 3A4 AND ANALYZING TRANSCRIPTIONAL REGULATION BY PREGANE X RECEPTOR (PXR).

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Cytochrome 3A4 (CYP3A4) is a major enzyme in hepato-intestinal detoxification and is estimated to metabolize over 50% of clinical drugs in use. Expression of CYP3A4 is transcriptionally regulated by the pregnane X receptor (PXR). PXR is activated by a wide range of endogenous and exogenous compounds, which induce CYP3A4. Regulations of CYP3A4 expression are of great interests to drug development and application. Here, we report the development of a human PXR-based luciferase reporter assay for screening compounds that activate hPXR and induce CYP 3A4 gene expression. Cell lines have been developed by stable transfection of human PXR and luciferase reporter gene into the HepG2 cells. The luciferase reporter gene was constructed using a combination of enhancer (-7836 to -7208) /promoter (-362 to +53) modules from CYP3A4 gene. The assay system is highly responsive to known CYP3A4 inducers such as rifampicin, clotrimezole, extracts of St. John's wort, but not to rodent CYP3A inducers such as pregnenolone-16a-carbonitrile and dexamethasone. Interestingly, utilizing the cell-based assay, we found synergistic activation of the luciferase reporter gene when the cells were co-treatd with the inducers of CYP3A4 and CYP1A1. For example, TCDD, benzo-(a)-pyrene markedly enhances rifampicin-induced luciferase reporter gene activity. These results reveal, for the first time, a potential interaction between the Ah receptor-regulated pathways and PXR-regulated pathways. In summary, the above bioassay offers a sensitive and cost-effective means for screening potential CYP3A4 inducers, while avoiding variability of human primary hepatocytes. In addition, this PXR-based bioassay has also been proven useful in analyzing the mechanism of transcriptional regulation by PXR. Supported in part by NIEHS Grant ES09859.

539 HEPATIC EFFECTS OF OCTAMETHYLCYCLOTETRASILOXANE (D4) IN FEMALE FISCHER 344 RATS AND FEMALE HARTLEY GUINEA PIGS FOLLOWING A 14-DAY ORAL ADMINISTRATION.

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This study was conducted to compare hepatic responses in the rat to that in the guinea pig following oral administration of octamethylcyclotetrasiloxane (D4). Female Fischer 344 rats and female Hartley guinea pigs were dosed with D4 (300 mg/kg/day, po), phenobarbital (positive control; 80 mg/kg/day, po), or vehicle (corn oil, po) for 14 consecutive days. On day 15 the animals were euthanized, organ weights were determined and tissues collected. In rats, D4 administration resulted in increased liver weight (59% increase in absolute and relative liver weight), induction of CYP2B (20 to 27-fold increase in enzyme activity and a marked increase in protein) and only minor changes in CYP1A (enzyme activity and protein), CYP3A (protein), CYP4A (protein), NADPH cytochrome c reductase activity, and epoxide hydrolase (protein). In contrast, liver weight was not significantly changed by D4 administration to the guinea pig. Only minor changes in CYP2B (enzyme activity and protein), CYP1A (enzyme activity and protein), NADPH cytochrome c reductase activity, and epoxide hydrolase (protein) were observed. Analysis of liver D4 content demonstrated a marked difference between species. Rat liver D4 content (0.86 ± 0.13 μg/g liver) was markedly lower than that for guinea pig (7.93 ± 7.44 μg/g liver). Overall, this study has confirmed that repeated administration of D4 to female Hartley guinea pigs, at a dose that causes increased liver weight and cytochrome P450 induction in female Fischer 344 rats, does not cause liver weight increases or significant induction of liver microsomal CYP2B, CYP1A and epoxide hydrolase in the guinea pig.

540 EPIGALLOCATECHIN GALLATE ELICITS SEX-DEPENDENT MODULATION OF CYP450 ISOFORMS IN THE SWISS WEBSTER MOUSE.

M. G. Goodin and R. J. Rosengren. *Department of Pharmacology and Toxicology, University of Otago, Dunedin, New Zealand.*

Epigallocatechin gallate (EGCG) is a potent cancer-preventative agent. The cytochrome P450 (CYP450) family plays a crucial role in bioactivation of carcinogens and EGCG is a potent *in vitro* inhibitor of CYP450 isoforms. However, EGCG's effect on CYP450 isoforms has not yet been investigated *in vivo*. Therefore, the aim of the present study was to characterize the modulatory effect of EGCG on CYP450 isoforms in male and female mice. Swiss Webster mice were dosed with EGCG (25 or 50 mg/kg, ip) for 7 days and hepatic microsomes were prepared on day 8. EGCG at 25 mg/kg was well tolerated by both females and males, as indicated by normal levels of liver transaminases and body weight gain. However, doses of 50 mg/kg caused 75% mortality in females compared to 30% in males. Following EGCG (25 mg/kg), CYP1A2 remained unchanged in females, while both CYP3A and CYP2E1 were increased. Specifically, CYP3A catalytic activity and polypeptide levels were increased 32 and 17% above vehicle control, respectively ($p<0.001$). Similarly, CYP2E1 catalytic activity was induced compared to vehicle control (*p*-nitrophenol hydroxylation values of: 2.03 ± 0.11 vs 1.54 ± 0.07 nmol/mg/min, $p<0.001$), while polypeptide levels were increased 24% above vehicle control ($p<0.001$). In males, EGCG (25 mg/kg) did not alter CYP1A2, 3A or 2E1. However, EGCG (50 mg/kg) inhibited CYP1A2 catalytic activity and polypeptide levels by 41 and 50%, respectively, compared to vehicle control ($p<0.005$). In conclusion, high doses of EGCG are hepatotoxic to the Swiss Webster mouse, and females are more susceptible than males. The mechanism of this toxicity is unknown. The toxic response may be species-specific as EGCG has been administered to rats without any reported mortality or toxicity. In males, EGCG inhibits CYP1A2 as demonstrated *in vitro*. However, EGCG is an inducer of CYP450 isoforms in female mice and suggests that EGCG could cause an increased activation of xenobiotics. Therefore, the anticarcinogenic action of EGCG is more likely to result from it's antioxidant properties.

541 MULTIGENERATIONAL POSTMORTEM FINDINGS IDENTIFY TARGET ORGANS OF RATS EXPOSED TO LOW LEVELS OF CHLORDANE.

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Technical chlordane (TC), a complex organochlorine mixture banned in 1988, is in the food chain, all environmental media & biota and in humans. It poses a risk to humans. Human target organs for chronic oral exposures at environmental levels are unknown. Our previous generational rat studies identified reproductive, developmental & CNS in-life effects. Sprague-Dawley rats were used in this oral two-generation study to identify target organs of TC toxicity at postmortem. F0, F1, F2 rats were exposed daily to TC at 0.00, 0.125, 0.25 and 1 mg/kg b.w. /day. Endpoints were terminal body weight (TBW) and weights & histopathology of brain, pituitary, liver, spleen, thymus, kidney, adrenals, uterus, testes, seminal vesicles, prostate, and epididymus; vaginal cytology for 21 days inclusive of day of sacrifice, sperm motility, velocity, morphology and concentration, blood parameters (testosterone, estrogen, chemistry and hematology) and liver cytochrome P450 (CYP1A1, 2B1, 2E1) enzymes. Gross pathology was unremarkable. Data different ($p<0.05$) from controls are reported. Absolute and relative (organ/brain & organ/TBW) liver weights increased in F0 females and F2 males at 1.0 mg/kg levels. Relative pituitary weights decreased in F1 females at 0.25 and 1.0 mg/kg and increased in F2 males at 1.0 mg/kg levels. At 0.25 mg/kg level, brain weight increased in F1 females & decreased relative to TBW in F0, males. TBW increased in F1 & F2 males. Relative adrenal weights decreased in F1 & F2 males at 0.25 &1.0 mg/kg. TC increased plasma estrogen & estrogen/testosterone ratio in Fo males. In F0, F1 & F2. TC induction of CYP1A1, 2B1& 2E1 was sex and dose-dependent. Histopathologically, TC increased seminiferous tubular & luminal diameters and epithelial heights of F1 & F2 and caused liver centrilobular hypertrophy in F1 males. Reproductive organs, liver, CNS & adrenals are targets of chronic TC exposures. (Supported by MHPF/ATSDR U50/ATU398948)

542 DEXAMETHASONE TREATMENT DECREASES HEPATIC ARACHIDONIC ACID EPOXYGENASE ACTIVITIES IN RATS AS A RESULT OF DECREASED CYP2C23 EXPRESSION.

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A sensitive and specific ELISA for 14, 15-dihydroxyeicosatrienoic acid (DHET) has been developed to facilitate investigation of the physiological and pathophysiological roles of epoxyeicosatrienoic acids (EETs) and DHETs, metabolites of arachidonic acid (AA) epoxygenase and soluble epoxide hydrolase (sEH), respectively. Male Sprague-Dawley rats were treated with corn oil or dexamethasone (DEX) (10 mg/kg for 4 days, i.p.) and hepatic microsomal AA epoxygenase activity was measured by ELISA after chemical hydration of 14, 15-EET to 14, 15-DHET. DEX treatment dramatically decreased hepatic microsomal AA epoxygenase activity. This result was confirmed by measurement of [14-C]EET formation activity of microsomal AA epoxygenase after incubation of microsomes with [14-C]AA and NADPH. The [14-C]EET was separated from other metabolites by HPLC. In rats, AA can be biotransformed to EET by the activities of several cytochromes P450, including CYP2C11, CYP2C23, CYP2J3 and CYP2J4. However, Western blot analyses revealed that, among these CYPs, only CYP2C23 levels were dramatically decreased by DEX treatment. These results suggest that decreased AA epoxygenase activities in liver obtained from rats after treatment with DEX was a result of decreased CYP2C23 expression. Contrary to decreased EET formation activities, free + esterified EET levels did not decrease after 4 days of DEX treatment. This result suggests that a long-term DEX treatment is necessary to affect the large EET pool in liver. Supported by NIEHS SBIR Phase II contract ES05459 (H.K. and J.H.C.), NIEHS grant ES07462 (X.D.) and NIEHS Center grant P30 ES06639.

543 EFFECTS OF BYSTANDER CELLS: IMPLICATIONS FOR LOW-DOSE EXTRAPOLATION OF CHEMICAL AND RADIATION-INDUCED CANCER RISK.

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Estimation of cancer risk at low doses of carcinogens has been dominated by the conceptualization of single cells as the target. Research has shown that some of the effects of ionizing radiation (including mutation) are seen not only in the cell that was hit by radiation, but in the neighboring cells (referred to as bystander effects). The fate of both hit and non-hit cells are diverse, ranging from cell death, cell cycle arrest and reproductive failure, mutation, or the development of an unstable genome. This symposium will focus on how bystander effects and other indirect effects contribute to two phenomena related to cancer, the adaptive response and genomic instability. The first presentation will provide an overview of bystander phenomena that have been observed in radiation biology and provide some insight into how they may be mediated. This will be followed by a demonstration of one mechanism by which selection pressures can give rise to an unstable genotype (via signals generated from mismatch repair). The subsequent talk will discuss the indirect mechanisms implicated in arsenic carcinogenesis and their implications for risk of cancer at low doses. The nature of dose-response curves that are plausibly generated through interactions between direct and indirect mechanisms of damage and adaptation will be discussed in the fourth presentation. The final presentation will discuss a general theory of carcinogenesis that includes genetic and epigenetic contributions to carcinogenesis in the context of the overall structure of the tissue. The symposium will provide a forum in which the necessity of addressing these phenomena in risk assessment and the difficulties in doing so can be discussed. The symposium should be of considerable interest to researchers interested in how basic biological responses observed at low doses should influence estimates of carcinogenic risk.

544 BYSTANDERS, ADAPTIVE RESPONSES AND GENOMIC INSTABILITY - POTENTIAL MODIFIERS OF LOW-DOSE CANCER RESPONSES.

R. J. Preston. *Environmental Carcinogenesis Division, University.S Environmental Protection Agency, Research Triangle Park, NC.* Sponsor: R. bull.

There has been a concerted effort in the field of radiation biology to better understand cellular responses that could have an impact on the estimation of cancer risk (and possibly other health risks) at low dose levels.It is unclear which of these responses are produced from chemical exposures. Bystander effects are a response in cells that are known not to have been traversed by a particle track.Low LET responses have been described.Such responses include induction of gene expression, mutagenic responses and cell transformation.Thus, the concept of effects only in "hit" cells does not hold and cells at risk per unit dose takes on a new definition. The bystander effects are the result of different cellular processes; cell-cell communication and diffusion mediated effects. Adaptive responses for a variety of cellular endpoints have been described for radiation and chemical exposures.The response is lower when a small adaptive dose is given prior to a much larger challenge dose than when the challenge dose alone is given.Thus, there is reduction of response per unit dose under adaptive conditions.However, adaptive responses are not universal and there is considerable interindividual variation. A number of studies have shown that genomic instability can occur at times quite far removed from a radiation exposure.This process also calls into question the concept of response per unit dose.Genomic instability is a hallmark of most tumor types.It remains to be established if it is induced or selected by exposure to radiation or chemicals. Adaptive responses and genomic instability can be induced in bystander cells. This further complicates the considerations of dose and response.There is the potential for all these cellular responses to modify the dose response for cancer at low exposure levels.The need is to further study the underlying mechanisms for these responses for radiation and to establish if similar effects can be operative for chemical exposures.(This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

545 THE SELECTION OF MISMATCH REPAIR DEFECTS: THINKING ABOUT EXPOSURE AND RISK ASSESSMENT.

R. fishel. *Kimmel Cancer Institute, Philadelphia, PA.* Sponsor: R. Bull.

Defects in human homologs of the bacterial DNA Mismatch Repair (MMR) proteins MutS and MutL have been found to be causative of Hereditary Non-Polyposis Colorectal Cancer (HNPCC). However, of the 9 known human MutS homologs (MSH) and MutL homologs (MLH), the majority of HNPCC mutations are confined to 2 genes, hMSH2 and hMLH1; in spite of the fact that hMSH2 and hMLH1 function as heterodimers with other MMR MSH and MLH partners. We have found that the primary human mismatch/lesion MSH recognition complex (hMSH2-hMSH6) functions as an adenosine nucleotide molecular switch. Mismatch/lesions provoke ADP→ATP exchange by MSH proteins that results in a large conformational transition and the formation of a hydrolysis-independent sliding clamp on the DNA. Our results suggest that stochastic loading of multiple ATP-bound MSH "signaling" sliding clamps, leads to "threshold" activation of the repair machinery. These studies support a new model for MRR in which MSH molecular switches signals the timing of downstream events in a manner that is similar to cellular signal transduction by G proteins. Recent studies on the mechanism of MMR signal transduction will be presented The recognition that MSH and MLH proteins constitute signaling molecules suggested the possibility of signaling interfaces and downstream effectors that are outside of the fundamental MMR process. It has always been assumed that elevated mutation rates, associated with an MMR defect, are the root cause of HNPCC (known as the "Mutator Hypothesis"). Identification of a role for the MMR machinery in signaling damage-induced apoptosis introduced the possibility that defects in the MMR process may not the cause of HNPCC. These and other data have led us to propose that there is a selection for MMR-defects that involves cellular survival following overwhelming DNA damage. Our results are consistent with an additional role for MMR in sensing and signaling cellular DNA damage. These studies have broad implications when considering the risk assessment following toxic exposures of populations containing variable levels MMR damage recognition machinery.

546 PROPOSED MECHANISMS FOR ARSENIC CARCINOGENECITY: IMPLICATIONS FOR THE SHAPE OF THE DOSE-RESPONSE CURVE.

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Epidemiological studies have established that inorganic arsenic is a significant human carcinogen that causes tumors predominantly in the skin and bladder following oral exposure and in the lung following inhalation. Despite numerous experimental studies and proposed hypotheses, there is no consensus on arsenic's mechanism of action. It does not behave as most classical chemical carcinogens, including other metals such as cadmium or chromium. In this respect, it does not induce bacterial or mammalian cell mutations at relevant concentrations nor does it produce tumors in standard one- or two-stage animal bioassays. Recent advances have allowed development of atypical rat or mouse models for arsenic carcinogenesis. These, in conjunction with *in vitro* studies, have suggested that arsenic may be inducing carcinogenesis by one or more mechanisms including it's ability to cause global hypomethylation leading to heritable changes in gene expression, act as a 'comutagen' by inhibiting DNA repair, to induce chronic growth signaling through

persistent activation of the MAPKinase pathway and induce oxidative damage through formation of dimethylarsenic radicals. Such mechanisms suggest the likelihood that the dose-response for arsenic may be non-linear in the low dose region and evoke the possibility that such events as genomic instability and by-stander effects may be involved.

547 BIOLOGICAL IMPLICATIONS OF ADAPTIVE RESPONSES AND BYSTANDER EFFECTS FOR INDIVIDUAL AND POPULATION DOSE-RESPONSE CURVES.

R. Conolly. *CIIT Centers for Health Research, Research Triangle Park, NC.*

The view that the dose-response for a carcinogenic stressor must be low dose linear when the stressor adds to an existing carcinogenic process has long held sway (Crump et al., Cancer Research 36:2973, 1977). As our understanding of biological complexity has increased, however, alternative possibilities have arisen. Interactions between the stressor and adaptive responses such as cell cycle checkpoint control and induction of DNA repair may lead to nonmonotonic dose response. In addition, data from radiation biology suggest that interactions between hit and bystander cells can modulate responses to radiation. The potential effects of adaptive responses can be readily illustrated with computational models. For example, a model describing a DNA-damaging agent and having the rate of DNA repair an inducible function of the level of DNA damage can generate a spectrum of dose-response shapes for DNA adduct burden. These shapes can vary from monotonically increasing to threshold to J-shaped depending on the potency with which the additional DNA damage induces repair and the degree to which repair can be induced. Another mechanism that can give rise to nonmonotonic dose-response involves low dose DNA damage that invokes cell cycle checkpoint control and high dose cytotoxicity, leading to a J-shape for the rate of cell division. The common theme in these mechanisms is the ability of the body to react to the environmental stress in a way that modulates the shape of the dose-response curve, responses that theoretical arguments for low dose linearity did not, at the time, have data to describe. Adaptive responses also have important implications at the population level. Cell cycle checkpoint controls and induction of DNA repair capacity are genetically mediated processes. Heterogeneity at the relevant loci could define segments of the population with different capacities for adaptive response and quite different dose-response behaviors in the low dose region. Study of these factors will inform considerations of uncertainty factors applied to intrahuman variability.

548 IMPLICATIONS OF EPIGENETIC EFFECTS FOR MODELING DOSE-RESPONSE.

C. J. Portier. *ETP, NIEHS, Research Triangle Park, NC.*

Dose-response analysis for cancer risks can be improved by the use of a broad array of data and biologically-based mechanistic models. Much of the mechanistic modeling around cancer risks has focused on the classical multistage model of carcinogenesis. The activity in this model is dominated by genetic events and then independent growth of individual cells. However, much of the recent work on carcinogenesis over the past few years has demonstrated that tissue structure and paracrine signalling pathways can play an important role in the development of tumors. There are two models in the literature that have addressed this issue; a model focusing on tissue structure and another on movement of clones of cells rather than individual cells. This talk will outline a general theory for carcinogenesis which includes both epigenetic and genetic events and the overall structure of the tissue. The existing models in the literature will be assessed against this broader based model and the implications for dose-response will be discussed.

549 GENOMICS AND PROTEOMICS IN REPRODUCTIVE AND DEVELOPMENTAL TOXICITY.

K. A. Treinen[1] and E. S. Hunter[2]. *[1]Reproductive Toxicology, Schering Plough Research Institute, Lafayette, NJ and [2]Developmental Biology Branch, NHEERL, USEPA, Research Triangle Park, NC.*

The focus of this symposium is to provide examples where proteomic and/or genomic data have been applied to better understand mechanisms of reproductive and developmental processes/toxicities. Unlike many biochemical studies previously used to characterize the effects of toxicants which often generate apical endpoints, the promise of genomic and proteomic analyses is to understand the underlying mechanisms that cause the specific cellular responses that mediate or are associated with the effects. A major challenge is determining the relationship of genetic/proteomic alterations, particularly the temporal relationship (primary or secondary effect) and generalized nature and quantity of the genes affected, to the end toxicity. Thus, the role and function of the gene products in normal as well as abnormal physiology must be addressed. The use of genomics and proteomics to determine general alterations in genes/proteins have been conducted for a few years now. The intent of this symposium is to provide a forum where the genes/proteins altered by toxicant exposure are consistent to what is known with respect to the altered biology, and likely play a role in the mechanism of toxicity.

550 CYBERTERATOLOGY: INVESTIGATING THE PHYSIOLOGICAL STATE OF THE EMBRYO *IN SILICO.*

T. B. Knudsen. *Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA.*

Functional genomics and computational biology provide new ways to unravel the complex responses of developing tissues to drugs and chemicals that increase or lower the risk for specific malformations. A major challenge in the application of microarray data to experimental teratology is relating the genome-wide changes in gene expression with cellular changes leading to pathogenesis. To address this issue, we generated a microarray dataset with RNA samples collected from tissues of early mouse embryos following exposure to different teratogens, doses, times, and intervention strategies. Computer analysis of the dataset revealed a higher order structure for genes functioning in the same biological process, sharing spatial-temporal expression domains, or mapping to chromosomal regions of conserved synteny. Many physiological changes predicted by higher order data structure could be confirmed utilizing independent experimental methodology. Some alterations were anticipated (receptor tyrosine kinase signaling pathway) and others not (coenzyme A and biotin-dependent carboxyl group transfers carried out by the mitochondrion). Therefore, a comprehensive gene expression matrix that captures the biological complexity of the embryonic transcriptome and its regulation provides a computational resource for deducing the physiological state of the embryo during development and disease. (Supported by grants AA13205 and ES09120 from the NIH and grant R 827445 from the EPA but does not reflect agency policy).

551 ZEN AND THE ART OF TERATOGENICITY SCREEN DEVELOPMENT: APPLICATIONS OF MECHANISTIC PROBLEM SOLVING TO GENERATION OF TAILORED TERATOGENICITY SCREENS.

K. Augustine. *Reproductive Toxicology, GlaxoSmithKline, King of Prussia, PA.*

Using problem solving approaches with a combination of *in vivo* and *in vitro* developmental systems, a platform of basic knowledge can be generated which facilitates teratogenicity screen development. Such screens have been utilized to achieve two goals: define the teratogenic mechanism and identify compounds with reduced teratogenic liability. The problem-solving tactics are multi-tiered, where at each tier, increasing knowledge is gained regarding the basis of compound-induced teratogenicity. These steps utilize data from the original *in vivo* embryo-fetal development study and then use a combination of *in vivo* critical drug-sensitivity window identification, applicable *in vitro* systems and finally genetic screens. This knowledge building approach enables a better understanding of the biological insults which result from embryo-fetal exposure to a teratogen as well as optimize selection of the most relevant developmental model systems and genetic pathways to pursue in generating sensitive and predictive gene expression screens. A case study of a compound identified to be a potent skeletal teratogen will be presented to illustrate how the multi-tiered approach has been applied in studying potential teratogenic mechanism(s) and development of screen design for identification of non-teratogenic back-up compounds for future drug development.

552 DISRUPTION OF PROSTATE GROWTH AND DEVELOPMENT BY DIOXIN.

R. E. Peterson and T. M. Lin. *School of Pharmacy, University of Wisconsin, Madison, WI.*

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (dioxin) inhibits prostate growth and development in the mouse. The earliest effect is inhibition of the initial step in prostate development: emergence of prostatic buds of basal epithelial cells from the fetal urogenital sinus (UGS). Using wild type and aryl hydrocarbon receptor (AHR) knockout (AHRKO) fetuses exposed to dioxin on GD 13 (5 ug/kg dam) we found that ventral prostatic bud formation was totally blocked and dorsal lateral bud numbers were reduced in wild type but not AHRKO fetuses on GD 18 demonstrating that the effect of dioxin was AHR-dependent. To investigate the gene expression profile induced by dioxin in the urogenital mesenchyme (UGM) and urogenital epithelium (UGE) of the ventral and dorsal regions of the UGS; wild type

and AHRKO fetuses exposed to either vehicle or dioxin on GD 13 (5 ug/kg dam) were sacrificed on GD 16 when prostatic bud formation is just beginning. Total RNA preparations were pooled according to tissue (dorsal UGM, dorsal UGE, ventral UGM, or ventral UGE) and treatment (vehicle or dioxin). Using an Affymetrix microarray system dioxin inducible, AHR-dependent mRNA expression was discovered to be both UGS region-specific (dorsal versus ventral) and UGS tissue-specific (mesenchyme versus epithelium). The numbers of genes whose expression was altered by dioxin in an AHR-dependent fashion in the UGM were: TCDD inducible, TGF-beta related, homeobox related and IGF related whereas those altered in the UGE were: cytoskeleton related, calcium related, extracellular matrix related, TCDD inducible, fatty acid metabolism, homeobox related, cell fate determination, retinoid related, inositol phosphate related, myc related, ras related and wnt related. The most abundant mRNAs affected by dioxin in the UGE belonged to the cytoskeleton and extracellular matrix families which is consistent with the observed disruption of prostatic bud formation by dioxin

553 SAGA OF A NOVEL SPERM BIOMARKER : DISCOVERY TO PROOF OF CONCEPT.

G. R. Klinefelter. *Reproductive Toxicology Division, NHEERL, USEPA, Research Triangle Park, NC.*

There has been concern that semen quality is declining in men. It has been suggested that such decline is linked to exposures to environmental chemicals. Since sperm acquire fertilizing ability during epididymal transit, chemicals that perturb the function of the epididymis could compromise fertilizing ability, and this could be associated with altered expression of functionally important sperm proteins. Indeed, the epididymal toxicants ethane dimethanesulphonate, epichlorohydrin, and hydroxyflutamide each decreased fertility, and each of these toxicants caused diminutions in sperm membrane proteins. Out of 120 identified proteins, one specific protein (SP22) was found to be highly correlated with the fertility of epididymal sperm. HPLC purification and sequencing revealed that SP22 was unique to reproductive biology. Two SP22 transcripts are expressed in the testis. The probe specific for the longer 1.5 kB transcript detects SP22 mRNA in pachytene spermatocytes and spermatids, while probe specific for the 1.0 kB transcript detects this mRNA in all cell types. SP22 antibodies have now been shown to: 1) localize SP22 on the equatorial segment of the sperm head in all species examined; 2) inhibit fertilization of epididymal sperm from the rat both *in vivo* and *in vitro*; 3) inhibit fertilization of epididymal sperm from the hamster *in vitro*; 4) inhibit the binding of human sperm to the zona pellucida; and 5) identify samples from idiopathic infertile men. Upon discovering that SP22 originates in the testis, we hypothesized that testicular toxicants, specifically those that target the maturing post-meiotic germ cells, also would result in reductions in fertility associated with decreased levels in SP22. Indeed, when adult rats were exposed to the testicular toxicant bromochloroacetic acid, a disinfection by-product of drinking water, diminutions in SP22 levels were again highly correlated with the observed decreases in fertility. SP22 has now been incorporated into an epidemiology study to determine whether SP22 levels on human sperm vary with varying levels of disinfection by-products.

554 CONDUCTING PARALLEL GENOMICS AND PROTEOMICS STUDIES: COMPARATIVE RESPONSES IN GENE EXPRESSION.

B. A. Merrick and J. E. Hartis. *National Center for Toxicogenomics, NIEHS, Research Triangle Park, NC.*

A major challenge in toxicogenomics will be the development of strategies for conducting genomic and proteomic studies to give a comprehensive or 'global' view of gene expression response. Understanding how changes in protein abundance and posttranslational events over time and experimental treatment are related to changes in transcript levels will provide a more comprehensive picture of gene expression than either transcriptomic or proteomic analysis alone, and may be a better approach for identifying specific genes or important biochemical pathways involved in toxicity. Specific research objectives, desired data density, gene discovery, biological sample type and animal species will influence the selection of specific proteomic technologies or 'platforms' for the global measurement of cellular transcripts and gene products. The spatial and interactive character of proteins and their intracellular-extracellular localization are also prominent factors in transcript and protein comparisons. The challenge for bioinformatics will be to integrate transcript and proteomics datasets into a common framework to interrelate the results of many varied experiments by different investigators. Examples of parallel genomic and proteomic datasets will be presented and compared for complementarity, uniqueness and integration for gene expression.

555 RED TIDES: A RECURRING PUBLIC HEALTH PROBLEM.

D. G. Baden[2] and J. Benson[1]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*HABLAB, University of North Carolina, Wilmington, Wilmington, NC.*

Red tide is a discoloring of coastal ocean waters caused by dense populations of dinoflagellates. Over 20 dinoflagellate species produce toxins that cause death or disease in marine animals. Red tide events occur world wide and are increasing in number and duration. The red tide occurring off the Gulf and Atlantic Coasts of Florida produces are the most extensive, long lived, and recurrent of all red tides. The dinoflagellate responsible for this red tide, *Karenia brevis*, produces potent neurotoxins called brevetoxins (PbTxs). In addition to causing Neurotoxic Shellfish Poisoning following ingestion, PbTxs aerosolized by wind and surf cause coughing, sneezing, watery eyes, rhinorrhea, and shortness of breath. There are anecdotal reports that inhalation exposure to PbTxs can trigger or exacerbate bronchoconstriction in asthmatics. Little is known about the long term health effects associated with inhalation of aerosolized PbTxs during red tide events. Examination of marine mammals dying as a result of a *K. breve* event suggest that the respiratory tract, nervous, immune, and hematopoietic systems are potential targets for toxicity upon repeated exposure, but dose-response relationships have not been established. An interdisciplinary group of scientists is working to evaluate health effects in occupationally and recreationally exposed individuals along *K. breve* affected beaches. In addition, laboratory studies are being conducted to determine inhalation toxicity in animal models, determine mechanisms of toxiicty, and investigate potential antidotes to PbTx-induced health effects. This symposium will summarize the problem, approach to human exposure assessment and evaluation of human health effects as well as results of laboratory studies.

556 OVERVIEW OF HARMFUL ALGAL BLOOM TOXINS: POSSIBLE REASONS FOR PRODUCTION, AND RESULTS OF EXPOSURE.

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The effects of Harmful Algal Bloom (HAB) toxins have been recorded over the past four centuries, and result from the interaction of specific chemical poisons with endogenous receptors in living affected systems. Ion channel blockers and activators, enzyme inhibitors, and central nervous system agonists characterize the currently known HAB toxin molecular mechanisms. Are the toxins produced to kill? Are there vital HAB organism physiological processes in which the HAB toxins participate and/or regulate? Do the HAB organisms actually produce the toxins, or are the toxins produced by a parasitic or commensal contaminant? Alternatively, with the myriad of receptors in living systems, and the countless numbers of low molecular weight ligands produced by micro-organisms, is it possible that such deleterious ligand-receptor interactions occur by pure serendipity? The recent findings that the HAB dinoflagellate Karenia brevis produces toxins which interact by at least two, and perhaps three widely dissimilar molecular mechanisms gives credence to the latter alternative of pure serendipity, a chance interaction between ligand and receptor which results in enzyme inhibition, sodium channel activation, and bronchoconstriction. The finding of new antagonistic natural products in the same organism further supports the idea of collaborating structural homologies. P01 ES10594.

557 CHARACTERIZATION OF AIRBORNE BREVETOXINS.

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Red tides, produced by the dinoflagellate Karenia brevis, occur almost annually in the Gulf of Mexico. K. breve produces brevetoxins that are potent neurotoxins. Exposure to brevetoxins (PbTxs) aerosolized by wind and surf results in respiratory symptoms. Anecdotal reports also suggest that inhalation exposure may exacerbate existing respiratory disease. The objectives of the ongoing study are to characterize the physicochemical properties of environmental brevetoxin -containing aerosols, and to assess occupational, and recreational exposure of individuals during red tide events. Results from monitoring aerosolized brevetoxins during two red tide episodes in the Gulf of Mexico are presented: one in the fall of 2000, at Corpus Christi, Texas, and the other in the fall of 2001 at Sarasota, FL. Two Hi-Vol samplers were deployed during the TX bloom, one equipped with a glass-fiber filter and the other with a five-stage impactor used to aerodynamically classify the particles into different size fractions. Low airborne concentrations of PbTx, between 1.6 and 6.7 ng m-3 and the mass median aerodynamic diameter (MMAD) was between 7 and 9 um. A few incidents of upper respiratory symptoms (throat and nasal irritation), but no reports of lower respiratory symptoms were associated with this event.

During the Sarasota study, several Hi-Vol air samplers were deployed along two different beaches. Measured brevetoxin aerosol concentrations were 4 to 480 ng/m3. During this study, personal samplers also were deployed on lifeguards and field workers. There was a great deal of variability of personal exposures depending on the level of activity, location, and time spent on the beach. Our data showed that in general the lifeguards received higher exposures than the field workers. The particle size distribution obtained at Sarasota was similar to that measured in Texas. These data suggest that low environmental concentrations exposure to PbTx could result in upper respiratory symptoms. This is consistent with the particle size measurement indicating the possibility for particle deposition within the lungs.

558 PATHOPHYSIOLOGIC AIRWAY RESPONSES TO INHALED RED TIDE BREVETOXIN IN ALLERGIC SHEEP.

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There is evidence to suggest that inhaled K. brevis (crude PbTx) causes shortness of breath and increased mucus production in humans, especially in those with underlying airway disease. To better understand these toxin-induced airway effects, we used sheep with airway hypersensitivity to Ascaris suum antigen (asthmatic sheep), as a surrogate for asthmatic patients, and studied changes in pulmonary airflow resistance (RL) and changes in tracheal mucus velocity (TMV), a marker of lung mucociliary clearance, after inhalation challenge with increasing concentrations of crude PbTx (0.1-1pg/ml), and with purified PbTx-2 and PbTx-3 (0.1-10 pg/ml). These two toxins were selected because they are present in highest concentration during the growth phase of K. brevis. Challenge with PbTx-2 and PbTx-3 produced 226± (s.e.m.) 21 % and 204±26% increases in RL over baseline, respectively at 10 pg/ml, whereas 1.0 pg/ml of crude PbTx induced a 201±9% increase in RL. Pretreating the animals with a histamine H1-antagonist, significantly blocked the constrictor effects of the crude and the purified toxins, suggesting a role for histamine containing (mast)cells in this response. The constrictor effects could also be blocked with B-Naphthoyl-PbTx-3, a synthetic brevetoxin derivative, or with AJB 6.0P, a natural antagonist, produced by the organism itself. Although PbTx-2 and PbTx-3 induced similar constrictor responses, only PbTx-3 significantly reduced TMV (59±12% of baseline); this reduction in TMV was blocked by both the B-Naphthoyl-PbTx-3 and the AJB 6.0P antagonists. We conclude that aerosols of K. brevis induce asthmatic-like airway responses in this sheep model, and so could adversely affect human health. The identification of agents that inhibit the effects of these toxins may lead to therapies for affected individuals.

559 BREVETOXIN-INDUCED ALTERATIONS IN NEURONAL Ca2+ DYNAMICS AND CELL SIGNALLING.

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Brevetoxins are potent allosteric enhancers of voltage-gated sodium channel function. These toxins are distributed to the central nervous system following inhalation exposure. They produce acute neuronal injury and death in cerebellar granule cells in primary culture following acute exposure. This neurotoxic response is abrogated by coapplication of either tetrodotoxin, an antagonist of voltage-gated sodium channels, or antagonists of the NMDA receptor. Brevetoxins also produce a rapid and sustained elevation in cytoplasmic [Ca2+] in cerebellar granule cells with a potency consonant with that for neurotoxicity (PbTx-1 EC50=6.64nM). The influence of brevetoxins on neuronal Ca2+ dynamics generalizes to cerebrocortical neurons inasmuch as PbTx-2 produces a concentration dependent augmentation of the spontaneous Ca2+ oscillation amplitude in primary cultures of these neurons. At concentrations greater than 300nM PbTx-2 produced a sustained rise in cytoplasmic [Ca2+]. Brevetoxin produces this modulatory effect by influencing glutamatergic signaling, inasmuch as antagonists of NMDA, AMPA/kainate and mGluR receptors inhibit the sustained rise in [Ca2+]i induced by PbTx-2. Activation of glutamate receptors is associated with an increase in [Ca2+]i which triggers activation of the MAP kinase family members ERK1/2. We therefore evaluated the effect of brevetoxin exposure on ERK1/2 activation. PbTx-2 induced a concentration-dependent increase in ERK1/2 activation. PbTx-2 exposure also produced a concentration-dependent rise in phosphorylated Akt. Thus sodium channel neurotoxins, by modulating the Ca2+ oscillations through glutamatergic receptor signaling mechanisms, can enhance the phosphorylation of ERK1/2 and Akt and therefore influence gene expression. The ability of brevetoxins to affect calcium dynamics and MAP kinase cascade singaling may influence neuronal mechanisms involved in development and learning.

560 AN EPIDEMIOLOGIC APPROACH TO THE STUDY OF AEROSOLIZED BREVETOXINS DURING RED TIDE EVENTS.

L. E. Fleming[2] and L. C. Backer[1]. *[1]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA and [2]Epidemiology and Public Health, University of Miami School of Medicine, Miami, FL.* Sponsor: J. Benson.

Red tides are caused by blooms of the marine dinoflagellates Karenia brevis. The organism produces potent neurotoxins (brevetoxins) that result in massive fish kills. Eating shellfish contaminated with brevetoxins causes neurotoxic shellfish poisoning [NSP]. Anecdotal reports of respiratory irritation and investigations of recent Florida manatee deaths suggest that environmental exposure to aerosolized brevetoxins also has adverse health impacts. Research using sheep and other laboratory animals has indeed confirmed that aerosolized brevetoxins can cause reversible bronchospasm. We have a unique approach to assess the human health effects from environmental exposure to brevetoxins. We have created an interdisciplinary team of scientists and a network of public and environmental health workers in Florida. In response to information that a red tide is onshore and inducing human symptoms, our team assembles to collect environmental samples (air, sea water, sea foam) and epidemiologic data (symptom questionnaires, pulmonary function tests, and personal breathing zone monitoring). We are studying the acute effects of aerosolized brevetoxins on lifeguards and the chronic effects on sensitive subpopulations (elderly people with underlying respiratory disease and children with asthma). Members of the team are evaluating the respiratory effects of brevetoxins in rat and sheep models, refining toxin measurements, developing and validating biomarkers for brevetoxin exposure and biological effects, and exploring the pathophysiology of respiratory exposure to brevetoxins. This multidisciplinary approach will serve as a model for investigating the human health effects from exposure to other natural toxins.

561 STRESS ACTIVATED SIGNAL TRANSDUCTION PATHWAYS.

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An intriguing finding over the past few years is activation of the signal transduction pathways that are known traditionally as proliferative responses by a variety of chemical toxicants. Examples of these chemicals include, but are not limited to, oxidants, quinones, arsenic, heavy metals and aromatic hydrocarbons. Although the number of toxicants found to activate signal transduction pathways is increasing and the number of signaling molecules being activated by toxicants is expanding, the functional significance of these signaling events has been a puzzle. Progress has been made recently using state-of-the-art techniques in understanding the biological consequence of three families of stress activated kinases: MAP kinases, phosphoinositide 3-kinase (PI3K), and NF-kB inhibitory subunit kinases (IKKs). The application of gene array, transgenic and pharmacological approaches has resulted in elucidation of the cascade of signaling events and dissection of transcription factors responsible for cell survival, apoptosis, or inflammatory response. This symposium will present novel and exciting findings in the field of signal transduction that are most relevant to the toxicological science. With an increased interest in using genomic approaches and kinase activities in profiling toxicants, this symposium will provide a timely update and important insights.

562 ROLE OF MAP KINASES IN AH RECEPTOR ACTIVATION.

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The aromatic hydrocarbon (Ah) receptor (AHR) is the only known cellular receptor of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and of many other widespread environmental contaminants that cause diverse toxic effects in animals and humans, including immune, reproductive and developmental toxicity, cancer, wasting syndrome and death. The biological effects of TCDD are mediated by activation of the AHR, which functions as a ligand-activated transcription factor whose homozygous ablation protects mice from dioxin toxicity. Dioxin also promotes signaling pathways, believed to be critical for the Ah receptor to be fully functional. The molecular connections between dioxin-activated signaling pathways and AHR function have yet to be established. Many environmental agents cause the activation of Mitogen-Activated Protein Kinases (MAPKs), leading to the modulation of transcription factor function and the alteration of gene expression. In this study, we show that dioxin and other AHR ligands induce the immediate activation of the extracellular signal-regulated kinases (ERKs) and the Jun N-terminal kinases (JNKs), but not the p38 MAPKs. MAPK activation by dioxin does not require the AHR, since it takes place equally well in AHR-negative CV-1 cells and in Ahr (-/-) mouse

embryonic fibroblasts as in AHR-positive cells. Dioxin-stimulated MAPKs are critical for the induction of AHR-dependent gene transcription, which is suppressed by the inhibition of ERK and JNK activities. Correspondingly, AHR transcription activity is largely potentiated by constitutively active MEK kinase 1 and Raf-1, known upstream regulators of the JNK and the ERK pathways, respectively. The effect of MAPKs on AHR activity is likely achieved by directly catalyzing receptor phosphorylation, which occurs as a consequence of dioxin treatment and induction of the MAPK activities. These data indicate that AHR ligands elicit AHR-independent non-genomic events that are essential for Ah receptor activation and that the MAP kinases regulate the activity and function of the Ah receptor as a transcription factor.

563 FUNCTIONS OF APOPTOSIS SIGNAL-REGULATING KINASE-1 REVEALED BY RNA INTERFERENCE.

J. Kyriakis and D. N. Chadee. *Diabetes Research Laboratory, Massachusetts General Hospital, Charlestown, MA.* Sponsor: Q. Chen.

Apoptosis signal-regulating kinase-1 (ASK1) and mixed lineage kinase-3 (MLK3) are two of a large group of mammalian protein kinases-kinase-kinases (MAP3Ks) that couple to mitogen-activated protein kinases (MAPKs) of the Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) family. ASK1 also activates MAPKs of the p38 group. Genetic and biochemical studies indicate that ASK1 is necessary for prolonged, redox-dependent activation of JNK/SAPK and p38 by proinflammatory stimuli. Agonists that recruit MLK3 are unknown, although pharmacologic studies suggest that it is an effector for Rho family GTPases and protein kinases of the Ste20 family. We have exploited the novel method of small interfering RNA (siRNA)-mediated RNA interference (RNAi) to suppress expression of different MAP3Ks and, by loss of function analysis, determine the biochemical and biological functions of these enzymes. Our findings indicate a complex signaling web by which extracellular stimuli link through MAP3Ks to different biological effects.

564 PI3K AND NRF2-DEPENDENT ARE-DRIVEN GENE EXPRESSION IN NEURONS AND ASTROCYTES: DEFINING PROGRAMMED CELL LIFE.

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The antioxidant responsive element (ARE) is found in the promoter regions of phase II detoxification enzymes such as glutathione S-transferases and NAD(P)H:quinone oxidoreductase. The expression of ARE-driven genes is mediated by the transcription factor Nrf2. Oxidative stress and small electrophilic molecules disrupt the interaction of Nrf2 with its cytoplasmic chaperone Keap1 leading to nuclear translocation of Nrf2. Chemicals that induce the nuclear translocation of Nrf2 and mice null for Nrf2 were used to test the hypothesis that increasing Nrf2-dependent genes confers resistance to apoptosis. A potent ARE activator, tert-butylhydroquinone (tBHQ) increased ARE-driven genes in primary neuronal and astrocytic cultures. Pretreatment of both culture types with tBHQ resulted in increased resistance to oxidative stress-induced apoptosis (hydrogen peroxide and glutamate treatment). Phosphatidylinostol 3-kinase (PI3K) is an upstream regulator of tBHQ-mediated Nrf2 nuclear translocation and ARE activation. The protective effect manifest by tBHQ treatment was partially reversed by inhibition of PI3K. In addition, primary cultures from Nrf2 -/- mice were more sensitive to oxidative stress-induced apoptosis (hydrogen peroxide, glutamate, rotenone and 1-methyl-4-phenyl-pyridinium treatment). To identify the Nrf2-dependent ARE-driven genes involved in this protective mechanism, primary cultures treated with tBHQ, derived from Nrf2 +/+ and Nrf2-/- mice, and infected with adenoviral Nrf2 vector were used for oligonucleotide microarray analysis. A comprehensive list of Nrf2-dependent ARE-driven genes was compiled leading to the identification multiple genes involved in preventing oxidative stress. This genomic fingerprint lends further support to the idea that genes regulated by Nrf2 through the ARE define programmed cell life. *Supported by NIEHS and the BWF New Investigator Award*

565 IKK - A MASTER REGULATOR OF INNATE AND ADAPTIVE IMMUNE RESPONSES.

M. Karin. *Department of Pharmacology, University of California at San Diego, La Jolla, CA.* Sponsor: Q. Chen.

The IκB kinase (IKK) complex is composed of 3 subunits: IKKα, IKKβ, and IKKγ. The catalytical subunits IKKα and IKKβ display a high degree of biochemical and structural similarity, both functioning as IκB kinases *in vitro*. The physiological function of each of the duplicated IKK subunits was probed by gene disruption and knockin experiments, which demonstrated a critical function for IKKβ in activation of NF-κB in response to a large number of proinflammatory stimuli, including TNFα, IL-1, dsRNA, LPS and ISS-DNA. IKKβ is also essential for prevention of TNFα induced apoptosis, indispensable for activation of innate immune responses, required for suppressing the apoptosis of TLR4-activated mouse macrophages, and an essential mediator of acute inflammatory response and tissue protection following exposure to certain physical stresses. All of these functions are mediated through the canonical NF-κB activation pathway involving IκB phosphorylation and degradation. The biological functions of IKKα were rather complex and perplexing. Although IKKα was not required for activation of the canonical NF-κB pathway in response to proinflammatory stimuli, it is essential for skin and bone morphogenesis. The role of IKKα in epidermal differentiation does not depend on its protein kinase activity nor on NF-κB. Recently, the IKKα kinase activity was found to be required for activation of a second NF-κB pathway based on the processing of NF-κB2/p100 to p52, a function seems to be triggered only by select members of the TNF family, is required for adaptive immune responses and proper organization or development of lymphoid organs, and appears to be unique for the B lymphocyte compartment. A third function of IKKα depending on its kinase activity is in development of the mammary gland. This function is exerted *via* the canonical NF-κB pathway but is not triggered by standard proinflammatory stimuli. In summary, duplication of the IKK catalytic subunits has enabled the assumption of diverse biological functions that are differentially dependent on IKKα and IKKβ.

566 METAL SPECIATION IN TOXICOLOGY: DETERMINATION AND IMPORTANCE FOR RISK ASSESSMENT - INTRODUCTION.

R. A. Yokel[1,2]. *[1]College of Pharmacy, University of Kentucky Medical Center, Lexington, KY and [2]Graduate Center for Toxicology, University of Kentucky, Lexington, KY.*

Speciation can influence the handling of metals (toxicokinetics) and their toxicity (toxicodynamics) and should be considered in quality risk assessment. The speciation of metals will be defined, as valence state and associated ligand. The metals that have only one biologically relevant valence state, for which speciation is a function of the associated ligand, will be identified. Metals that have more than one biologically relevant valence state, for which speciation is a function of both valence state and the associated ligand, will also be identified. An overview of the five presentations will be provided and the audience reminded that there will be time for discussion at the end of the workshop.

567 THE ROLE OF METAL SPECIATION IN TOXICOLOGY: METAL TOXICOKINETICS.

R. A. Yokel[1,2]. *[1]College of Pharmacy, University of Kentucky Medical Center, Lexington, KY and [2]Graduate Center for Toxicology, University of Kentucky, Lexington, KY.*

Speciation can have a substantial impact on the fate and toxicity of metals. This will be illustrated by some methods that determine or predict the impact of speciation on the handling of metals. The importance of metal species on absorption/uptake, distribution and elimination in plants, invertebrates, fish and mammals, and the impact on toxicodynamics, will be illustrated with examples, including the influence of citrate on Al toxicokinetics. The interaction of metal speciation with diffusion and carrier-mediated processes on absorption/uptake, distribution and elimination will be considered. Methods to determine and predict the impact of metal speciation on oral absorption will be presented, including determination of oral bioavailability and application of the free ion activity model. Examples will include the complexation of some essential metals by phytates and other dietary ligands that reduce oral bioavailability, the complexation of Al by small carboxylic acids secreted by Al-tolerant plants to reduce Al uptake, and ongoing work to assess the impact of Al speciation on its oral absorption. The impact of chemical speciation on metal distribution within the organism will be illustrated with examples of metal-ligand complexes that enter the brain as substrates for carriers. Examples will include transport of the methylmercury-L-cysteine complex by the large neutral amino acid carrier due to its structural similarity to L-methionine and ongoing work with manganese. The role of biotransformation to change species that result in changes in metal absorption, distribution and elimination and resultant toxicity will be addressed. The rates of non-enzymatic biotransformation processes will be considered. The impact of metal speciation on selection of chelation/absorption methods to treat metal accumulation/toxicity will be illustrated with the choice of therapy to treat mercury to conclude this presentation of the potential importance of metal species on the handling of metals.

568 THE ROLE OF METAL SPECIATION IN TOXICOLOGY: METAL TOXICODYNAMICS.

S. M. Lasley. *Biomedical & Therapeutic Sciences, University of Illinois College of Medicine, Peoria, IL.*

An important relationship between chemical speciation and toxicity exists for several metals of toxicological importance. Such a relationship is highly relevant to an understanding of the mechanisms of toxicity and their reversal. This information is also important for sound risk assessment of metal toxicity. Data supporting this relationship are exemplified by toxicity that is a function of valence state (e.g., Hg, As) or the nature of metal binding ligands (e.g, Pb, Al). The metal that best illustrates the diversity of effects caused by different chemical species is Hg. This metal exists in three valence states (Hg^0, Hg^+, Hg^{+2}) and also can form stable organic compounds. Each oxidation state and organic species exhibits characteristic toxicities. Arsenic also is found in three valence states (As^0, As^{+3}, As^{+5}), and organic arsenicals are formed in the body and by aquatic organisms. Pentavalent As is rapidly converted *in vivo* to the trivalent species, the most toxic form. In contrast to Hg, organic As compounds are less toxic than their inorganic counterparts. Divalent Pb is the most biologically significant form and exhibits a complex speciation with anions in electrolyte buffers. Almost all blood Pb is located in erythrocytes with ~70% bound to hemoglobin; only a small fraction is found in plasma with half bound to albumin. Otherwise, speciation of Pb in physiological systems is largely undefined. Organic forms of Pb are more toxic than inorganic Pb compounds. Trivalent Al is the only biologically significant form of the metal, and binds strongly to O_2-donor ligands such as citrate and phosphate. Al chemistry is complicated by a tendency to hydrolyze and form polynuclear species, many of which are sparingly soluble. Most serum Al is bound to proteins, primarily to transferrin. Citrate enhances Al gastrointestinal absorption, so this combination promotes Al toxicity. In conclusion, it is evident that knowledge of valence and ligand speciation has considerable predictive value with respect to toxicity. In many cases the degree and severity of the hazard can be reliably identified.

569 SEPARATION AND DETECTION METHODS TO SPECIATE TOXIC METALS.

J. A. Caruso. *Chemistry, University of Cincinnati, Cincinnati, OH.* Sponsor: R. Yokel.

Determining specific metal species is important. The realization that some metal species are essential while others are toxic raises the question of which metal species are we dealing with? Such analyses require careful sample preparation, sophisticated separation techniques and then ultra-trace metal detection. This talk will discuss metal speciation focusing on extractions for sample preparation, chromatography and capillary electrophoresis for separation and inductively coupled plasma mass spectrometry (ICPMS) and electrospray MS (ESMS) for detection. Unlike total metal analyses, speciation analyses must preserve the original species. Mild extractions from hot water to enzymatic extractions are used, depending on the species to be determined. Chromatographic methods typically include size-exclusion chromatography (SEC) for separating by molecular size, gas chromatography (GC) for volatiles, liquid chromatography (LC) for species in solution, and capillary electrophoresis when sample injection volume is low. SEC may be part of sample preparation wherein the separated species of a molecular size range are further separated by ion-exchange or ion-pairing reversed phase chromatography. ICPMS provides sub-ppb detection levels and can serve as an excellent prescreening technique for ESMS when further species identification is necessary. By combining chromatography with ICPMS or ESMS detection analytical advantage is quickly realized in that we have both temporal and mass selectivity. For example, capillary GC produces very high chromatographic resolution, but that even for peaks that overlap in time, information at different masses may be obtained, enabling determination of most metal species. Selenium is essential and toxic depending on the species and the intake level. Discussion will include specific species determination within a common group such as selenoamino acids from proteins or peptides. Arsenic is generally thought of as toxic. Speciation studies for As are advanced, specifically As speciation from inorganic As, organoarsenicals, arsenosugars and arsenolipids. Appropriate illustration will be given.

570 MODELING METHODS TO DETERMINE Al AND Mn SPECIATION FOR TOXICITY ASSESSMENT.

W. R. Harris. *Chemistry and Biochemistry, University of Missouri-St. Louis, St. Louis, MO.* Sponsor: R. Yokel.

When labile metal ions enter the body, they interact with a large pool of biological ligands to form a variety of metal complexes. Computer programs are available that use known binding constants to calculate the concentration of each metal complex. Such calculations are most commonly used for divalent and trivalent first-row transition metals and a few metallic main group elements such as Pb(II), Al(III) and Ga(III). Kinetic inertness and/or a limited database of binding constants restricts the use of this method for Cr(III), Co(III), and less metallic main group elements such as arsenic. The basic features of this type of calculation will be described, and speciation calculations on Mn(II) and Al(III) will be presented as examples. From a computational view, these calculations are relatively simple and can be completed within a few seconds on a typical PC. The most critical factor in the design of an accurate model is the selection of the metal-ligand stability constants to be used. The literature often contains multiple, conflicting values for key stability constants. In choosing the "best" value, one must consider the experimental conditions, such as temperature and ionic strength, and the reliability of the experimental methods. It may be necessary to estimate stability constants for key complexes that have not been evaluated experimentally. It is critically important to include the ligand protonation constants, so that competition between the metal ion and the hydrogen ion for the ligand is included in the calculation. It is also important to include competitive metal ions such as calcium(II) and magnesium(II) that are present in serum at relatively high concentrations. The speciation models will be discussed with respect to the free ion activity model for metal transport across biological membranes, which emphasizes the transport only of the free metal ion. Speciation models can also be used to identify neutral complexes that may cross membranes by passive diffusion or other complexes that might be suitable substrates for selective membrane transport systems.

571 METAL SPECIATION IN HUMAN HEALTH RISK ASSESSMENT: CHALLENGES POSED BY MANGANESE, IRON, AND OTHER ESSENTIAL NUTRIENTS.

D. C. Dorman. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Risk assessment (RA) considers pharmacokinetic and pharmacodynamic processes and often results in the setting of exposure standards. As has been shown in earlier presentations, the oxidation state of a metal can dramatically influence the pharmacokinetics, pharmacodynamics, and toxicity of a metal. In some cases, different exposure standards are established for different metal species. For example, the TWAs for chromium (Cr) differ depending on whether the metal is in the Cr(II) or Cr (VI) valence state. *In vivo* and *in vitro* toxicity data available for iron (Fe) and manganese (Mn) suggest that the species of the metal can influence toxicity. This observation could imply that exposure standards for these metals should consider speciation. However, this approach would ignore the fact that Fe exists in multiple valence forms in the body, and redox cycling between ferric (Fe3+) and ferrous (Fe2+) states is basic for many biological processes. Enzyme systems (e.g., methemoglobin reductase) exist to maintain Fe in the valence state needed for its normal functions. Like Fe, Mn can exist in a number of oxidation states, and also undergoes changes in its valence states within the body. The valence of Mn in most enzymes is Mn3+, while most Mn taken into the body exists as either Mn2+ or Mn4+. Mn2+ is the predominant form in biological systems. Biologically based dose response (BBDR) models can incorporate information about the pharmacokinetics and pharmacodynamics of different species of metals. BBDR models also can address issues related to metal speciation. This presentation will use Fe and Mn to illustrate how BBDR models that consider the essentiality of these metals as well as their toxicity could be developed and used to improve the RA process.

572 APPLICATION OF LASER CAPTURE MICRODISSECTION FOR THE CHARACTERIZATION OF GENOMIC MARKERS FOR KETOCONAZOLE TOXICITY IN DOG LIVER.

J. McNulty, F. M. Goodsaid, C. Pisani, L. Obert, G. Mandakas, R. Smith, H. Zairov and I. Y. Rosenblum. *Genetic and Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

A search for genomic toxicity markers focuses on an identification of changes in gene expression levels correlating with the development of histological endpoints. We have studied quantitative gene expression changes in livers of dogs dosed with Ketoconazole, a marketed antifungal drug. Identification of candidate predictive biomarkers may be defined as an early gene expression change intensifying with detection of histopathology. Quantitative gene expression normalized to total RNA with 18S endogenous control was used to identify candidate genes consistent with this definition, regardless of mechanism of toxicity. HMGCoA reductase, Annexin V, and Gap Junction Protein A were identified in whole tissue as biomarkers for ketoconazole-induced liver necrosis. In addition to analysis of whole tissue, we applied Laser Capture Microdissection (LCM) to map the distribution of gene expression for HMGCoA reductase within liver cells in normal tissues, necrotic lesions, or cells adjacent to lesions. Portal triad and vascular structures were not included in this analysis. HMGCoA reductase was selected for gene expression analysis in LCM samples because its basal expression levels were high enough (CT < 25) for an accurate measurement of gene expression in LCM samples. Mean expression

levels for HMGCoA reductase were highest in cells adjacent to lesions and lowest in lesions. A narrow range (less than 2-fold) in gene expression level was measured for cells in control and normal tissues, while a much wider range of expression values was measured for cells in lesions or adjacent to lesions. In addition, temporal changes in gene expression for histologically normal cells sampled by LCM closely matched those sampled in whole tissue. These data demonstrate the usefulness of LCM in isolating specific cells of interest within target organs. LCM sampling, in conjunction with qRT-PCR can identify gene expression changes that are temporally and spatially associated with lesion formation.

573 ALTERATIONS IN THE EXPRESSION OF TRANSLATION FACTORS AS MOLECULAR MARKERS OF CARCINOGENESIS AND CHEMICAL TOXICITY.

P. Joseph, Y. Lei, C. O'Kernick and T. Ong. *NIOSH, Morgantown, WV.*

Translation factors control the translation of all proteins including those vital for cell growth and differentiation as well as those involved in cellular response to chemical toxicity. Previously, we have identified, cloned, and characterized translation initiation factor 3 (TIF3) and translation elongation factor-1δ (TEF-1δ) as two novel cadmium-responsive proto-oncogenes. Cadmium-induced cell transformation and tumorigenesis were found, at least in part, to be mediated through overexpression of TIF3 and TEF-1δ. Further studies have been performed in our laboratory to determine whether alterations in the expression of various translation (initiation, elongation, and termination) factors can be used as molecular markers for carcinogenesis and chemical toxicity. By employing the real time PCR technique, we have investigated the expression levels of various translation factors in human cancer cell lines and their corresponding controls. Similarly, expression levels of the translation factors were investigated in human prostate epithelial cells, RWPE1, exposed to a cytotoxic concentration of cadmium chloride. Among the translation factors studied, the expression level of translation elongation factor 1A2 (TEF1A2) exhibited the highest alteration (approximately 2000-fold overexpression) in the cancer cell lines compared with the corresponding controls. Translation initiation factor 5A2 (TIF5A2) and translation elongation factor 1δ (TEF1 δ) also exhibited significant overexpression (10- and 5-fold overexpression, respectively) in the tumor cells compared with the corresponding control cells. Exposure of RWPE1 cells to a cytotoxic concentration of cadmium chloride also resulted in overexpression of several translation factors. These results thus demonstrate that translation factors may be cellular targets for carcinogenesis and chemical toxicity, and that alterations in their expression levels may be used as molecular marker for carcinogenesis and chemical toxicity.

574 MURINE EMBRYONIC STEM CELLS AS A MODEL TO IDENTIFY BIOMARKER PROFILES OF PPAR ACTIVATORS, THIAZOLIDINEDIONES AND FIBRATES.

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Murine embryonic stem (ES) cell differentiation represents a dynamic biological system for investigating compound induced effects on a wide range of cell types and tissue-tissue interactions *in vitro*. Our goal in this study is to identify biomarker profiles that are characteristic of PPAR activators and to characterise the pathways contributing to organ injury. The PPAR activators included PPARγ-agonists (troglitazone, pioglitazone and rosiglitazone), PPAR αagonists (WY14643, fenofibrate, bezafibrate), and the mixed PPARα-γ agonists (KPR297 and AZ242). Initially concentration profiles eliciting a 50% decrease in cell number at the end of a 6-day differentiation embryoid body (EB) assay using the murine embryonic stem cell line CCE-Lena (derived from 129Sv mouse strain) were determined. The effect of each compound on ES differentiation and EB development on cultures exposed for either the 6-day duration or for the last 3-days following an initial 3-day EB development period is being investigated. At the concentrations tested the PPARγ agonists inhibited cell growth by 22-42% during 3-days of exposure and 56-68% over 6-days. The PPARα agonists WY14643 and bezafibrate decreased cell proliferation up to 37% at 6-days, while fenofibrate elicited a similar response as the thiazolinediones, inhibiting cell proliferation 33% and 65% with 3-days and 6-days of exposure. The mixed PPARα-γ agonist KPR297 inhibited cell proliferation 55% over 6-days, while AZ242 decreased cell proliferation only 12%. ES differentiation is being evaluated by gene expression profiles of 3-and 6-day compound exposures.

575 REGULATION OF THE HUMAN *CYP3A4* GENE IN LUNG CELLS.

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CYP3A4 and CYP3A5 are closely related P450 enzymes with distinctly different patterns of expression. CYP3A4 is the major human hepatic P450 enzyme, while CYP3A5 is highly expressed in human lung cells. The biochemical mechanisms responsible for the selective expression have not been fully characterized. Expression, as measured by luciferase reporter constructs, of these two genes in human lung A549 cells are dramatically different. A large (~3kb) clone of the *3A5* 5′-region activated luciferase gene expression by 5-fold in A549 cells, while an analogous fragment of the *3A4* gene did not. Comparing sequences of the 5′-flanking regions of *3A4* and *3A5* shows that a 58 bp insertion in the *3A4* promoter region (–70 to –127) corresponds to the luciferase data obtained by deletion analyses. Introduction of this 58 bp region significantly reduced luciferase activity of *3A5* clones. EMSA analyses of this region suggested two E-box motifs were the *cis*-acting elements within this region. These two binding regions may associate with as many as three A549 nuclear proteins. Preliminary EMSA supershift experiments with antibodies to known E-box binding factors (TAL1, δEF1, E2A, and HEB) failed to yield a supershifted band. Thus, these results demonstrate that the 5′-upstream region of *3A4* contains transcriptional motifs that are not present in the 5′-upstream region of the *3A5* gene, which may direct lung-selective expression of these two *CYP3A* genes. (Supported by PHS Grant HL60143).

576 MULTIPLEX PCR AND TOXICOGENOMICS.

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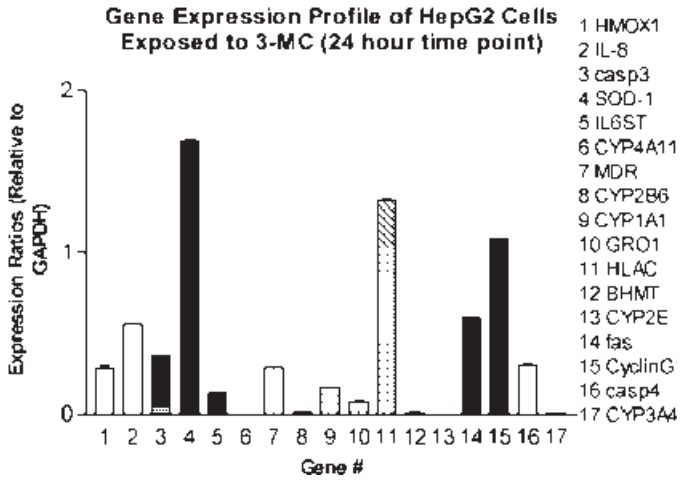
a) Multiplex PCR is a process that is very complementary to the further development of toxicogenomics. Multiple primer pairs were designed for genes whose expression has been determined to be impacted by toxic compounds as well as some pathway specific genes. These primers are then used in a single multiplex PCR reaction to monitor the expression of up to 23 genes at once. The research presented here displays the use of multiplex PCR to determine the effect of compounds on the expression of genes relevant to potential toxicological effects and of other genes of interest for early lead compound screening. b) HepG2 cells were treated with 3-methylcholanthrene (3-MC). RNA was extracted and purified by standard methods. 100ng of total RNA was used as templates in RT reactions to produce cDNA. PCR reactions were carried out on each cDNA. PCR reactions were diluted and loaded onto the Applied Biosystems 3100 Genetic Analyzer. The data points obtained representing each PCR product were then normalized against a control data point and graphed. c) see Figure d) Multiplex PCR is an effective way to use gene expression analysis to prescreen compounds to determine their toxicological impact as well as their impact on pathways of interest.



577 ROLE OF CONSTITUTIVE ANDROSTANE RECEPTOR IN THE INDUCTION OF XENOBIOTIC TRANSPORTERS BY *trans*-STILBENE OXIDE.

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Trans-stilbene oxide (TSO) induces Phase I and II drug metabolizing enzymes such as cytochrome P450 2b1/2 (Cyp2b1/2), epoxide hydrolase, and glutathione S-transferase. Coordinate regulation of phase I and phase II enzymes with xenobiotic transport proteins has been shown after treatment with microsomal enzyme inducers. The purpose of this study was to determine whether TSO also regulates xenobiotic transporter expression, and to determine the role of constitutive androstane receptor (CAR) in TSO induction. Total RNA was isolated from liver excised from male Sprague-Dawley rats treated with TSO for 4 days (200 mg/kg, ip, bid) and the mRNA for each gene was quantified by the branched DNA signal amplification assay. Increased levels of Cyp2b1/2, Cyp3a1/23, UGT1A6, and UGT2B1 mRNA

in liver were detected after TSO treatment. Coordinately, mRNA levels for Multidrug Resistance Proteins 2, 3, and 4 (Mrp2, 3, 4) and Multiple Drug Resistance Proteins 1a and 1b (Mdr1a, Mdr1b) were also increased in liver after TSO treatment. The expression of Mrp1, Mrp5, Mrp6, Mdr2, Oatp1, Oatp2, and Bsep transporters was unchanged by TSO treatment. Next, to determine whether TSO activates gene expression *via* CAR, male and female Wistar-Kyoto rats were treated with TSO for 4 days. Wistar-Kyoto females express lower levels of CAR protein, such that they exhibit significantly lower induction of Cyp2b1 by phenobarbital as compared to Wistar-Kyoto males treated with phenobarbital. TSO induced Cyp2b1/2, UGT2b1, and Mdr1b in males significantly higher than in females, suggesting that TSO activates their gene expression *via* CAR. However, TSO induced Cyp3a, UGT1A6, and Mrp3 equally in both genders, indicating that induction of each gene occurs independently of CAR. In summary, TSO coordinately increases liver xenobiotic transporter mRNAs, along with mRNA for phase I & II enzymes *via* both CAR-dependent and CAR-independent mechanisms. (Supported by NIH grants ES-09716, ES-09649, ES-07079, and ES-11239)

578 NRF2 DEFICIENCY IS LINKED TO LUPUS-LIKE AUTOIMMUNE DISEASE.

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Nuclear factor E2 p45-related factor 2 (Nrf2), a bZIP transcription factor, plays a central role in the regulation (basal and inducible expression) of antioxidant genes and phase II drug metabolizing enzymes through the antioxidant response element found in their promoters. Accumulating evidence indicates that Nrf2 is important for protection against carcinogenesis and oxidative stress through comparative studies on Nrf2 wild-type and knockout mice. Recently, our laboratory found 70% of the aged (over 12 months) female Nrf2-deficient mice displayed weight loss, splenomegaly, and multiple organs failure compared to 0% of the aged male Nrf2 deficient littermates. Pathological analysis demonstrated extensive mononuclear infiltration in liver, kidney, and heart, resulting in lupus-like vasculitis. Immunofluorescent staining of IgG, IgM, and C3b in multiple organs displayed massive mesangial deposits exclusively in the aged Nrf2 knockout female mice. Serum ELISA for autoantibody detection also indicated a high prevalence of anti-double-stranded DNA antibody in these mice. Taken together this suggests the aged Nrf2-deficient female mice develop lupus-like autoimmune disease. Microarray analysis had been applied for genetic dissection of the gene expression profiles linked to Nrf2 deficiency. Both genesets generated from five-month male and female knockout mice showed significant downregulation in certain antioxidant genes and phase II detoxifying enzymes. However, compared to the wild-type counterpart, female knockout mice demonstrated a dramatic increase in gene expression of anti-DNA immunoglobulin light chain IgG, Ig Kappa chain, Sm antigen, Sjogren's syndrome antigen, and other immunoregulatory factors that may be linked to lupus as early as seven months prior to pathological changes. These results suggest that Nrf2 may be involved in autoimmune regulation and may be one of the genes determining genetic susceptibility to lupus-like autoimmune disease.

579 ALTERATION OF PACLITAXEL DISPOSITION IN RATS BY ANTISENSE MORPHOLINO OLIGOMERS TARGETED TO CYTOCHROME P450 3A2.

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Phosphorodiamidate Morpholino Oligomers (PMO) are non-ionic DNA analogs that are highly resistant to various nucleases and proteases, as well as extremely efficient inhibitors of translation *via* a non-RNase H, sequence-specific steric-blockade process. PMOs also have favorable organ distribution following systemic administration at all organ sites rich in cytochrome P450 (CYP) activity, namely the liver, kidneys and small intestines. CYP3A2 causes paclitaxel metabolism by a hydroxylation at the para position of the C3 phenyl group on the C13 side chain. The hypothesis for the current studies states that CYP3A2 inhibition by intraperitoneal antisense PMO pretreatment in rats will decrease metabolism of paclitaxel and reduce its clearance, thereby increasing its bioavailability. Vehicle, control or antisense PMOs targeted to CYP3A2 were injected intraperitoneally into male rats at the dose of 2.5 mg/kg/day for three days. PMO administration was started 48 hours prior to administration of paclitaxel, which was administered as a single bolus intravenous injection at the dose of 8.0 mg/kg. Antisense pretreatment resulted in sequence-specific decrease of (53.9 ± 8.2)% in CYP3A2 activity, as determined by erythromycin N-demethylation. Antisense pretreatment further resulted in a statistically significant alteration in various plasma pharmacokinetic parameters of bolus intravenously administered paclitaxel: increased area under curve and distribution half-life combined with decreased plasma clearance. It is concluded that CYP3A2 inhibition by antisense PMO in rats can alter paclitaxel disposition in a predictable manner.

580 BUCCAL-LUNG COMPARISON OF QUANTITATIVE EXPRESSION OF CARCINOGEN AND OXIDANT METABOLISM GENES IN HUMAN SUBJECTS.

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Buccal cells lining the inner cheek of tobacco smokers are carcinogen and oxidant-exposed, easily accessible, and potentially valuable surrogates for lung. Lung cancer susceptibility phenotyping might include carcinogen- and oxidant-metabolism gene expression. METHOD: For buccal cell analysis, we adapted techniques of RNA extraction, and employed our novel RNA-specific RT-PCR strategy that is unconfounded by gDNA pseudogene sequence. Precise real-time quantitation of the expression of these genes scales target to reference housekeeper (LightCycler, Roche). Genes analysed include Ahr, cytochromes P450 CYP1A1, CYP1B1, glutathione-S-transferases GSTM1, GSTM3, GSTP1, GSTT1, NAD(P)H-quinone oxidoreductase (NQO1), superoxide dismutase (CuZn-SOD), catalase (CAT)and glutathione peroxidase (GPx). For any given individual among our initial group of human subjects, we have compared buccal gene expression to laser capture microdissected lung quantitated gene expression. RESULTS: We have found close concordance of phase I, II and antioxidant genes in constitutive expression (CYP1A1, GSTM1, GSTM3 not expressed; GSTP1, GSTT1, NQO1, SOD, CAT, GPx all expressed) between the two tissues. There is often buccal-lung discordance for Ahr. However, for all 11 transcripts studied in our first 8 individuals to date, 94% of the gene-specific buccal-lung expression comparisons show concordant expression. CONCLUSION: In summary, we have in preliminary fashion shown that 1) gene expression is quantifiable in cytologically-collected buccal cells and 2) there appears to be good concordance between those carcinogen and oxidant metabolism genes expressed in the buccal mucosa and those expressed in the laser microdissected human lung epithelium. We have enlarged the group undergoing buccal-lung comparison, and commenced stratification of the expression data for covariation with tobacco exposure.

581 UV RADIATION ENHANCES *MYCOBACTERIUM ULCERANS* INFECTION IN A Crl:IAF(HA)-hrBR HAIRLESS GUINEA PIG MODEL OF BURULI ULCER DISEASE.

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UV pre-exposure enhances intracellular mycobacterial infections, however its effect upon the pathogenesis of the extracellular *Mycobacterium ulcerans* parasite had not been previously examined. Groups of 5 animals were exposed to cumulative UVB doses of 0 (control), 3 or 30 kJ/m^2 of followed 3 days later by subcutaneous infection with 3 x 10^4 CFU of M. ulcerans in order to induce the nodular form of the disease. The resultant nodules were measured for the next 22 days. The experiment was then repeated using intradermal infection with 2 x 10^6 CFU in order to induce the ulcerative form of the disease. The resultant ulcers were measured for the next 30 days. In both experiments, the animals were tested for DTH reactivity to Burulin-S as a marker of the onset of the reactive phase of the disease. Following low inoculum infection, distinct, well demarcated, subcutaneously-situated skin nodules were present at infected skin sites between 7 and 22 days post-infection. Between days 14 and 21, the mean nodule diameters of the UV irradiated groups were significantly ($p < 0.03$) greater than that of the control group. UV pre-exposure resulted in significant ($p < 0.035$) suppression of DTH responses to Burulin-S challenge. High inoculum intradermal infection resulted in the development of ulcerative lesions. Between 10 and 30 days post-infection, the mean lesion diameters and mean ulcer development times of UV irradiated groups were significantly ($p < 0.05$) greater than those of the controls. However, UV irradiation did not affect DTH responses to Burulin in the high inoculum experiment. These results demonstrate that UV pre-exposure results in enhanced M. ulcerans infection in the hairless guinea pig model of Buruli ulcer disease and suggest that UV exposure may be a relevant factor in the pathogenesis of human forms of the disease.

582 CELL CYCLE DISRUPTION BY HYDROQUINONE AND CATECHOL.

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Cigarette smoke induces profound suppression of T cell responses in the lungs. We have reported that the two major phenolic components of cigarette tar, hydroquinone (HQ) and catechol, block DNA synthesis in proliferating lymphocytes by

reversibly inhibiting ribonucleotide reductase. However, exposure of resting lymphocytes to HQ or catechol at the time of stimulation also inhibits blast transformation and prevents normal progression through G0- and G1-phases of the cell cycle, despite up-regulation of IL-2, CD25 and CD69. Specifically, HQ or catechol exposure impaired induction of RNA synthesis and E2F-dependent genes, and diminished cell surface levels of transferrin receptors (TfR). The purpose of these experiments was to investigate the molecular basis of HQ- and catechol-induced cell cycle arrest. Peripheral blood mononuclear cells (PBMC) were activated with anti-CD3 and PMA in the presence or absence of 50 μM HQ or 50 μM catechol. Total TfR RNA and protein levels were decreased in HQ- and catechol-treated cells. Additionally, binding of iron-regulatory proteins (IRP) to iron-responsive-element (IRE)-containing RNA, which is known to increase TfR message stability, was impaired by HQ but not catechol. HQ or catechol exposure also prevented normal induction of CDK4 protein. Cyclin-dependent kinases (Cdk) inactivate the Rb family of pocket proteins (including p130), which leads to accumulation of E2F transcription factor activity. Consistent with these effects, HQ and catechol exposure prevented dissociation of E2F4 from p130. Lastly, HQ and catechol inhibited c-Myc protein levels by greater than 50%. Targets of c-Myc function in regulating protein translation and cell size, iron metabolism, and cell cycle control. As such, the absence of c-Myc protein alone could explain the pattern of inhibition seen in HQ- and catechol-treated cells. Such studies will provide the further indication of the regulatory pathways by which components of cigarette smoke inhibit T cell function in the lungs of smokers. Supported by NIEHS grants ES05673 and ES11062.

583 PERINATAL EXPOSURE TO ATRAZINE SUPPRESSES JUVENILE IMMUNE FUNCTION IN MALE, BUT NOT FEMALE SPRAGUE-DAWLEY RATS.

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The ability of the chloro-triazine herbicide atrazine (ATR) to induce transitory hypoprolactinemia or hypothyroidism suggests that ATR may have perinatal immunomodulatory potential either directly or through the immunoregulatory properties of prolactin and thyroid hormones. Although there is no evidence of ATR immunotoxicity in juvenile or adult rats, we have previously shown that perinatal exposure to ATR decreased DTH responses in male offspring. Therefore, to further evaluate ATR as a developmental immunotoxicant, pregnant Sprague-Dawley rats were subjected to 4 separate treatment regimes from gestational day 10 through post-natal day (PND) 23: 1) ATR (35mg/kg/day) in methylcellulose *via* gavage, 2) bromocryptine (0.2mg/kg/2x/day) *s.c.* to induce hypoprolactinemia, 3) propylthiouracil (2mg/kg/day) *s.c.* to induce hypothyroidism, or 4) methylcellulose *via* gavage for control dams. Offspring were then tested after PND 50 for: 1) natural killer (NK) cell function, 2) delayed-type hypersensitivity (DTH) response to bovine-serum albumin (BSA), and 3) antibody response to SRBC. Exposure of pregnant dams to ATR decreased IgM production and DTH responses in male offspring; therefore, these ATR-affected measures were examined in 6 month old rats to test for persistence. No immunosuppression was found in 6 month old rats. These results demonstrate that perinatal exposure to ATR produced temporary gender specific changes in adult immune function. Although transitory hypoprolactinemia increased IgM production in female rats, neither hypothyroidism nor hypoprolactinemia altered NK or DTH responses, suggesting that immune changes associated with ATR exposure are not mediated through suppression of these hormones. *(This abstract does not reflect EPA policy and was supported in part by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010 with North Carolina State University).*

584 PROPANIL (DICHLOROPROPIONANILIDE; DCPA) REDUCES NORMAL MACROPHAGE FUNCTION.

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DCPA is an herbicide known to alter macrophage function, including proinflammatory cytokine secretion. To determine if other macrophage functions are affected, we measured the direct effects of DCPA on phagocytosis, respiratory burst and bactericidal activity in primary mouse peritoneal exudate cells (PEC) and a human cell line (THP-1). DCPA caused significant reduction in the ability of THP-1 and PEC cells to phagocytize fluorescent beads ($p<0.05$) . The weighted phagocytic index for THP-1 cells was reduced from 0.730 (+/-0.303) in the ethanol control to 0.267 (+/-0.068) in the 100 microM DCPA group, a 63% decrease in phagocytosis. Significant differences ($p<0.05$) were also evident with both the 50 microM and 100 microM DCPA treatments in the PEC. Another function of macrophages is destruction of phagocytosed organisms *via* generation of free radical oxygen and nitrogen species. We employed a respiratory burst detection method utilizing luminol to amplify chemiluminescence generated by PMA-stimulated THP-1 cells and PEC. Our results demonstrate that 100 microM DCPA virtually shuts down the respiratory burst activity of both cell types, whereas 50 microM DCPA gave an intermediate response. The response of THP-1 cells treated with 25 microM DCPA was similar to ethanol control cells. The respiratory burst of PEC was more sensitive to the effects of DCPA, resulting in a significantly different dose-dependant decrease in relative light units upon exposure to 5, 50 or 100 microM DCPA ($p<0.05$). Finally, we measured the effects of DCPA on the ability of macrophages to kill the intracellular bacterium Listeria monocytogenes as a tangible indicator of a potential biological consequence. DCPA treatment at 100 microM abrogated the listericidal abilities of both THP-1 cells and PEC. These results demonstrate that DCPA causes a decrease in the functional activity of macrophages, and further support the earlier findings regarding the immunotoxic effects of DCPA. Supported by NIEHS grant ES7512 and ES11311

585 THE EFFECT OF DICHLOROPROPIONANILIDE (DCPA) ON C-JUN.

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DCPA, the active ingredient in post-emergent herbicides such as WHAM!EZ™ is used extensively in the United States in the cultivation of rice. Using *in vivo* and *in vitro* exposure models, our laboratory has demonstrated that DCPA inhibits several important immune system functions including both T-independent and T-dependent antibody responses, cytokine production and natural killer (NK) cell function. Exposure of either the human T lymphoma cell line, Jurkat, or human peripheral blood lymphocytes (PBLs) to DCPA results in a dose-dependent decrease in the level of IL-2 secreted by these cells. This decrease in IL-2 production is due in part to a decrease in the DNA binding activity of several transcription factors essential for IL-2 gene transcription. One of these transcription factors, AP-1, is a heterodimer composed of the two proteins c-jun and c-fos. In order to elucidate the mechanism by which DCPA exposure alters AP-1 DNA binding activity, we examined the level of c-jun mRNA, protein and phosphorylation state in DCPA treated Jurkat cells compared to vehicle control. Preliminary data indicate that DCPA exposure decreases the level of total c-jun protein as well as the level of phosphorylated c-jun. In addition c-jun mRNA expression is delayed by DCPA exposure. Previous studies in the laboratory have demonstrated that c-fos transcription is not affected by DCPA exposure. Together these data suggest that DCPA exposure alters AP-1 DNA binding activity by decreasing the level of total c-jun protein and the level of phosphorylation of c-jun compared to vehicle control and may be the mechanism by which IL-2 production is reduced. Supported by NIEHS grants ES7512 and ES11311.

586 PESTICIDE MIXTURES INCREASED IMMUNOTOXICITY IN C57BL/6 MICE, *IN VIVO.*

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There is great uncertainty about how complex mixtures of xenobiotics affect human and animal health. Numerous risk assessment studies have been done to evaluate the severity of the threat that each toxic chemical posed; yet the exposure to chemicals is rarely limited to a single compound. We have studied the combined effects of lindane (L; an organochlorine), malathion (M; an organophosphate), and permethrin (P; a pyrethroid) on murine (C57Bl/6) immune system, *in vivo*. Animals were randomly divided into groups of six and injected intraperitoneally with three different doses (one-half, one-third, one-fourth, or one-eight of LD_{50}) of individual pesticide. The vehicle and positive (cyclophosphamide) control groups were included. Animals were injected with pesticides on day-1 and day-3, challenged with sheep red blood cells (sRBC) on day-4, and sacrificed on day-8. Exposure to individual pesticides did not alter the thymus/ and spleen/body weight ratios; thymic and splenic cell counts, or CD4/CD8 and CD45/CD90 phenotyping of cells. However, anti-sRBC plaque forming cell (PFC) counts were significantly lowered even at the lowest doses of pesticide exposure. Thus, the mean PFC counts/ 10^6 splenocytes were found to be 58 for corn oil; 39, 37, 37 for L 1/3, 1/4, 1/8 of LD_{50}; 36, 38, 46 for M 1/2, 1/3, 1/4 of LD_{50}; and 41, 34, 42 for P 1/2, 1/3, 1/4 of LD_{50}, respectively. Two other groups of animals were injected with the mixtures (L+M or L+P) of one-third of $LD_{50}$of each pesticide. Exposure to pesticide mixtures did not alter CD4/CD8 and CD45/CD90 cell subpopulations. However, the thymus/ and spleen/body weight ratios, thymic and splenic cell counts, and PFC counts were significantly lowered in pesticide mixture exposures. These data indicate that L, M, and P are potent immunotoxicants and mixtures of these pesticides cause significantly higher toxicity compared to individual exposures.* *The views expressed in this paper are those of the authors, and do not necessarily reflect the views and policies of the University.S.EPA.*

587 HCK- AND PKR-DEPENDENT MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHORYLATION AND AP-1, C/EBP AND NF-KAPPAB ACTIVATION PRECEDE DEOXYNIVALENOL-INDUCED TNF-ALPHA AND MIP-2 EXPRESSION.

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The mechanism by which deoxynivalenol (DON, vomitoxin) upregulates TNF-alpha and MIP-2 expression in murine macrophage-like RAW 264.7 cells was investigated. PP1, a Src-family-selective tyrosine kinase (Hck) inhibitor and 2-AP, an inhibitor of dsRNA-dependent protein kinase (PKR) additively inhibited DON-induced TNF-alpha and MIP-2 production as well as phosphorylation of mitogen activates protein kinases (MAPKs). PP1 and 2-AP also inhibited DON-induced nuclear translocation and binding activity of AP-1, C/EBP and NF-kappaB. Furthermore, pretreatment with the p38 inhibitor SB203580, JNK inhibitor SP600125 and ERK inhibitor PD98059, ablated DON-induced binding activity of the transcription factors and subsequent production of TNF-alpha and MIP-2. Thus, DON induced a signalling cascade in the macrophage that sequentially involved Hck/ PKR →JNK/ ERK/ p38→ AP-1/ C/EBP/ NF-kappaB and that resulted in elevated TNF-alpha and MIP-2 gene expression (Supported by NIEH Grant ES-03358 and ES-09521)

588 ROLE OF AROMATIC HYDROCARBON (Ah) RECEPTORS IN MURINE SPLEEN CELL MITOGENESIS AND RESPONSES TO BENZO(a)PYRENE (BaP) AND ITS MAJOR METABOLITES.

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Benzo(a)pyrene (BaP) is an environmental polycyclic aromatic hydrocarbon (PAH) that produces immunotoxicity resulting in either increased or decreased lymphocyte activity. Our lab has shown that BaP and certain BaP-metabolites (BaP-M) alter antigen/mitogen receptor and Ca2+-dependent signaling. Metabolism of BaP through P450-dependent (CYP1A1, CYP1B1) and independent (EPHX1, peroxidases, DD1) pathways generate products that each differentially regulate signaling. The purpose of these studies was to examine the effects of BaP and BaP-M on B and T cell proliferation in murine spleen cells, as well as to determine the role of AhR-dependent pathways in the immunotoxicity of BaP. The results of these studies show that while BPDE suppressed T and B cell proliferation at a concentration of 1 μM, BaP increased B and T cell proliferation by factors of 2-5 fold. In control WT (C57BL6/N) mice, BaP produced a dose-dependent increase in PHA-induced T cell proliferation and LPS-induced B cell proliferation. However, BaP-M produced a differential effect on murine spleen cells, with 1, 6-BPQ and 6, 12-BPQ increasing T cell proliferation, and BP-diol increasing B cell proliferation. These results suggest that murine splenic B and T cells may differentially metabolize BaP leading to proliferative effects. Because the AhR is important in the induction of BaP metabolism, we studied the effects of AhR on B and T cell proliferation and responses to BaP/BaP-T in WT, AhR +/-, and AhR -/- mice. AhR -/- mice had increased T and B cell proliferative responses to PHA and LPS in control cultures, suggesting that AhR may play a role in regulating cell proliferation. Knocking out AhR did not decrease BPQ-induced T cell proliferation. However, BP-diol-induced B cell proliferation was reduced in AhR -/- mice. Thus, we conclude that the AhR plays a differential role in B and T cell proliferation and that BaP-induced immunotoxicity is in part AhR-dependent. Supported by RO1 ES-05295 to SWB and RO1 ES-10433 to MKW.

589 PRENATAL AND LACTATIONAL EXPOSURE OF C57BL/6 MICE TO 2, 3, 7, 8 TETRACHLORODIBENZO-P-DIOXIN IMPAIRS THE IMMUNE RESPONSE TO INFECTION WITH INFLUENZA A VIRUS.

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Despite substantial investigation into the immunotoxic effects observed in adult animals exposed to TCDD, little is known about the immune function of mice exposed *in utero* and lactationally. This is of particular interest due to concerns regarding maternal transfer of Ah receptor ligands across the placenta and *via* breast milk in humans. The current study is part of an ongoing project to characterize primary and secondary immune function in mice exposed to TCDD during development. Impregnated C57Bl/6 mice were given 4 doses of TCDD: 0.25 ug/kg on gestational days (GD) 0 and 7, followed by either 1ug/kg (low dose) or 5 ug/kg (high dose) on GD 14 and postnatal day 2. Some neonatal mortality was observed, especially in the high dose group, which was likely due to decreased lactation in the dams. The immune response of 6-7 week old pups was tested by intranasal challenge with a dose and strain of influenza A virus which is not lethal in immunocompetent mice. Mice were sacrificed 8 days post-infection. In female pups, *in utero*/lactational exposure to TCDD was associated with decreased production of anti-viral antibodies, decreased numbers of CD8+ T cells in the lung, and reduced numbers of mediastinal lymph node cells. In addition, the number of IFNg-producing cells in the MLN was significantly suppressed in the TCDD-exposed female pups. In contrast to females, male offspring were generally resistant to TCDD-induced defects in response to infection with influenza virus. No effect of TCDD exposure on mortality or morbidity was observed in mice of either sex. Thus, female mice appear to be more sensitive to immune suppression induced by *in utero*/lactational exposure to TCDD, although additional studies using other antigens are necessary to verify if these effects are sex-specific. In summary, we have found that exposure to TCDD during development induces persistent deficits in immune function in female offspring.

590 SHORT-TERM EXPOSURE TO INHALED DIESEL EXHAUST PARTICLES ENHANCES ASTHMA-LIKE SYMPTOMS IN THE LOW IGE RESPONDER C57BL/6 MOUSE.

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Although several epidemiological studies indicate a correlation between exposure to ambient particulate matter and adverse health effects in humans, there is still a fundamental lack of understanding of the mechanisms involved. Here we have established a mouse asthma model to study the adjuvant effects of diesel exhaust particles (DEP). We developed this asthma model using the C57BL/6 mouse strain as this will allow us to study the effects of DEP on several gene knock-out mice, all of which are on the C57BL/6 background. Furthermore, this mouse model is unique in that it represents a short-term model of exposure to DEP (10 days) compared with other models in the literature (usually 6 weeks or more). C57BL/6 mice (6/group) were exposed *via* inhalation to 2000 ug/m3 DEP for 10 days and to 1% ovalbumin (OVA) for 6 days. Mice treated with DEP+OVA had a 10-fold increase in total cells in the bronchoalveolar lavage (BAL) fluid compared to OVA only treated animals. Additionally, the percent of eosinophils in the BAL fluid of DEP+OVA treated mice was 80 %, compared with only 18 % in OVA only treated animals. Airway hyperresponsiveness (AHR) as measured by methacholine challenge was also significantly increased in the OVA+DEP mice compared to OVA only treated mice. In all endpoints measured, DEP treatment alone had no effect. Thus we have developed a mouse model with two key components of asthma: an increase in total cells and eosinophils in the lung and an increase in AHR. Supported by US Public Health Service Grant AI50495.

591 EFFECTS OF CO-EXPOSURES OF CONCENTRATED AMBIENT PARTICLES AND ALLERGEN ON THE LUNGS OF BROWN NORWAY RATS.

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Health effects of co-exposures of air pollutants and airborne allergens have not been thoroughly investigated. The purpose of our study was to determine the effects of inhalation exposure of concentrated ambient particles (CAPs) on the lungs of rats that were concurrently exposed to a pulmonary allergen (ovalbumin; OVA). A mobile air research laboratory, equipped with inhalation exposure chambers and ambient particle concentrators, was used to conduct the studies. The lab was parked in a residential site in Claremont, CA. OVA-sensitized, male, Brown Norway rats were exposed to filtered air (controls), concentrated ambient coarse (2.5-10μm; CCAPs), fine (0.15-2.5μm; FCAPs) or ultrafine (0.01-0.15μm; UFCAPs) particles for 5 h/day (11am-4pm) for 3 consecutive days. Immediately prior to each daily exposure, rats were intranasally challenged with saline alone or a 0.5% solution of ovalbumin in saline. Rats were exposed to average mass concentrations of 554, 515 and 45μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively. 24 hours after the end of the exposures, rats were sacrificed, their airways lavaged with saline, and their lungs processed for microscopic or mRNA analyses. OVA-instilled rats had an allergic bronchiolitis with mucous cell hyperplasia and an allergic alveolitis with increases in eosinophils in the bronchoalveolar lavage fluid (BALF). OVA-instilled and air-exposed rats had 538% more eosinophils in the BALF, 104% more stored mucosubstances in the bronchiolar epithelium, and a 6-fold increase in mucin gene expression in bronchiolar airways than saline/air controls. Exposures to FCAPs or UFCAPs, but not CCAPs, caused attenuation (50-100%) of the OVA-induced allergic alveolitis, mucous cell metaplasia and mucin gene expression. These results indicate that fine and ultrafine particulate matter may interfere with allergen-induced airway responses during co-exposure of these airborne agents. (Research funded in part by USEPA Grant #R-82921601)

592 ENVIRONMENTAL TOBACCO SMOKE (ETS) ELICITS A TIME-DEPENDENT ALLERGIC AIRWAY RESPONSE TO INHALED ANTIGEN.

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Epidemiologic evidence supports a critical role for (ETS) in the occurrence and severity of allergies/asthma. However, neither the precise combination of ETS and allergen exposure nor the mechanism(s) by which these factors interact and contribute to asthma induction is known. Our hypothesis is that ETS inhalation provokes an asthmatic response by overcoming the normal airway tolerance to antigens. Our protocol combines daily ETS exposure with nose-only (N-O) sensitization to ovalbumin (OVA). Female A/J, Balb/c and C57Bl/6 mice inhaled steady-state ETS from 3 1R4F cigarettes, 5 hr/day, 9 wk. Mice were sensitized by N-O exposure (20 min/d, 10 d) to 1% aerosolized OVA at wks 2-3, 5-6 or 8-9 during ETS exposures. At 6-8 wks post-OVA sensitization, mice received a single OVA challenge (5%, 5 min). Airway responsiveness was assessed immediately after OVA challenge. Bronchoalveolar lavage (BAL) differentials, immunoglobin levels and lung inflammation were determined 24 hrs later. ETS exposure of A/J and Balb/c mice for >4 weeks prior to OVA sensitization elicited aggravated pulmonary responses, increased eosinophilia and production of OVA antibodies. The combined effect of inhaled ETS and OVA was more pronounced in Balb/c than in A/J mice. ETS exposure for only 1 week prior to OVA sensitization failed to elicit these responses. None of these responses was enhanced in C57Bl/6 mice regardless of the timing of ETS exposure and OVA sensitization. Together these results indicate that ETS exposure can elicit an asthmatic response to an allergen only a) in susceptible mice and b) when allergen sensitization follows prolonged ETS exposure. This is consistent with epidemiological data that ETS exposure exacerbates, rather than induces, asthmatic responses. Further, the results demonstrate that an asthmatic response can be elicited effectively by ETS + antigen administration *via* inhalation only, thereby overcoming aerosol tolerance. (Supported by PM External Research Group).

593 RESPIRATORY ALLERGY AND INFLAMMATION DUE TO AMBIENT PARTICLES (RAIAP), A EUROPEAN-WIDE ASSESSMENT. ALLERGY SCREENING.

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An objective of the RAIAP project is to examine whether qualitative differences in particulate air pollution at different locations in part explain differences in prevalence or severity of respiratory allergies throughout Europe. Ambient particulate matter (coarse and fine) was collected in Amsterdam, Rome, Lodz, and Oslo, during the spring, summer, and winter 2001/2002, as well as from a Dutch sea-side background location. In the part of the study presented here, these particles are screened for their capacity to induce, in an *in vivo* mouse model, either reactions associated with an immune response, using the popliteal lymph node assay, or an allergen specific IgE response, using an ELISA-based assay. The particulate matter is coded with respect to sampling site during the screening. So far, only the spring particles have been examined. Diesel exhaust particles from NIST (SRM 1650a) are included as a positive control. It turned out that both the coarse and fine fractions from all four sampling sites gave a significant increase in weight, cellularity, and proliferation responses to the model allergen ovalbumin, indicating that all particles have an adjuvant effect. No significant differences between the four sampling sites were observed. There seems, however, to be a locality difference in the irritant effect of the coarse particles. The adjuvant effect of the collected particles was confirmed by their ability to significantly increase the production of allergen specific IgE and IgG1. Although only significant for one location, the fine fractions seem to elicit stronger IgE responses than the coarse ones. A significantly stronger IgE response with fine particles from one, as compared to two other locations is seen. Thus, all RAIAP particles, both the coarse and fine ones, have an adjuvant effect in the doses examined (100-200 μg per mouse). However, no clear differences between the locations have been observed so far. (A European Commission Shared-Cost Research Project, QLK4-CT-2000-00792.)

594 INHALATION DOSIMETRY DIFFERENCES IN BALB/C AND B6C3F1 MICE INFLUENCE AIRWAY RESPONSIVENESS TO METHACHOLINE CHALLENGE.

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The airway response to bronchoconstrictive agents is commonly used to measure airway reactivity. While the effect of aerosol concentration and size are well characterized factors in the inhalation toxicology field, the delivered dose of inhaled aerosols in the pulmonary system is often overlooked. It is common practice in these challenge procedures to report the airway reactivity as the concentration of methacholine in the solution being nebulized into an aerosol that causes a 200% increase in airway resistance (PC200R). However, the response may be due to differences in actual deposition in the upper airways of the animals. To investigate the effect of inhalation dosimetry, BALB/c and B6C3F1 mice were placed in an unrestrained barometric whole-body plethysmograph and subjected to challenge with aerosols generated from isotonic saline (control) and 7 methacholine solutions of increasing concentration (2.5 to 320 mg/ml) for 3 min. Measurements of airway resistance were recorded and averaged for 3 min following each aerosolization period. The concentration of aerosol in the plethysmograph was measured with filters using fluorscein as a tracer, and aerodynamic particle size distribution was determined with a MOUDI cascade impactor. The methacholine aerosol size distribution data were then used to estimate upper airway delivered dose according to the deposition curves for the two strains reported by Oldham and Phalen (Anat Rec, 268, 59-65, 2002). Based on PC200R, the BALB/c mouse was 14 times more sensitive than the B6C3F1 mouse. When the concentration of methacholine in the whole-body plethysmograph, total volume inhaled, and deposition fraction were included in the estimated inhaled dose, the BALB/c mouse was only 2.4 times more sensitive than the B6C3F1 mouse. Therefore, individual subject inhalation dosimetry may differ significantly from the dose normally indicated by PC200R and may explain a wide range of PC200R-based methacholine challenge sensitivities.

595 INCREASED LUNG DISEASE TO RESPIRATORY SYNCYTIAL VIRUS BY INHALED DIESEL ENGINE EMISSIONS.

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The role of air pollutants and engine emissions in the susceptibility to respiratory viral infections is not well understood. Respiratory syncytial virus (RSV) is the leading cause of wheezing and bronchiolitis in lungs of infants and young children. To determine the impact of relevant levels of whole diesel engine emissions (DEE) on RSV-induced lung disease, a resistant mouse model of RSV infection was exposed to inhaled DEE before infection and markers of lung inflammation, lung epithelial host defense, and homeostasis assessed. Inhaled DEE concentrations consisted of 30 $\mu g/m^3$, 200 $\mu g/m^3$, and 1000 $\mu g/m^3$ of total particulate matter (PM). RSV infection did not induce notable lung histopathology, inflammation, or viral persistence in air-exposed mice. Prior exposure to inhaled DEE (6 h/d, 7 consecutive days) prolonged the expression of RSV gene subsets, consistent with increased lung viral burdens. Lung inflammation to RSV was increased in DEE-exposed mice concomitant with increased viral gene expression. Lung inflammation endpoints exhibited a concentration-dependent response, with the highest inflammation in RSV-infected mice exposed to 1000 $\mu g/m^3$ PM DEE. Lung histopathology and specifically lung epithelial morphology were markedly altered during RSV infection by prior DEE exposure. Mucus production was increased in the airways of DEE-exposed, RSV-infected mice in a concentration dependent manner. Immunohistochemical studies of distinct lung epithelial cell subsets indicate spatial-temporal alterations in airway epithelial Clara cells and alterations in lung surfactant protein homeostasis, including important host defense proteins. These studies indicate that relevant levels of inhaled DEE exposure can exacerbate the susceptibility to RSV-induced lung disease and may provide a useful model for elucidating the molecular mechanisms of increased susceptibility to acute respiratory infections.

596 AIRWAY REACTIVITY IN ALLERGIC MICE AND RATS AFTER SHORT-TERM EXPOSURE TO CONCENTRATED AMBIENT PARTICULATE MATTER.

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The present study was designed to find out whether exposure to ambient particulate matter (PM) causes exacerbation of the symptoms of asthma. We have used mouse and rat models in which characteristics of asthma, such as increased immune globulin levels and increased airway reactivity. Animals were exposed to concentrated ambient particulate matter (CAPs < 2.5 um; 500 - 2000 ug/m3) during 3 consecutive days. Allergic animals had an increase in eosinophils, IgE and IgG1 in the lung as well as a hypertrophy of the bronchiolar epithelium. However, CAPs did not only marginally affect the pathological conditions. The smooth muscle contraction due to increasing concentrations of metacholine exposure was assessed. Only slight effects of CAPs on the airway reactivity could be observed. We concluded that CAPs used in this study did not significantly affect airway pathobiology and reactivity. These data do therefore not provide support to the hypothesis that PM2.5 can exacerbate a pre-existing asthmatic condition.

597 EXACERBATION OF BRONCHIAL HYPERRESPONSIVENESS (BHR) BY URBAN PARTICULATE MATTER.

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Recent epidemiological studies have demonstrated a correlation between exposure to increases in urban particulate matter (PM) and exacerbation of bronchial hyperresponsiveness (BHR). However, the mechanism by which PM enhances the immune response contributing to the BHR is unknown. In this study, DO11.10 mice, which are transgenic for an ovalbumin-specific T-cell receptor, were used to assess the acute and delayed effects of PM on BHR. Based on preliminary dose-response studies, mice at 4 weeks of age were instilled intranasally with 750 μg of PM1648 (a model PM available from NIST) with or without 150 μg ovalbumin. Enhanced pause (Penh) was examined following methacholine challenge at 4 and 72 hours post-instillation using whole-body plethysmography (Buxco). BHR was increased at 4 hours following co-administration of PM and antigen compared to age-matched controls. Exacerbation of BHR also occurred at 72 hours with separate administration of PM and antigen. This data demonstrates that exposure to PM1648 increases both the acute and delayed BHR to antigen. These results could be explained by PM acting as an adjuvant-like cofactor to the antigen. Alternatively, in the acute model, PM may be acting as a carrier for the antigen, facilitating its presentation to the immune system. This work was supported by NIEHS grant ES 11120 and EPA grant R826782.

598 MOTOR ACTIVITY IN DEVELOPMENTAL NEUROTOXICITY TESTING: A CROSS-LABORATORY COMPARISON OF CONTROL DATA.

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The USEPA Developmental Neurotoxicity Study Test Guideline (OPPTS 870.6300) calls for a battery of functional and neuropathological assessments in offspring during and following maternal exposure. Motor activity is measured on post-natal days (PND) 13, 17, 21, and 60±2; inclusion of 3 time points during lactation allows assessment of the pattern of overall activity levels during this time and of the development of habituation. Although the Guideline specifies use of an automated motor activity recording apparatus, the type of device or duration of the testing period are not specified; many devices and session lengths are seen among the studies that have been submitted to the Agency. To evaluate whether some types of devices/testing conditions yield more reliable data than others, we compared motor activity data generated in control animals from 21 studies in terms of: 1) variability of data within a study, across time points; 2) variability of data across studies within a laboratory, by time point; 3) patterning of mean baseline motor activity counts over time, across studies within a laboratory; 4) variability for particular devices, across laboratories. Data from ten laboratories were evaluated; the number of studies available from a single laboratory varied from 1 to 5. Preliminary analysis found higher variability for earlier lactation time points than for later lactation time points in most laboratories. Variability for the adult time point was usually lower than for the lactation time points. Variability did not consistently correlate with type of device or with session duration. The pattern of baseline activity counts across postnatal age and studies varied among laboratories. For all laboratories, mean baseline activity counts were higher on PND17 than on PND13; for most laboratories, mean counts on PND21 were lower than on PND17. Further consideration of how to reduce variability of these motor activity measurements is warranted. *This abstract does not necessarily reflect the policy of the US Environmental Protection Agency.*

599 LACK OF EFFECT OF PERINATAL EXPOSURE TO A POLYBROMINATED DIPHENYL ETHER MIXTURE (DE-71) ON THE HABITUATION OF MOTOR ACTIVITY IN ADULT RATS.

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants and are becoming increasingly evident in environmental media, wildlife and human breast milk. Published research in mice has shown that early postnatal exposure to some PBDEs attenuates the habituation of motor activity when the pups were tested as adults. This experiment determined the effect of perinatal exposure of rats to the PBDE mixture DE-71 on the habituation of motor activity in adult offspring. Pregnant Long Evans rats (N=8/group) received (p.o.) either corn oil (1 ml/kg) or DE-71 (5, 30 or 100 mg/kg) from GD6 to PND21. Doses were based on prior research showing alterations in thyroid hormone status. As adults, one male and one female from each litter (total: 8 rats/treatment/gender) underwent habituation testing. Test sessions (45 min) were carried out in photocell devices that separately recorded horizontal and vertical activity in five 9-min intervals. In general, motor activity was highest early in the session, but dropped by approximately 65-75% by the end of the session. Perinatal exposure to DE-71 did not affect either overall levels of motor activity or the within-session habituation of activity in either gender. These results suggest that effects in mice postnatally exposed to individual PBDE congeners may not predict effects in adult offspring of rats perinatally exposed to DE-71. This abstract does not necessarily reflect USEPA policy.

600 PERSISTENT IMPAIRMENTS IN SHORT-TERM, BUT ENHANCED LONG-TERM, SYNAPTIC PLASTICITY IN HIPPOCAMPAL AREA CA1 FOLLOWING DEVELOPMENTAL HYPOTHYROIDISM.

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Thyroid hormones (TH) are critical for nervous system development. Deficiency of TH during development impair performance on tasks of learning and memory that rely upon the hippocampus, but the mechanism underlying this impairment is not well understood. The present study was designed to identify hypothyroid-induced changes in hippocampal function using neurophysiological assessments in area CA1 of hippocampal slices. Hypothyroidism was induced by administration of 3 or 10ppm propylthiouracil (PTU) to pregnant and lactating dams *via* the drinking water from GD6-PN30. Synaptic transmission and plasticity using the long-term potentiation (LTP) model were assessed in area CA1 of hippocampal slices from treated offspring. Input/output and paired-pulse functions were collected to assess the integrity of synaptic transmission at PN85-101. Baseline synaptic transmission was not altered in PTU-exposed animals. However, significant dose-dependent reductions in paired-pulse facilitation of the EPSP were observed, indicative of perturbations in presynaptic transmitter release function. An increase in magnitude of LTP of the PS and increased phosphorylation of MAPK/ERK as a function of LTP induction were also observed in the high dose group. Neurophysiological assessments in the preweaning period and in adults in a different hippocampal subregion (i.e., dentate gyrus) demonstrate a different pattern of effects and more profound deficits (see accompanying Gilbert and Sui abstract). These results indicate that neonatal hypothyroidism produces long-term changes in hippocampal function that can be observed using field potential analysis *in vitro* and *in vivo*; that site is critical in determining the nature of the deficit; and that neurophysiological alterations may contribute to behavioral deficits associated with developmental hypothyroidism. (Does not reflect USEPA policy).

601 DEVELOPMENTAL HYPOTHYROIDISM IMPAIRS HIPPOCAMPAL LEARNING AND SYNAPTIC TRANSMISSION *IN VIVO*.

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A number of environmental chemicals have been reported to alter thyroid hormone (TH) function. It is well established that severe hypothyroidism during critical periods of brain development leads to alterations in hippocampal structure and learning deficits, yet evaluation of modest perturbations of the thyroid axis coupled with more subtle alterations in brain function has been lacking. The present study examined the effects of developmental hypothyroidism on hippocampal function using behavioral and neurophysiological assessments in the intact animal. Thyroid hormone function was reduced in pregnant dams by propylthiouricil (PTU) *via* the drinking water (0, 3, 10 ppm) from GD 6 to weaning. This regimen reduced serum TH concentrations on PN14. All animals had returned to a euthyroid state at time of testing. At 5-6 mon of age, spatial learning was assessed in male offspring in a Morris water maze. Between 4-7 mon of age, neurophysiological examination of perforant path-dentate gyrus field potentials was performed *in vivo*. Input/output (I/O) functions probed the integrity of synaptic transmission. Paired pulses were delivered to examine inhibitory function (PPD) and presynaptic transmission (PPF). Long-term potentiation (LTP) was induced by theta burst stimulation to assess neuroplasticity. PTU induced dose-dependent impairments in spatial learning in the absence of cue learning deficits or reductions in swim speed. Electrophysiologically, population spikes and EPSP slopes were dose-dependently reduced, and LTP was impaired. In contrast, paired pulse tests revealed a shift to more facilitated responses as a function of PTU treatment. These findings corroborate earlier data from our laboratory demonstrating alterations in synaptic function following a higher dose of PTU exposure occurring primarily during lactation and suggest that hippocampal neurophysiological assessments may prove valuable in defining the lower end of the dose-response function for chemicals that alter thyroid function early in development. (Does not reflect USEPA policy).

602 PERINATAL EXPOSURE TO A POLYBROMINATED DIPHENYL ETHER MIXTURE (DE-71): DISRUPTION OF THYROID HOMEOSTASIS AND NEUROBEHAVIORAL DEVELOPMENT.

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Polybrominated diphenyl ethers (PBDEs), produced commercially as mixtures, are used as flame-retardants in numerous consumer products. Previous work has demonstrated that the DE-71 induces hypothyroxinemia in both adults and developing rats. In these studies, primiparous rats were orally administered DE-71 (1 - 100mg/kg/day) in 1.0 ml/kg corn oil from gestation day 6 (GD6) to postnatal day 21 (PND21). Offspring were evaluated at various ages for survival, body weight, serum thyroid hormones, age of eye opening, motor activity development, hepatic enzyme activity (EROD, PROD, UDPGT), auditory startle, auditory thresholds, and fear conditioning. There was no effect of DE-71 on body weight gain or survival in either the dams or offspring. There were dose-dependent decreases in thyroxine in offspring relative to controls on PND 5 (73.3, 49.3 and 43.5 % of control) as well as on PND 14 (75.6, 33.6 and 29.9%) for 5, 30 and 100mg/kg respectively. DE-71 caused increases in hepatic EROD, PROD and UDPGT activity in both offspring and dams. Motor activity and startle were unaffected by treatment. Tests of fear conditioning revealed a dose-dependent decrease in cue- but not context-based performance in male offspring tested as adults. There was no effect on fear conditioning performance in offspring tested as weanlings. Auditory thresholds are currently being evaluated. These data suggest that developmental exposure to DE-71 induces hepatic enzymes and hypothyroxinemia in both dams and offspring, and alters some aspects of offspring neurobehavioral development. This is an abstract of a proposed presentation and does not necessarily reflect University.S.EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

603 DEVELOPMENTAL NEURO- AND CARDIOTOXICITY OF TERBUTALINE: DNA SYNTHESIS AND NEURAL BIOMARKERS.

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Beta-2-adrenergic receptor (B2AR) agonists, such as terbutaline, are widely used to arrest preterm labor, despite the fact that this is not on FDA's list of approved uses. B2AR agonists cross the placenta to stimulate fetal BARs that control neural and cardiac cell differentiation. We administered terbutaline to developing rats in two different exposure periods corresponding to human development in the third trimester (postnatal days PN2-5) and in the perinatal period (PN11-14). We examined DNA synthesis in the heart and in brain regions with different maturational timetables. Exposure on PN2-5 reduced DNA synthesis in the heart and brainstem of females, with sensitization of the effect upon repeated terbutaline administration. Terbutaline treatment on PN11-14 decreased cerebellar DNA synthesis after one or four daily injections; in contrast, cardiac DNA synthesis decreased after the first injection but increased after the fourth injection. We next focused on neural biomarkers to determine which cell populations are being affected by terbutaline exposure. Terbutaline administered on PN2-5 resulted in immediate (PN6) increases in glial fibrillary acidic protein (GFAP) in all brain regions, suggestive of gliosis. Terbutaline exposure on PN11-14 did not significantly alter GFAP. Our results indicate that excessive BAR stimulation by terbutaline alters cell development in brain regions and peripheral tissues, with the net effect depending on sex and on the timing of exposure. These effects may contribute to the higher incidence of neuropsychiatric, cognitive, cardiovascular and metabolic abnormalities reported in the offspring of women who underwent therapy with BAR agonist tocolytics. We are currently investigating the effects of terbutaline on neuronal biomarkers and on cellular morphology in the brain and heart. (Supported by NIH HD09713)

604 SHORT-TERM NICOTINE EXPOSURE IN ADOLESCENT RATS ELICITS BRAIN CELL DAMAGE.

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Adolescence is when cigarette use typically begins. Recent studies suggest that the adolescent brain is particularly vulnerable to nicotine dependence. We investigated the effects of short-term nicotine exposure on indices of brain cell damage in adolescent rats. Beginning on postnatal day 30, rats were given a one week regimen of either continuous nicotine infusions (to simulate regular smoking) or twice-daily injections (to simulate intermittent smoking), at three doses (0.6, 2 and 6 mg/kg/day) set to achieve plasma nicotine levels found in occasional to regular smokers. During and after nicotine exposure, we assessed indices of nicotinic acetylcholine receptor activation (nAChR upregulation), cholinergic synaptic function (3H-hemicholinium-3 binding to the presynaptic choline transporter), cell number (DNA content), cell packing density (DNA concentration), cell size (protein/DNA ratio) and membrane surface area (membrane/total protein). Nicotine infusions elicited robust increases in nAChRs even at the lowest dose, with effects persisting for one week after the end of treatment. In the nicotine group, we observed reductions in cell number and packing density in the cerebral cortex and midbrain, accompanied by net increases in cell size for the remaining cells. Membrane surface area was reduced relative to cell size, suggesting a loss of neuritic projections, and indeed, [3H]hemicholinium-3 binding was reduced. Many of the effects persisted for at least one month posttreatment and were significant at 2 mg/kg/day, which produces plasma levels of nicotine found in light smokers. Intermittent nicotine exposure (injection route) elicited significant effects that were less consistent than those seen with infusions. Our results indicate that even short-term exposure to low doses of nicotine disrupts adolescent brain development; the degree of nAChR upregulation and cell damage are dependent on the pattern of exposure (continuous versus intermittent). Supported by a grant and fellowship from the Philip Morris External Research Program and by a fellowship from CNPq Brazil.

605 FETAL CHLORPYRIFOS EXPOSURE: ADVERSE EFFECTS ON BRAIN CELL DEVELOPMENT AND CHOLINERGIC BIOMARKERS.

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Fetal and childhood exposures to the widely-used organophosphate pesticide, chlorpyrifos (CPF), have raised concerns about developmental neurotoxicity. Previously, biomarkers for brain cell number, cell packing density and cell size indicated that neonatal rats were more sensitive to CPF than were fetal rats, yet animals exposed prenatally still developed behavioral deficits in adolescence and adulthood. We administered CPF to pregnant rats on gestational days 17-20, using regimens devoid of overt maternal or fetal toxicity. We then examined subsequent development of acetylcholine systems in forebrain regions, and compared the effects to those on general biomarkers of cell development. Choline acetyltransferase, a marker for cholinergic nerve terminals, showed only minor CPF-induced changes. In contrast, hemicholinium-3 binding to the presynaptic choline transporter, which is responsive to nerve impulse activity, displayed marked suppression that persisted into adulthood. There was no compensatory upregulation of cholinergic receptors, as m2-muscarinic cholinergic receptor binding was unchanged. CPF also elicited alterations in biomarkers of cell development, with reductions in cell packing density, increases in relative cell size and contraction of neuritic extensions; however, neither the magnitude nor timing of these changes were predictive of the cholinergic defects. The current findings indicate a wide window of vulnerability of cholinergic systems to CPF, extending from prenatal through postnatal periods, occurring independently of adverse effects on general cellular neurotoxicity. Support: NIH ES10356 & ES10387.

606 CHANGES IN SEROTONIN RECEPTORS IN BRAIN REGIONS OF RATS AFTER NEONATAL EXPOSURE TO CHLORPYRIFOS.

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Chlorpyrifos (CPF), a commonly used pesticide, is a developmental neurotoxin, and animal studies indicate cognitive impairment after fetal or neonatal exposure. However, little or no attention has been paid to potential effects on serotonergic (5HT) systems, which similarly participate in cognitive function, but also regulate mood, appetite and other behaviors. Neonatal rats received 1 mg/kg CPF on postnatal days (PN) 1-4, or 5 mg/kg on PN11-14, doses that lie below the threshold for systemic toxicity. Twenty-four hours and 6 days after the last dose, we measured 5HT1A and 5HT2 receptor binding in cell membranes prepared from regions containing 5HT terminals (forebrain) and cell bodies (midbrain/brainstem). With exposure on PN1-4, 5HT receptor binding showed receptor upregulation at PN5 across both regions and both sexes. The elevations were still significant on PN10 in males, but in females deficits emerged in the midbrain/brainstem. With later exposure during the peak period of axonogenesis/synaptogenesis (PN11-14), the initial (PN15) increase in receptor binding occurred only in females. By PN20, significant deficits appeared in both sexes, both regions and for both receptor subtypes. Superimposed on the decrease in 5HT receptors, binding of paroxetine, a ligand for the presynaptic 5HT transporter, also showed a decrement. These results suggest that CPF exposure during a critical window centering around axonogenesis/synaptogenesis elicits deficits of 5HT synaptic biomarkers that are compatible with po-

tential emergence of affective dysfunction. Future work will address the persistence, underlying mechanisms and behavioral consequences of these effects of CPF exposure. (Supported by NIH ES10387 and ES10356)

607 PRENATAL EXPOSURE TO DIOXIN AND INHALED BENZO(a)PYRENE: REDUCED CAPACITY FOR LONG-TERM POTENTIATION IN THE F1 GENERATION.

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The purpose of this study was to assess the transplacental effects of exposures to 2, 3, 7, 8, tetrachlorodibenzo-p-dioxin (TCDD) or benzo(a)pyrene [B(a)P], or to a successive exposure to both compounds [TCDD On GD14 & B(a)P on GD14-17] in timed pregnant dams, on the characteristics of long-term potentiation (LTP; a cellular correlate of learning and memory) in F1 generation animals. After birth, F1 generation pups were weaned, randomly selected for treatment groups (i.e., unexposed control; TCDD; B(a)P; and TCDD/B(a)P groups), and housed separately by sex. On postnatal day 60-62 the animals were anesthetized and prepared for recording from the hippocampus. F1 rats exposed *in utero* to TCDD alone or in sequence with B(a)P, failed to develop LTP under the same conditions that produced robust LTP in the unexposed F1 control rats. In control cases, LTP was accompanied by a decrease in onset latencies and leftward shifts in input-output curves. F1 rats exposed *in utero* to B(a)P alone developed a weak but enduring potentiation. The results indicate that midgestational exposure to TCDD and B(a)P decrease the capacity for synaptic plasticity in F1 generation rats.

608 METABOLISM OF INHALED BENZO(a)PYRENE IN THE DEVELOPING CENTRAL NERVOUS SYSTEM AND PARTIAL ABLATION OF LONG-TERM POTENTIATION IN F1 GENERATION ANIMALS.

D. B. Hood, J. Wu, A. Ramesh, D. Wormley, T. Nayyar and M. Greenwood. *Pharmacology, Meharry Medical College, Nashville, TN.*

This study was undertaken to determine the bioavailable dose associated with the transplacental disposition of inhaled benzo(a)pyrene [B(a)P] metabolites and the resulting effects on synaptic plasticity in F1 generation pups. In this study, laparotomy on GD 8 was performed on timed-pregnant rats followed by dosing *via* nose-only exposure for four hours a day for ten days (GD 11- GD 20) to three concentrations of a B(a)P : carbon black aerosol (25, 75 and 100 micrograms/m3). A dose-dependent decrease in birth index was observed in the B(a)P: carbon black aerosol exposed group when compared to the unexposed control or carbon black exposed group ($p<0.05$). Analysis of cerebrocortical extracts from F1 generation pups revealed a dose-dependent increase in total B(a)P metabolites. The developmental expression profiles for AhR and CYP1A1 were analyzed from cerebrocortical and hippocampal tissue by RT-PCR and Northern Blotting. The results, using 18sRNA as the internal standard, demonstrate that inhaled B(a)P upregulates AhR and CYP1A1 during the first postnatal week when the regulatory synergism between neuronal-glial interactions is essential for programmed growth and differentiation. On postnatal day 60-62 randomly selected male rats were anesthetized with urethane and small access holes were made in the skull through which electrodes were lowered to stimulate the entorhinal cortex and analyzed for long-term potentiation (LTP) in the dentate gyrus. It was found that robust LTP was readily produced in the control group, in contrast, rats exposed to B(a)P at the highest dose did not develop LTP. Overall, our results suggest that gestational exposure to B(a)P decreases the capacity for synaptic plasticity in the F1 generation.

609 BENZO(a)PYRENE AND DIOXIN INDUCED DOWNREGULATION OF HIPPOCAMPAL NMDAR1 EXPRESSION AND DEFICITS IN FIXED-RATIO PERFORMANCE IN F1 GENERATION RATS.

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Dioxin and B(a)P are widely distributed environmental contaminants which are suspected to impair advanced brain function in animal models and humans. The NMDAR1 receptor is a well characterized molecule that is responsible for plasticity and the development of synaptic connections in the central nervous system (CNS). To access the neurotoxicological effects of prenatal exposure to these compounds in F1 generation offspring, timed-pregnant F-344 rats were exposed to inhaled B(a)P aerosol (100µg/m3) for 4 hours a day on GD14 to 17 or to dioxin (700ng/kg BW) by gavage on GD 14. The mRNA and protein expression of NMDAR1 from the hippocampus of F1 generation pups was quantified by Real-time PCR and western blot analysis on PND 0, 5, 10, 20 and 30. Real-time PCR results revealed similar developmental expression profiles for the control and exposed groups from PND 0 to 30, where the mRNA expression level on PND 30 was increased to 150% relative to that on PND 0. The quantitation further revealed down-regulation in the B(a)P (10%) and Dioxin (15%) exposure groups as compared to controls. Western blot analysis revealed a significant 38% and 48% reduction in NMDAR1 protein levels on PND 30 subsequent to B(a)P and Dioxin exposure, relative to controls. On PND 80, the Fixed-Ratio (FR) Performance was conducted to evaluate the acquisition process of learning and memory. The analysis revealed that as the complexity of the task increased from a FR1 to FR5, the exposed animals showed deficits in the acquisition process of learning and memory that did not occur in the controls. The reduction in hippocampal NMDAR1 expression correlates well with the hippocampal based Fixed-ratio performance behavioral study. These results suggest that prenatal exposure to B(a)P and dioxin cause deficits in learning and memory and these deficits appear to persist through adulthood long after the end of exposure.

610 EYEBLINK CLASSICAL CONDITIONING OF RABBITS AS A POTENTIAL POSTNATAL BEHAVIORAL-FUNCTIONAL EVALUATION.

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Although most behavioral-functional evaluations are performed with rats or mice, some agents may require use of alternate species, for which there is little validated testing and/or control data. Our laboratory validated eyeblink classical conditioning in rabbits, a test of learning and memory, to address this deficit. Saline (S), scopolamine (SC, 1.0 mg/kg/day) or d-amphetamine (A, 1.85 mg/kg/day) were given subcutaneously on 8 consecutive days, 30 minutes pretest. Each group consisted of 4 rabbits/sex, 5-6 months of age. Each trial was 600 milliseconds (ms) in duration and had a conditioned stimulus (CS, a 100 ms tone), a "blank period" (400 ms without stimulus) and an unconditioned stimulus (US, a 100 ms corneal airpuff). The CS-US interval was 500 ms. Rabbits were restrained and adapted to classical conditioning equipment (San Diego Instruments) for a period of 3 consecutive days (15 min. on day 1, 30 min. on day 2 and 60 min. on day 3). Baseline data were collected without stimulation on day 3. A total of 60 trials/day were determined over the 8-day treatment period. Mean conditioned responses on day 8, when learning should be present, were 31.1% and 58.6%, 15.5% and 16.0% and 48.8% and 79.1% for male and female rabbits in the S, SC and A groups, respectively. In each group, female rabbits learned more rapidly than male rabbits. As compared with the S group, SC-treated rabbits had impaired learning, possibly associated with undetected sedation due to central cholinergic effects. A-treated rabbits learned faster and remembered better than S-group rabbits, possibly as the result of a combination of associative and nonassociative learning. Thus, this classical conditioning paradigm demonstrates changes in learning and memory in rabbits and can serve as a behavioral test in this species for safety evaluations.

611 EVALUATION OF THYROID FUNCTION USING SERUM T3, T4 AND TSH LEVELS (OBTAINED BY USING RIA KITS) IN CRL IGS MATERNAL, FETAL AND NEONATAL RATS.

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Early neonatal hypothyroidism affects development of the brain and reproductive organs. EPA has recommended inclusion of thyroid hormone evaluations (T3, T4 and TSH serum levels and histopathology) in multigeneration and developmental neurotoxicology studies. Such endpoints have been minimally studied in neonatal rodent of commonly used strains. To identify time-dependent and/or sex-related differences in serum T3, T4 or TSH levels in maternal, fetal and neonatal rodents, control data were compiled from studies in which CRL IGS Sprague-Dawley rats were used. All values were obtained from serum, using RIA kits. Maternal values are for individuals. Fetal and pup values are for sera pooled by litter, except for LDs 21/22 values, which are for individual male and female weanling rats. Values are tabulated below. These findings show that RIA kits can be used to determine serum levels of T3, T4 and TSH in maternal and fetal, neonatal and juvenile rats and provide a relatively consistent, sensitive and appropriate means for detecting functional changes in the thyroid in rodents.

Serum Levels			
	T3 (ng/dL)	T4 (ug/dL)	TSH (ng/mL)
Dams			
GD21	99.7 (5)	2.31 (16)	5.05 (16)
LD 1	79.0 (5), 99.4 (16)	3.05 (5), 2.03 (16)	4.98 (5), 5.07 (16)
LD 21	57.8 (30), 61.5 (30)	2.03 (30), 2.22 (30)	2.05 (30), 1.62 (30)
LD 22	20.3 (15)	3.52 (15)	3.97 (15)
Pups			
GD 21	25.4 (31)	1.59 (16)	7.22 (16)
LD 5	62.1 (15)	2.08 (21)	4.86 (16)
LD 30	79.5 (16)	3.73 (16)	8.64 (16)
LD 21-22 M	105.9 (27), 106.3 (20), 91.0 (15)	2.41 (27), 3.23 (20), 3.03 (15)	1.24 (27), 0.82 (20), 0.63 (15)
LD 21-22 - F	106.0 (28), 108.2 (20), 103 (15)	2.27 (28), 3.42 (20), 3.02 (15)	1.24 (28), 0.94 (20), 0.26 (15)

GD - Gestation Day, LD - Lactation Day, M - Male, F - Female, () - Numbers per Group

612 LOW DOSE PERINATAL VINCLOZOLIN EXPOSURE IN THE LE RAT ALTERS *EX COPULA* PENILE ERECTIONS AND REDUCES PUP SIRING FOLLOWING GROUP MATING.

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Vinclozolin is a dicarboximide fungicide applied to industrial park landscapes, golf turf, and numerous crops intended for human consumption. Health concerns have arisen because metabolites of this compound have been shown to bind to the rat, monkey, and human androgen receptor. Following perinatal exposure, vinclozolin permanently disrupts sex-specific development as indicated by significantly reduced male rat reproductive organ weights. Unfortunately, little is known about the functional implications of reduced organ weights measured post mortem. To fill this void, two androgen-mediated reproductive endpoints were examined in exposed male rat offspring. Pregnant Long-Evans rats were administered 0.0, 0.75, 1.5, 3.0, or 6.0 mg/kg vinclozolin p.o. from gestation day 14 to postnatal day 3. Endpoints following parturition such as pups per litter, sex ratio, gestational length, mortality, or body weights throughout the preweaning period were not affected. There was a dose-related increase in the number of male pups with visible areolas on postnatal day 12. Vinclozolin reduced the frequency of reflexive penile erections but not penile movements in adult males in a dose-related fashion during ex copula tests. Low intensity erections, characterized by distension of the glans and tumescence of the base, were the most sensitive to exposure. Exposure also reduced the ability of males to deliver sperm and sire offspring during five consecutive days of naturalistic group mating where an experimental male interacted with three free-cycling females and an unexposed competitor male. Exposed males were less likely than controls to initiate copulation, perhaps due to a lowered social status, impaired control of the penis, or a combination of both factors.

613 NEONATAL PBDE 99 EXPOSURE CAUSES DOSE-RESPONSE RELATED BEHAVIOURAL DERANGEMENTS THAT ARE NOT SEX OR STRAIN SPECIFIC IN MICE.

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Flame-retardants are used to suppress or inhibit combustion processes in order to reduce the risk of fire. One class of flame-retardants, polybrominated diphenyl ethers (PBDEs), have been found to increase in the environment and in human mother's milk. In recent studies we have seen that neonatal exposure to some brominated flame-retardants, such as PBDE 47, PBDE 99, PBDE 153 and PBDE 209, can cause permanent aberrations in spontaneous behaviour in male NMRI mice, an effect that worsen with age, and affect the cholinergic system. The present study was undertaken to investigate whether these behavioural defects are sex dependent and/or strain dependent. Male and female C57 Bl/J mice were exposed to 0.4, 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg b.wt., as one single oral dose, on postnatal day 10. Spontaneous behaviour was observed in both male and female mice at the age of 2, 5, and 8 months. Male mice exposed to 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg b.wt. on postnatal day 10 showed significantly impaired spontaneous motor behaviour at all three testing occasions. This effect was dose-response related and the deranged behaviour worsened with age. Female mice exposed to 0.8, 4.0, 8.0, or 16.0 mg PBDE 99/kg b.wt. on postnatal day 10 showed significantly impaired spontaneous motor behaviour at all three testing occasions. This effect was dose-response related and the deranged behaviour worsened with age. In conclusion, neonatal exposure to PBDE 99 induces behavioural derangements in adult C57 Bl/J mice of both sexes, an effect that is dose-response dependent and seem to worsen with age. PBDE 99 causes similar developmental neurotoxic effects in both C57 Bl/J and NMRI male mice.

614 PRENATAL LEAD (Pb) EXPOSURE AND SCHIZOPHRENIA: PRELIMINARY FINDINGS.

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Schizophrenia, a mental disorder with unknown etiology affecting more than 2 million Americans, is characterized by hallucinations, delusions, social withdrawal, and disorganized thinking. Prior studies suggest that environmental disruptions in prenatal development may be risk factors. A cohort established for the study of maternal and child health in Oakland, CA, enrolled 19, 044 live births between 1959 and 1966. Maternal serum samples were collected throughout pregnancy, and stored at -20°C. Potential cases of schizophrenia spectrum disorders (SSD) were identified from inpatient hospital records within a computerized database. Potential cases were diagnosed following a clinical interview or by chart review. A group of 71 SSD cases were identified. Matched controls were selected on the basis of gender, number of available sera samples, date of birth, and timing of membership and blood draw. Serum δ-aminolevulinic acid, a biological marker of Pb exposure, was measured in 2nd trimester samples of 44 cases and 75 controls using HPLC. The data was converted into exposure categories of high maternal blood lead (>15 μg/dL) and low maternal blood lead (<15 μg/dL). The odds of SSD given exposure, when measured using conditional logistic regression, gives an estimated odds ratio (OR)=1.83 (95% CI: 0.87-3.87, p=0.1.) A random effects model, adjusted for gravida's age at termination of pregnancy gives similar results, with an OR=1.7 (95% CI: 0.93-3.10, p=0.08). This finding suggests that prenatal Pb exposure may be associated with adult psychotic disorders.

615 INTERACTIONS OF PRE/POSTNATAL LEAD (Pb) EXPOSURE AND MATERNAL STRESS.

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Children that continue to sustain the highest levels of Pb exposure in the US are also those that are socioeconomically disadvantaged and medically underserved, adding to the list of risk factors that hinder them from the ability to achieve their maximal potential. Pb could be predicted to interact significantly with such life stresses: both Pb and stress, for example, activate brain mesolimbic dopamine (DA) systems. Yet, current understanding of Pb largely reflects its study in isolation. The interaction of maternal stress with Pb exposure was studied here. Dams exposed to 0 or 150 ppm Pb (mean blood Pb= 38 ug/dl) in drinking water for 3 months prior to breeding and throughout lactation were subjected to 45 min restraint stress 3 times/day on gestational days 16-17. Both Pb and stress increased corticosterone levels measured after the final restraint stress on day 16 in dams, with highest levels produced by Pb+stress. Behavioral effects in male offspring (control, control/stress, Pb, Pb/stress) were examined on a fixed interval (FI) reinforcement schedule previously found sensitive to Pb and mediated by mesolimbic DA. Maternal stress alone (control/stress) had no evident effects on FI performance across 9 weeks of testing. Pre/postnatal Pb exposure (Pb group) produced sustained decreases (35%) in FI response rates that were modulated by maternal stress (Pb/stress), which increased FI rates back to levels equivalent to controls. A restraint stress imposed prior to FI testing in week 9 suppressed FI response rates in all groups. These reductions were attenuated by maternal stress (control/stress) and by Pb exposure (Pb group). Maternal stress modulated challenge restraint stress effects of Pb, decreasing FI rates (Pb/stress) relative to those produced by Pb alone. These findings support the assertion that the behavioral toxicity associated with developmental Pb exposure can be modulated by its interactions with environmental stress, and that stress responses to environmental challenges can be modulated by Pb exposure. ES05017, ES05903 and ES01247.

616 DEVELOPMENTAL EFFECTS OF MATERNAL EXPOSURE TO NICOTINE AND CHLORPYRIFOS, ALONE AND IN COMBINATION IN RATS.

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In the present study, we evaluated the neurotoxic effects in offspring following maternal exposure with low doses of nicotine and chlorpyrifos. Pregnant rats (300-350 g) were treated daily with nicotine (1 mg/kg, s.c., in normal saline) and/or chlorpyrifos (0.1 mg/kg, dermal, in ethanol) for the gestational days 5-20. Male offspring from the mothers treated with nicotine alone gained significantly lower weight on PND 30 as compared with the control. On PND 7, there was a significant increase in the brain acetylcholinesterase (AChE) activity in the pups from nicotine and chlorpyrifos groups, whereas plasma butyrylcholinesterase (BChE) activity showed a significant increase (~167 and 176% of control) in the pups from chlorpyrifos alone or in combination with nicotine, respectively. There was no change in the ligand binding for muscarinic acetylcholine receptors. On PND 30 male pups showed a significant increase in the AChE activity in the brainstem (~134-148% of control) and cerebellum (~299-345% of control) in all the treated groups. PND 30 females showed a significant increase in the AChE activity in the brainstem of chlorpyrifos alone group and in the cerebellum of the pups from the combination of nicotine and chlorpyrifos. A significant increase in the immunostaining for glial fibrillary acidic protein was observed in the cortex, the CA1 and CA3 subfields of hippocampus, and dentate gyrus on PND 30 in the pups from nicotine and chlorpyrifos treated mothers. These data suggest that maternal exposure during the entire gestational period to low doses of nicotine and chlorpyrifos, in combination, may lead to neuropathological changes in different brain regions. Supported, in part by an EPA grant # R829399-01-0.

617 MULTIGENERATIONAL IN-LIFE DATA IDENTIFY TARGET ORGANS/SYSTEMS OF RATS EXPOSED TO CHLORDANE AT ENVIRONMENTAL LEVELS.

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Technical grade chlordane (TGC), a pesticide mixture of four main isomers (heptachlor, cis-chlordane, trans-chlordane, and trans-nonachlor), banned in 1988 is still persistent in the environment. Target organs/systems for long-term exposure of TGC at environmental levels used in this study are unknown in humans and other mammals. Sprague-Dawley rats (F0, F1 F2) were exposed to continuous levels of TGC at 0.0, 0.125, 0.25 and 1.0 mg/kg body weight/day by oral gavage and reproductive, developmental and neurobehavioral endpoints were used to identify in-life target organs/systems of TGC toxicity. TGC increased ($P<0.05$) F1 males and females pre-mating body weight at 0.25 and 1.0, and feed intake at 0.25 mg/kg. TGC increased F1 live male/female sex ratio; male anogenital distance at 0.125 & 0.25 mg/kg for F1 and 0.25 &1.0 mg/kg for F2, and pre-weaned body weight of F2 females on PND7 and PND21. TGC at 0.25 mg/kg advanced eye opening, incisor eruption, and preputial separation in F2 rats. Also, at 1.0 mg/kg incisor eruption was advanced in of F2 rats. Surface righting increased at 0.25 mg/kg for F2 females on PND3. TGC delayed negative geotaxis in F1 males at 0.125 on PND7 and 1.0 on PND8-9; F1 females at 0.125, 0.25 and 1.0 mg/kg on PND8; and F2 females at 0.125 on PND11. Percent success in olfactory discrimination was reduced at 0.125 mg/kg for F1 females on PND9. Data identified the reproductive system, developing neonates and the CNS as in-life targets for oral TGC exposure levels in this study. (Supported by MHPF/ATSDR - #U50/ATU398948)

618 *IN VIVO* ETHANOL DECREASES PHOSPHORYLATED MAPK AND P70S6 KINASE IN THE DEVELOPING RAT BRAIN.

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Exposure to ethanol during pregnancy is detrimental to fetal development, and individuals affected by the Fetal Alcohol Syndrome present a number of central nervous system dysfunctions including microencephaly and mental retardation. Recently, it has been suggested that ethanol-induced inhibition of glial cell proliferation may be relevant in the causation of microencephaly. In this study, we measured the developmental changes of MAPK(ERK1/2) and p70S6 kinase, which are considered to play a prominent role in cell proliferation, and their phosphorylated proteins in brain, and examined the effects of *in vivo* ethanol administration. MAPK and phospho-MAPK increased gradually after birth, and reached adult levels on postnatal day 21. In contrast, levels of both p70S6 kinase and phospho-p70S6 kinase decreased after birth. Exposure to ethanol (2-6 g/kg, from postnatal day 4 to 7) had no effects on MAPK or p70S6 kinase levels, but caused a dose-dependent decrease of both phospho-proteins. These results suggest that phosphorylation of MAPK and p70S6 kinase may represent relevant targets for the developmental neurotoxicity of ethanol, and may involved in microencephaly.

619 EFFECTS OF REPEATED DEVELOPMENTAL EXPOSURE TO CHLORPYIFOS AND METHYL PARATHION ON CHOLINE ACETYLTRANSFERASE AND MUSCARINIC RECEPTORS IN RATS.

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The effects of early post-natal oral exposures to two organophosphorus insecticides on cholinergic parameters in whole brain and brain regions were investigated. Chlorpyrifos, a diethyl phosphorothionate, and methyl parathion, a dimethyl phosphorothionate, were studied. Incremental daily exposure regimens were used from post-natal day (PND)1 to 21: chlorpyrifos, low dosage, 1 mg/kg PND 1-21; medium dosage, 1 mg/kg PND 1-7, 2 mg/kg PND 8-14, 4 mg/kg, PND 5-21; high dosage, 1.5 mg/kg PND 1-7, 3 mg/kg PND 8-14, 6 mg/kg PND 8-14; methyl parathion, low dosage, 0.2 mg/kg PND 1-21; medium dosage, 0.2 mg/kg PND 1-7, 0.4 mg/kg PND 8-14, 0.6 mg/kg PND 15-21; high dosage, 0.3 mg/kg PND 1-7, 0.6 mg/kg PND 8-14, 0.9 mg/kg PND 15-21. Cholinesterase (ChE) activity was measured in freshly thawed and ground tissue with a 5-minute continuous assay because of the rapid reactivation of the dimethyl phosphorylated ChE. During the time of treatment, animals from the high dosages displayed about 60% brain ChE inhibition, about 50% for the medium dosage, and about 20% for the low dosage. Muscarinic receptor densities, as monitored with 3H-quinuclidinyl benzilate, were reduced about 20% during the times of greatest ChE inhibition in the whole brain preparations, with M1/M3 receptor levels [monitored with 3H-4-diphenylacetoxy-N-(2-chloroethyl) piperidine] affected more greatly than M2/M4 receptor levels (monitored with 3H-AF-DX-384); receptor densities were the same as controls after the cessation of treatment. Choline acetyltransferase activity, monitored radiometrically, showed a slight decrease after the cessation of treatment at PND 30. The minor reductions in choline acetyltransferase activity observed at PND 30 were not observed in brain regions at PND 40. The changes in receptors and enzyme activities observed during development did not appear to be permanent alterations of brain neurochemistry. (Supported by NIH R01 ES 10386).

620 BRAIN β1 INTEGRIN PROTEIN EXPRESSION IN RAT PUPS EXPOSED TO MONOMETHYLTIN (MMT).

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Integrins are cell adhesion proteins composed of α and β protein subunits. These heterodimeric transmembrane proteins have been shown to be important in a number of critical functions during brain development, including cell migration, axonal elongation and myelination. As such they have the potential to act as markers of effect for developmental neurotoxicants. MMT is one of a family of organotins used as stabilizers in plastics' manufacture. Evidence exists that these compounds leach into drinking water from PVC and CPVC pipes and potentially represent a health risk. Cognitive deficits have been reported in rat pups exposed during gestation and early postnatal development to MMT (12-120 mg/L) in drinking water in an early study by Noland, et al. (1982). However, no NOEL (no-observable effect level) was reported and these data have not been reproduced. This study seeks to confirm and expand the findings of the Nolan study. Long-Evans rat dams were exposed to 0, 10, 50 or 245 mg MMT/liter in drinking water from 2 weeks prior to mating to pup weaning at 21 days postpartum. On postnatal day 22 rat pups were weighed, anesthetized, and brains removed, weighed and different brain areas dissected. Tissues were extracted for total protein and Western analysis carried out for β1 integrin subunit. No differences were found in any dosing group in body or brain weights. A non-significant decrease (15-20%) was seen in β1 integrin subunit protein expression in the hippocampus and cerebellum at the two higher doses with no difference in the frontal cortex. This abstract does not necessarily reflect USEPA policy.

621 DEVELOPMENTAL EXPRESSION, PHOSPHORYLATION AND BINDING ACTIVITY OF CREB IN LEAD EXPOSED RAT BRAIN.

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Developmental lead exposure results in learning and memory deficits in children. The NMDA receptor is a molecular target for lead effects and its activation is essential for the transcription of genes important for learning and memory. A crucial

nuclear protein involved in this process is the cyclic-AMP response element binding protein (CREB) which is phosphorylated by calcium-sensitive kinases and initiates transcription by binding to the cyclic AMP response element (CRE) in the promoter region of target genes. We recently reported that adult rats exposed to lead during development express altered CREB phosphorylation (Toscano et al, Toxicology Sciences 60 (1), 187, 2001). In the current study, developing rats exposed to lead were used to assess CREB expression, phosphorylation and binding activity in cortical and hippocampal nuclear extracts at postnatal (PN) day 7, 14, 21 and 50. Further, we developed a novel filter binding assay that provides a quantitative measure of the interaction of CREB in nuclear extracts with a CRE containing oligonucleotide. In control rats, CREB and phospho-CREB (pCREB) expression is highest at PN7 and decreases steadily until PN 50. Developmental lead exposure does not affect total CREB levels in either the cortex or hippocampus but decreases pCREB levels at PN14 and 50 in the cortex and at PN50 in the hippocampus. Electrophoretic mobility shift assay (EMSA) demonstrates a decrease in CREB binding activity in the hippocampus at PN 7 and 14 and an increase at PN50. No changes in CREB binding activity were observed in the cortex at PN7, 14 or 21 but binding was decreased at PN50. Our novel filter binding assay will allow us to further characterize the EMSA results by allowing quantification of Bmax and Kd for the CREB-CRE interaction. These disruptions in pCREB expression and binding activity during the ontogeny of the rat brain begin to decipher the effects of lead downstream from the NMDA receptor. [Supported by grant #ES06189 to TRG]

622 ACUTE ETHANOL EXPOSURE INCREASES CATALASE ACTIVITY, BUT DOES NOT ALTER GLUTATHIONE LEVELS IN POSTNATAL DAY 4 RAT PUPS.

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Rats exposed to ethanol show selective cerebellar cell loss when the exposure occurs during postnatal days 4-6 (PD4-6), an effect not seen in other brain regions during this developmental period. While the exact mechanisms underlying vulnerability to ethanol-induced cell loss are unknown, evidence suggests that oxidative stress may play a role in ethanol pathogenesis. Recent work has shown that vitamin E supplementation provides protection against ethanol toxicity. In addition, studies measuring endogenous antioxidant levels following *in utero* ethanol exposure indicate significant ethanol-induced alterations in glutathione, superoxide dismutase, catalase, and vitamin E levels. The purpose of the current study was to investigate ethanol's effects on antioxidant levels following a single day of ethanol exposure during a period of development known to be vulnerable to ethanol-induced cerebellar cell death. PD4 Long Evans rat pups were matched for sex and rats within each pair received either sham intubations or 5.25g/kg ethanol (11.9% (v/v) solution in milk formula) in 2 intubations, 2 hrs apart. At 0, 4, 6, and 12 hrs after the initial intubation, the cerebellum, hippocampus, cortex, and liver were collected and assayed for either reduced glutathione levels or catalase activity (only cerebellum and hippocampus). Ethanol treatment resulted in a linear increase in catalase activity over time in the cerebellum [$F(1, 13)=9.506$, $p<0.01$] and the hippocampus [$F(1, 13)=6.010$, $p<0.05$]. Cerebellar catalase activity at 12 hrs was significantly higher in the ethanol group than the sham group ($t(3)=3.593$, $p<0.05$). In contrast, acute ethanol exposure on PD4 had no effects on glutathione levels. While these data do not rule out other mechanisms for cell loss, they do suggest a role for oxidative stress in ethanol-induced cerebellar cell death.

623 SUCCIMER CHELATION SIGNIFICANTLY AMELIORATES THE LASTING COGNITIVE AND AFFECTIVE DYSFUNCTION PRODUCED BY EARLY LEAD EXPOSURE IN A RODENT MODEL.

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Early lead exposure has been shown to cause changes in cognitive and affective functioning that last long past the period of exposure. The present study was designed to assess the efficacy of succimer chelation in alleviating these lasting areas of dysfunction. A 2 X 3 factorial design was used, including three levels of lead exposure (no lead, low lead, high lead) and two succimer conditions (succimer or placebo). Lead exposure was started at birth and continued until postnatal day 30. Succimer chelation was initiated immediately thereafter and lasted for 21 days. In adulthood, 120 rats (20 per group) were tested on a battery of tasks, designed to tap various aspects of cognition and emotional reactivity. First, early lead exposure produced lasting cognitive and affective changes that varied with the intensity of lead exposure. Specifically, both lead exposure groups showed deficits on learning, whereas rats in the higher exposure group showed additional dysfunction in attentional function and reactivity to committing an error on the previous trial. Second, succimer treatment prevented some, but not all, of these lead-induced alterations. Specifically, succimer ameliorated dysfunction in associative function and reactivity to errors in both groups, but to a greater extent in the lower exposure group. In contrast, succimer did not significantly ameliorate the attentional dysfunction seen in the higher exposure group. Finally, this study provided evidence that succimer treatment alone, in the absence of lead exposure, is not benign. Rats exposed to succimer, but not lead, showed deficits in learning and attention. These findings provide encouragement for the clinical use of succimer in ameliorating lead-induced cognitive and affective deficits, but urge caution in the widespread use of the drug in the absence of lead exposure or at very low levels of exposure. Supported by NIEHS grants ES07457, ES05950 & DK07158-27.

624 INFLUENCE OF CAGE DESIGN ON MEASUREMENT OF MOTOR ACTIVITY IN PRE-WEANLING RAT PUPS.

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The purpose of this investigation was to optimize cage design for the detection of motor activity in pre-weanling rat pups. Motor activity was measured in pups on postnatal days 13, 17, and 21 in one of 4 cage types using a Coulbourn®automated motor activity system. Ten male and 10 female rat pups were treated with the vehicle, 80 mg/kg carbaryl, or 2 mg/kg amphetamine approximately 30 min prior to motor activity measurement. All 4 cages were stainless steel wire mesh. The 4 cage types were: 1)the standard adult cage (16" x 10" x 6") with the infrared sensor positioned 3" above the cage floor; 2)the adult cage with the infrared sensor in a lower position (located 0.5" above the cage floor); 3)a modified adult cage containing a semicircular insert in the rear of the cage and a semicircular insert in front of the infrared sensor (located 0.5" above te cage floor); and 4)a mouse cage (4" x 7" x 5") with the sensor 0.5" above the cage floor. Cage size, shape, and sensor height affected the total motor activity data (duration of movement and number of movements). Carbaryl decreased motor activity in day 13, 17, and day 21 pups. Increased motor activity was observed in day 21 pups treated with amphetamine; however, motor activity in day 13 and 17 pups was decreased or unchanged by amphetamine. The mouse cage provided the best results for day 13 and 17 pups, and the standard cage with the sensor in a lower position provided the best results for day 21 pups.

625 MATERNAL AND FETAL ACETYLCHOLINESTERASE ACTIVITIES AFTER REPEATED DERMAL EXPOSURE OF PREGNANT RATS TO METHYL PARATHION.

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It is unclear if exposure to organophosphates such as methyl parathion during pregnancy affects cholinergic activity in the fetus and neonate. Methyl parathion has been noted to cross the placenta after oral or IP administration and, in some cases, was associated with physical anomalies in the fetus. Since dermal exposure can cause more prolonged toxicity, it is interesting that after a single dermal administration of radiolabeled methyl parathion to pregnant rats, radioactivity was detected in the fetus within 2 h and disappeared with a half-life of 22 h. One aim of the present studies was to determine if repeated dermal exposure of pregnant rats to methyl parathion adversely affects the fetus. Dames were treated dermally with methyl parathion (1 or 2 mg/kg/day) beginning on gestation day 3-5 and ending on gestation day 18-19. Acetylcholinesterase activities in maternal blood and cerebral cortex, placenta, and fetal cerebral cortex were assayed as indices of toxicity. Administration of methyl parathion caused dose-dependent increases in blood concentrations in the dame (16.1±6.6 and 98.5±11.1 ng/ml; mean±SE) that were associated, respectively, with 25 % and 60% decreases in blood (control = 31.1±4.1 nmol/10 min/mg Hb) and cortical cholinesterase (control = 87.6±7.1 nmol/min/mg prot) activities. Placental cholinesterase activity was not affected at the lower dose of methyl parathion, but was decreased 30 % by 2 mg/kg/day. However, neither dose affected cholinesterase activity in the cerebral cortex of the fetus. Also, neither methyl parathion or its active metabolite, methyl paraoxon, were detected in the fetus. These data indicate that the placenta can protect the fetus from toxicity resulting from repeated low-level dermal exposure (1-2 % of the dermal LD50) to methyl parathion and perhaps other organophosphorus agents. The contributions of placental acetylcholinesterase and placental metabolism to the inactivation of methyl parathion or methyl paraoxon remain to be defined. (CDC Grant R06/CCR419466).

626 ASSESSING NEURODEVELOPMENTAL EFFECTS OF ENVIRONMENTAL EXPOSURES TO ANTI-THYROID AGENTS: HOW RELEVANT ARE HIGH DOSE RAT STUDIES?

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Substantial interest has arisen regarding the possibility of neurodevelopmental effects following low-level environmental exposures to chemicals that can have antithyroid effects. In studies of rats, maternal exposure to agents that cause severe hy-

pothyroidism, including propylthiouracil (PTU) and methimazole (MMI), has produced a range of biochemical, neurodevelopmental, and neurobehavioral outcomes in offspring. However, debate continues about the threshold at which these effects occur, the relevance of these studies to low dose environmental exposures, and in turn, the biological significance of small differences in these measures when seen at environmentally-relevant doses. We present a review of the published literature on biochemical/molecular, behavioral, and morphological endpoints in rat offspring *in vivo* when dams are treated through pregnancy with known anti-thyroid agents. Our findings reveal: the preponderance of studies have examined animals induced with severe hypothyroidism (*e.g.*, thryoxine [T_4] levels of 50% or more below control values); timing of exposure and age of testing significantly impact reported results; the most sensitive behavioral events appear to be auditory effects; at high doses an apparent morphological effect is a decrease in migration of external granule cells to the internal granular layer of the brain; and the few relatively lower dose studies suggest effect thresholds consistent with at least moderate or more significant hypothyroidism. Interpretative problems in assessing hazards associated with environmental exposures arise when small differences in these measures are reported at very low environmental doses and/or corresponding to small or transient changes in thyroid hormone or thyroid stimulating hormone (TSH) levels.

627 A ZEBRAFISH MODEL FOR STUDYING THE NEUROBEHAVIORAL IMPACTS OF DEVELOPMENTAL CHLORPYRIFOS EXPOSURE.

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Chlorpyrifos is a widely used insecticide, which interferes with neurobehavioral development. Rat models have been key in demonstrating that prenatal chlorpyrifos exposure causes learning deficits and motor alterations, which persist into adulthood. Complementary non-mammalian models can be useful in determining the molecular mechanisms underlying these persisting behavioral effects. Zebrafish with their clear chorion and extensive developmental information base provide an excellent model for assessment of molecular processes of toxicant-impacted neurodevelopment. We have developed methods for assessing spatial discrimination learning in zebrafish, which can differentiate response latency from choice accuracy in a three chambered fish tank. Low and high doses of chlorpyrifos (10 and 100 ng/ml) were administered to zebrafish embryos on days 1-5 post-fertilization. The 100 ng/ml dose caused significant short-term slowing of swimming activity on days 6 and 9 post-fertilization and had persisting effects of impairing spatial discrimination and decreasing response latency in adulthood. The 100 ng/ml dose (1-5 days post-fertilization) also significantly accelerated mortality rates of the fish during the adult study from 20-38 weeks of age. Developmental exposure to 10 ng/ml of chlorpyrifos did not cause a significant change in locomotor activity during the period soon after hatching but did cause a significant slowing of response in adulthood. This dose also caused a significant learning impairment in adulthood, but did not cause increased mortality. Chlorpyrifos exposure during early development caused clear behavioral impairments, which lasted throughout adulthood in zebrafish. The molecular mechanisms by which early developmental chlorpyrifos exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebrafish model. This research was supported by NIH grants #ES10387, ES10356 and ES9808.

628 THE MORRIS MAZE AS A TEST OF LEARNING AND MEMORY IN RATS - A CASE STUDY DEMONSTRATING THE VALUE OF RE-TESTING THE SAME SET OF ANIMALS.

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Female Han Wistar rats derived from mothers treated with a novel pharmaceutical or vehicle control were tested in the Morris water maze at Day 31±1 of age, as part of the requirement for functional testing of selected F1 animals on a study of pre- and post-natal development. The animals were placed individually in the maze which contained an escape platform at a fixed point in the circular pool. The platform was concealed below the surface of the water which had been made opaque using Opacifier 621. Each animal received 3 trials on each of 4 consecutive days of testing using standard methods. All groups of animals showed a consistent improvement in mean trial times and sector entries, but the performance of animals derived from parent females treated at 10 or 50 mg/kg/day was somewhat inferior to that of controls. Although performance was similar in all groups on Day 1 of testing, trial times and sector entries for offspring derived from treated parent females were greater than in controls on Days 2, 3 and 4. The same animals were re-tested, using identical test procedures, 18±1 day after Day 1 of the original test. Animals in all groups showed excellent recall of the first test, with performance on the first day being similar to that on the 3rd or 4th day of the first test. In addition, all groups of animals continued to show further improvements in performance over the 4 days of testing on the second occasion. The re-test demonstrated that animals derived from treated parent females had learnt the position of the escape platform by Day 4 of the original test and that they were able to recall this information on Day 1 of the second test (a useful measure of long-term memory). In conclusion, the results demonstrate the value of re-testing animals in the Morris maze subsequent to some deficits in performance being detected on a previous occasion.

629 ESTABLISHMENT AND PERSPECTIVES OF AN AGING PRIMATE COLONY FOR THE STUDY OF GERIATRIC DISEASES.

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According to a recent review, life expectancy of women is predicted to reach 100 years by 2050 (1) and the proportion of people older than 60 years is estimated to exceed 20% of the population by 2050. Inevitably, the prevalence of age-related diseases and deficiencies will gain further significance and the health status of the aged will have a great bearing on society and economy. This also presents a real challenge to industry for developing sufficient and appropriate medications for the elderly and the aged population. For the successful development of new therapies for geriatric diseases, relevant animal models will be of vital importance. The aim of the present work was to assemble a colony of aged nonhuman primates and to initiate a screening program for the prevalence of selected age-related diseases. Mauritian male and female cynomolgus monkeys (Macaca fascicularis) were retained as retired breeders (> 13 years old) from a breeding colony. Among 222 animals screened for fasted blood sugar levels, 16 females but no males had levels in the diabetic range. Among 56 females, 22 had blood estradiol levels clearly below normal or in the range of ovariectomized animals. Fundus examination of 50 animals for ocular pathology revealed abnormal findings in 37 animals. Screening for prostate-specific antigen in 56 males did not yield evidence for prostate pathology. In the cerebrospinal fluid of very old animals a clear increase of amyloid-beta-42 was detected. The screening program is still ongoing but our initial findings already suggest that aging cynomolgus monkeys can be used to develop models for spontaneous osteoporosis, diabetes and ocular disease and potentially also for Alzheimer disease. 1. Oeppen J, Vaupel JW (2002) Science 296 :1029-1031

630 COMPARISON OF DUAL-ENERGY X-RAY ABSORPTIOMETRY AND PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY FOR BONE MINERAL DENSITY ANALYSIS IN THE CYNOMOLGUS MONKEY.

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Bone density is a pivotal parameter utilized to monitor the effectiveness of osteoporosis therapeutics as well as the side effects of other pharmaceutical compounds. Dual-energy X-ray absorptiometry (DXA) is the standard technique for measuring bone mineral density (BMD). Peripheral quantitative computed tomography (pQCT) is a more recent development that allows separate determination of cortical and trabecular BMD in the peripheral skeleton. Controversies exists which technique, DXA or pQCT, might be preferable in future, and whether separate analysis of trabecular and cortical bone by pQCT will permit earlier detection of BMD changes than DXA technology. The purpose of this study was to compare both techniques by measuring BMD using pQCT and DXA in intact and castrated male and ovariectomized female cynomolgus monkeys (Macaca fascicularis), a frequently used primate model in osteoporosis research. The results indicate that pQCT seems to be more sensitive than DXA, since pQCT measurements revealed an decrease in BMD in ovariectomized females, whereas DXA measurements did not. However, in elderly animals sites other than femoral neck should also be measured, i.e. radius and/or proximal tibia/distal femur, when pQCT measurements are performed without any DXA measurements. These comparative measurements allow the conclusion that both techniques are suitable and that, whenever possible, both techniques should be used for BMD measurements in preclinical studies to optimize results.

631 IMPACT OF BODY WEIGHT (BW) CHANGE ON THE EDSTAC TIER I MALE AND FEMALE PUBERTAL PROTOCOLS.

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A primary concern with the Endocrine Disruptor Screening and Testing Committee's (EDSTAC) Tier I male and female pubertal protocol is that a nonspecific reduction in bw may potentially confound the interpretation of effects on the

endocrine endpoints of interest. To assess the relationship between bw and puberty, male and female Wistar rats were underfed to 90, 80, 70 and 60% of the ad libitum food consumption observed in controls. The females were underfed postnatal day (PND) 21-42. This regimen resulted in decreased terminal bw of 1.8, 4.1, 12.1, and 18.8% of the controls. Vaginal opening, uterine weight (wt) and thyroid hormone values were not altered in any of the underfed female groups. Although decreases in bw of 12.1 and 18.8% did significantly lower absolute ovarian wt, relative ovarian wt was not affected. Thus, there were no significant differences in any of the female reproductive endpoints measured in accordance with the female pubertal protocol. In the male, underfeeding from PND 23-53 resulted in terminal bw decreases of 2.0, 6.0, 9.0, and 19.5% of the controls. Preputial separation, epididymal or testes wts were not different from control in any of the underfed males. Although a decrease in the absolute wt of the ventral prostate and seminal vesicle was noted in those males with a 19.5% decrease in bw, the relative wts of these tissues were not different than controls. A decrease in thyroid stimulating hormone levels was observed in males with the 19.5% reduction in bw, while both T3 and T4 were significantly reduced in the males with a 9 and 19.5% reduction in bw. Importantly, there were no significant alterations of the male reproductive endpoints at less than 9.0% decreased bw. These data demonstrate that reduced bw over a range of 2-9% do not confound the reproductive endpoints in either pubertal protocols and that keeping the dose range below the MTD (defined as a 10% reduction in bw) will provide valid data concerning a compound's potential effect on these endocrine dependent events. (This abstract does not necessarily reflect EPA policy)

632 CALCIUM LIABILITY OF THE VITAMIN D ANALOG RO 65-2299 (BAL2299) IS INDEPENDENT OF THE DIETARY VITAMIN D3 INTAKE IN THE RAT.

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Calcium-related toxicity, especially hypercalcemia, is the dose limiting factor for vitamin D (VitD) analogs in repeated dose toxicity studies. We examined whether the calcium liability of the VitD analog Ro 65-2299 in rats can be counteracted by lowering VitD supplement in the diet. In a first experiment, female rats were fed either a VitD3 free diet or a diet containing 400 IU or 800 IU VitD3/kg during a 5-day treatment period with Ro 65-2299 at 0.25 and 0.5 mg/kg/day given orally by gavage. At both doses, Ro 65-2299 caused a significant increase in urinary Ca excretion and hypercalcemia. The effect on serum Ca was dose-dependent but independent of the diet. A slightly smaller effect on urinary Ca excretion was observed in rats fed either a VitD3 reduced or a VitD3 free diet at a dose of 0.5 mg/kg/day, but not at 0.25 mg/kg/day. In a second experiment, female rats were fed a VitD3 free diet for more than 5 weeks before start of treatment. Thereafter, the feeding and treatment regimen of the first experiment was repeated but only a dose of 0.25 mg/kg/day was applied. Similar to the first experiment, there were increases in serum Ca and urinary Ca excretion without any relevant differences between rats having received different VitD3 supplements. In conclusion, lower dietary VitD3 supplement failed to counteract Ca liability of the VitD analogue Ro 65-2299. The reduction of VitD3 intake does not allow the use of higher doses of VitD analogs in repeated dose toxicity studies.

633 BROWN ADIPOSE TISSUE PROLIFERATIVE RESPONSE TO XENOBIOTICS: THE NEGLECTED ENDOCRINE TISSUE.

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BAT controls thermogenesis in all mammalian species. Yet, BAT has not been routinely included in regulated safety assessment study protocols. Recent observations of proliferative and neoplastic changes of BAT in chronic toxicity studies have called attention to this neglected endocrine tissue. The present study is an attempt to characterize BAT responses to Phentolamine (PHEM). The finding that led to these investigations was the observation in the 24-mo. bioassay in S-D rats of hibernomas, mainly mediastinal, in males, in a comparable low incidence in all 3 dose groups. The tumor incidence was not related to dose or duration, despite the fact that there was dose-dispropor- tional high PHEM exposure in the high dose animals. The substance was negative in a 6-mo. p53-deficient heterozygous mouse model. To elucidate the pathogenesis of hibernomas, a one-year mechanistic study was conducted in younger (6 wk at start) and older rats (36 wk at start). The data obtained, including endocrine parameters, rectal temperature and PCNA, suggest that after chronic exposure in younger rats (15 mos old) and older rats (24 mos), PHEM induced sustained pharmacological effects which were reversible with 1 mo. recovery, indicating their adaptive nature. PHEM did not interfere with the rate of replication of BAT or adrenal cells. Thus, PHEM does not produce any BAT effect to lead to hibernomas. In addition, in both control and treated rats, the males have a higher UCP/Actin ratio value than females in both younger and older rats. Younger rats across all groups and genders have bigger vacuoles than older rats, and interscapular adipocytes have bigger vacuoles than either mediastinal or retroperitoneal adipocytes. Moreover, the rate of proliferation is at a steady state over time in all control groups. Thus, all these indices denote that the rate of BAT involution is very slow under normal or slightly hyperthermic conditions, and that BAT is very useful to monitor in safety assessment.

634 CYPROHEPTADINE ALTERS TRANSLATION INITIATION IN RINm5F CELLS.

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The antihistaminic, antiserotonergic drug cyproheptadine (CPH) is known to inhibit insulin synthesis *in vivo* and *in vitro*. This inhibition of insulin synthesis occurs without a commensurate decrease in preproinsulin mRNA (PPImRNA) levels, suggesting a post-transcriptional mechanism of action. Using a subcellular fractionation technique followed by real-time RT-PCR, we have previously shown that a translational event, characterized by a decrease in the percentage of cellular PPImRNA associated with endoplasmic reticulum (ER) bound polysomes and increases in the percentage of translationally uninitiated PPImRNA and the percentage of PPImRNA associated with monoribosomes, is involved in CPH induced inhibition of insulin synthesis. Experiments in this study were performed to further investigate the effects of CPH on translation initiation in RINm5F cells. Polysome profile analysis after a 2 hour 10 μM CPH treatment confirmed an increase in the monoribosome peak. Further investigation into the CPH induced initiation block was undertaken by examining the phosphorylation state of the translation initiation factors eIF4E, 4E-BP1 and eIF2α after CPH treatment. After a 2 hour treatment with 10 μM CPH, decreased phosphorylation of both eIF4E and 4E-BP1 was observed. Hypophosphorylation of both eIF4E and 4E-BP1 is consistent with decreased translation initiation. Further, a 24 hour treatment with 10 μM CPH resulted in increased phosphorylation of eIF2α, also consistent with a block of initiation and suggesting the possible involvement of the unfolded protein response in CPH induced β-cell toxicity. Taken together, these results suggest that the inhibition of insulin synthesis elicited by CPH treatment in RINm5F cells involves alterations of initiation factor phosphorylation leading to decreased initiation of PPImRNA.

635 ADRENOCORTICOTROPIC HORMONE-LOADING TEST IN CONSCIOUS CYNOMOLGUS MONKEYS.

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The adorenocorticotropic hormone (ACTH)-loading test is known to evaluate adrenal gland function. However, there were no comparative studies to evaluate the most appropriate administration route of ACTH. In previously conducted studies the animals were anesthetized. In this study, we conducted ACTH-loading in conscious cynomolgus monkeys *via* two routes, intravenous and intramuscular. Ten males and ten females, weighing 2.41 to 4.71 kg, were used. The animals received either 5 mcg/kg of tetracosactide acetate intravenously or 12.5 mcg/kg of tetracosactide zinc acetate intramuscularly. Half of the animals were initially administered ACTH intravenously and then intramuscularly at 3-day intervals. The other animals were initially administered intramuscularly and then intravenously at the same intervals as stated above. Serum samples for hormone analysis were collected periodically. Hormones analyzed were endogenous ACTH, cortisol and aldosterone. Endogenous ACTH decreased after administration by both routes, and recovered to basal levels after intravenous administration but not completely after intramuscular administration. Cortisol reached a maximum 2 hours after administration, and were approximately 1.5 to 2-fold basal levels after intravenous administration. Aldosterone reached a maximum 1 hour after administration, and were 2.5 to 4.5-fold of basal levels after intravenous administration. Conversely, cortisol and aldosterone peaks were lower and more prolonged after intramuscular administration. The 3-day interval diminished the reactivity of the adrenal hormones to exogenous ACTH. These results suggest that the effect of intramuscular ACTH-loading lasts longer than that by intravenous administration and the effect remains even after a 3-day interval. In conclusion, the intravenous ACTH-loading test is more appropriate than intramuscular administration. Furthermore, this test in conscious animals has the potential for inclusion as an evaluation parameter in general toxicity studies.

636 TH9507: EMBRYOFETAL AND FERTILITY STUDIES OF A GROWTH HORMONE-RELEASING FACTOR (GRF) ANALOGUE.

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TH9507 is a GRF analogue which has been stabilized by anchoring a hydrophobic moiety to the peptide, while preserving its amino acid sequence. TH9507 is in Phase II trials (doses range from 1.7 to 33.3 µg/kg) for multiple indications (muscle wasting in COPD, recovery after hip fracture surgery, sleep maintenance insomnia and immune dysfunctions). The mode of action involves induction of growth hormone (GH) release, followed by GH-induced release of Insulin-like Growth Factor–1 (IGF-1). Acute and sub-chronic toxicology studies have permitted continuation of clinical trials and helped establish doses for the rat embryofetal & fertility studies reported below. A SC range-finding embryofetal study was conducted in rabbits at 100 to 600 µg/kg/day (from gestation Day 7 to 19). In-life and Day 29 uterine and fetal examinations revealed no evidence of maternal toxicity, embryolethality, fetotoxicity or teratogenicity at any dose level. A subsequent definitive embryofetal development study in rabbits employed doses of 100 to 600 µg/kg/day and confirmed these results. A fertility and embryofetal development study was also conducted in rats. F0 males & females were treated at 0, 100, 300 or 600 µg/kg/day. Males were treated for at least 28 days prior to mating and throughout mating until necropsy. Females were treated for 14 days prior to mating, during mating and up to gestation Day 17, inclusive. F0 males & females exhibited dose-dependant increases in body weight and food intake which were attributed to pharmacological effects of TH9507. No adverse effect on F0 male & female reproduction was observed and there were no major external, internal or skeletal malformations in the F1 population. At 600 µg/kg/day increased fetal weight, accompanied by indications of advanced ossification, may have been related to the maternal effects.

637 NON-SPECIFIC ALTERATION OF STEROIDOGENESIS IN MA-10 LEYDIG CELLS BY SUPRA-PHYSIOLOGICAL CONCENTRATIONS OF THE SURFACTANT IN ROUNDUP® HERBICIDE*.

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A decrease in progesterone synthesis in MA-10 Leydig cells stimulated with a cAMP derivative and treated with supra-physiological concentrations of Roundup led Walsh, *et al.* 2000 to suggest that Roundup had endocrine disrupting potential. The authors demonstrated that the formulation, not the active ingredient, was responsible for the concentration-dependent but reversible decline in progesterone synthesis. We repeated this experiment with the inclusion of a sensitive cytotoxicity assay visualizing the loss of mitochondrial potential using fluorescent cationic JC-1 dye. It was shown that the concentration-dependent decrease in progesterone synthesis in hCG-stimulated MA-10 cells resulted from mitochondrial membrane damage by the polyethoxylated tallowamine (POEA) surfactant in Roundup. Decreased hormone-stimulated progesterone synthesis in MA-10 cells was only demonstrated at concentrations of POEA that caused loss of mitochondrial potential. In a separate set of experiments, a number of cationic (benzalkonium chloride), anionic (lauryl sulfate, linear alkyl benzene sulfonate) and non-ionic (alcohol ethoxylates) surfactants commonly found in household products were tested. It was found that all of these surfactants produced a comparable decrease on hCG-stimulated progesterone synthesis in MA-10 cells, at and below concentrations caused by Roundup. The results of this study underscore (1) the non-specific action of a variety of surfactants on cellular function in an *in vitro* test system and (2) how this secondary activity can confound the results when surface active agents are used in *in vitro* test systems. Based on routes and estimates of human exposure to Roundup from agricultural and residential uses, it is apparent that POEA will not disrupt testicular steroid synthesis *in vivo*. * *Roundup herbicide* refers to Roundup Concentrate Weed and Grass Killer (180 g/L).

638 EVALUATION OF THE MALE PUBERTAL ASSAY TO DETECT EFFECTS OF p, p'-DDE AND KETOCONAZOLE IN CD RATS.

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The male pubertal assay has been recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as an alternative Tier I screening assay to detect potential endocrine-active chemicals. The present study was performed to evaluate the reliability of this assay. Two dosing regimens (20- and 30-day) were used to confirm and extend previous works on the assay's ability to detect a weak androgen receptor antagonist (p, p'-DDE, experiment 1) and steroid synthesis inhibitor (ketoconazole, experiment 2). In experiment 1, weanling male rats were dosed by gavage from day 22 to 41 of age (20-day treatment) or day 23 to 52 (30-day treatment) of age with vehicle (corn oil) or p, p'-DDE (100mg/kg/day). In experiment 2, male rats were dosed by gavage from day 21 of age (20-day treatment) or day 31 of age (30-day treatment) to 50 days of age with vehicle (0.25% methylcellulose) or ketoconazole (25 mg/kg/day). p, p'-DDE was positive for antiandrogenic effects, evidenced by decreases in androgen dependent tissue weights, and delayed preputial separation (PPS, 30-day dosing only). Organ weight changes after 20- and 30-day dosing were equivalent. Although a tendency for decreases in the weights of some androgen dependent tissues and serum testosterone levels were observed after 30-day dosing of ketoconazole, none achieved statistical significance. The 30-day dosing of ketoconazole resulted in a delay in PPS and a temporary decrease of anogenital distance. These findings suggest that this assay is reliable for detecting androgen receptor antagonists and steroid synthesis inhibitors if suitable protocol is used.

639 COMPARISON OF THE EFFECTS OF TWO AR ANTAGONISTS ON TISSUE WEIGHTS AND HORMONE LEVELS IN MALE RATS AND ON EXPRESSION OF THREE ANDROGEN DEPENDENT GENES IN THE VENTRAL PROSTATE.

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The fungicides vinclozolin (V) and procymidone (P) are AR antagonists that alter androgen-dependent genes *in vitro* and induce antiandrogenic effects *in vivo*. V has been shown to alter AR-dependent genes; increasing TRPM-2 (testosterone repressed prostatic message) and repressing C3 (prostatein subunit C3) mRNA levels *in vivo*. The goal of this study was to determine if adult male rats exposed to P display effects similar to V on TRPM-2, C3 and AR message levels using real time reverse transcription polymerase chain reaction (QPCR). An extension of this study using microarray analysis is presented separately (Rosen, et al., 2003 SOT). Castrated SD rats (72) were assigned to treatments: corn oil vehicle + testosterone implant (T), oral V (200 mg/kg/d) + T implant (VT), oral P (200 mg/kg/d) + T implant (PT) and vehicle with an empty implant (Oil). Rats were necropsied after 20 hr, 4 days, and 7 days. Blood was collected for T and LH, organs were weighed and a portion of the ventral prostate (VP) was fixed for immunohistochemistry. RNA was extracted from ventral prostate (VP) tissue and TRPM-2, C3, and AR quantified using QPCR. As expected, castrate (Oil), VT, and PT decreased androgen dependent tissue weights and increased serum LH. Serum T levels did not differ among the T, VT and PT groups. TRPM-2 was increased at all time points in Oil, VT and PT groups versus the T controls. At 7 days, C3 mRNA levels were reduced in the Oil, VT and PT groups (significant for Oil only). In contrast to the AR mRNA levels, which were elevated, immunohistochemical evaluation indicated that AR protein was reduced by castration. These results indicate that VT and PT produce nearly identical morphological, endocrine and molecular alterations of androgen-dependent processes, which are similar to, but less robust than those produced by elimination of T by castration. Abstract of a proposed presentation and does not necessarily reflect EPA policy.

640 INCREASED EXPRESSION AND ACTIVATION IN ADULTHOOD OF CASPASE-3 AND -6 IN RAT GERM CELLS EXPOSED *IN UTERO* TO FLUTAMIDE.

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In utero exposure to the antiandrogen flutamide induces a wide range of abnormalities in the adult male reproductive system including moderate to severe hypospermatogenesis. However, the cellular and molecular mechanisms underlying this testicular effect remain to be investigated. In this study where pregnant rats were treated with flutamide (0.4, 2, 10 mg/kg/day) by gavage, the resulting hypospermatogenesis observed in the adult male rats (which have been exposed *in utero*) was found to be related to an increase in apoptosis in germ cells. In addition, the number of apoptotic germ cells visualized by the TUNEL approach was increased with increasing concentration of flutamide. The chief effectors of the apoptotic cell death pathway are the caspases which are synthesised as inactive (pro-caspase) precursors and which are then cleaved to generate active enzymes. Pro-caspases-3 and 6 were immunodetected in Leydig and germ cells in both untreated or flutamide-exposed rats while cleaved active caspase-3 was immunodetected exclusively in

germ cells from *in utero*-exposed rats. No immunoreactive active caspase-3 was detected in control adult rat testes. Caspase-3 and -6 mRNA and pro-caspase-3 and -6 protein levels were increased in the adult rat testes exposed *in utero* to flutamide in a dose-dependent manner. Western blots analyses confirmed that *in utero* exposure to flutamide also induced effector caspase activation since cleaved active caspase-3 and -6 proteins were absent in control testes whereas they were detected in *in utero* exposed testes and their levels were increased with increasing concentration of flutamide. In summary, the present findings suggest that the hypospermatogenesis induced by an *in utero* exposure to the antiandrogen flutamide is related to a sustained increase in germ cell apoptosis which could be due to a permanent increase in the expression and activation in germ cells of two effector caspases, namely caspase-3 and -6.

641 *IN VITRO/IN VIVO* EVALUATION OF THE ANTI-ANDROGENIC ACTIVITY OF BENZO(a)PYRENE AND METHOXYCHLOR.

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The androgen receptor (AR) transactivation and Hershberger assays are currently being developed for large scale screening of chemicals for endocrine activity. The goal of this study was to evaluate the correlation between the *in vitro* and *in vivo* anti-androgenicity of two compounds (methoxychlor [MXC] and benzo(a)pyrene [BAP]) previously reported to exhibit anti-androgenic activity. For the AR transactivation assay, AR(+) LNCaP cells were transfected with an inducible luciferase reporter construct with concatenated androgen-responsive elements (pGudLuc7ARE) and exposed for 24 h to concentrations of MXC and BAP up to 10 uM in the presence and absence of 1 nM of the AR agonist, R-1881. Neither MXC nor BAP produced significant agonist activity up to the concentrations evaluated. However, both BAP and MXC produced significant anti-androgenic activity *in vitro* as evidenced by their inhibition (37% and 88%, respectively) of the response to R-1881 at the highest concentrations tested. For assessment of anti-androgenic activity *in vivo*, the Hershberger assay utilized a 10-day oral gavage-dosing regimen in castrated CD rats, necropsied 24 h after the final dose of test material. MXC (10, 50, 100 and 200 mg/kg/day) or BAP (1, 10, 50 and 100 mg/kg/day) were dosed (8 animals/group) in the presence of 0.4 mg/kg/day of testosterone propionate (TP) with 0.5% methylcellulose as the vehicle. Neither compound produced significant decreases in ventral prostate, seminal vesicle, glans penis, Cowper's glands or levator-ani muscle weights relative to the TP control. However, the highest doses of MXC and BAP resulted in statistically significant decreases in body weight (p=0.019) and increased liver weight (p=0.003), respectively, relative to TP controls. These limited data demonstrate poor correlation between the *in vitro* and *in vivo* end points evaluated for anti-androgenic activity and further stress the importance of a weight of evidence approach in assessing endocrine activity of test materials.

642 RAINBOW TROUT ANDROGEN RECEPTOR ALPHA AND HUMAN ANDROGEN RECEPTOR: COMPARISONS IN THE COS WHOLE CELL BINDING ASSAY.

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Typically, *in vitro* hazard assessments for the identification of endocrine disrupting compounds (EDCs), including those outlined in the EDSTAC Tier 1 Screening (T1S) protocols, utilize mammalian receptors. However, evidence exists that fish sex steroid hormone receptors differ from mammalian receptors both structurally and in their binding affinities for some steroids and environmental chemicals. Most of the binding information available to date comes from studies conducted using cytosolic preparations. We sought to compare competitive binding using rainbow trout androgen receptor alpha (rtAR) and human androgen receptor (hAR) expressed in transfected COS cells. In this system, we can investigate the binding affinities of individual receptors without the potentially confounding effects of other steroid receptors present in cytosolic tissue extracts. Saturation ligand binding and Scatchard anaylsis using [3H]R1881, a synthetic androgen, revealed a Kd of 0.24 nM for the rtAR. In the same system, we found a Kd of 2.27 nM for the hAR. Binding studies in competition with [3H]R1881 were conducted using steroids and a selection of environmental chemicals shown to bind mammalian AR. All the chemicals and steroids studied competed for binding in both rtAR and hAR. The relative order of binding affinities of natural and synthetic androgens for the rtAR was methyltrienolone > trenbolone > 11-ketotestosterone > dihydrotestosterone(DHT) > testosterone(T) > androstenedione. The rank order for the hAR was similar except that DHT and T had higher affinity than 11-ketotestosterone. We also found in our system that androstenedione bound with lower affinity than what has been reported in the literature for the rtAR. Other steroids and antiandrogens were studied and their relative binding order was similar for the two species. Studies such as these will facilitate the identification of EDCs that affect many species and support future risk assessment protocols. [This abstract does not necessarily reflect EPA policy.]

643 INVESTIGATION OF HORMONAL CHANGES IN TESTICULAR TOXICITY INDUCED BY NEFIRACETAM IN DOGS.

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Nefiracetam, a neurotransmission enhancer, induces testicular toxicity in rats, dogs and monkeys. In rats, nefiracetam decreased serum and testicular testosterone (T) levels 1-4 hours after single or 1-week repeated oral administration, but its reduction returned to the control level 8 hours later. We, therefore, considered that the transient decrease in the T level contributes in part to the testicular toxicity. In the present study, to know whether the testosterone level could be decreased in dogs and to consider the mechanism of the decreased T level, male adult beagle dogs were orally administered 300 mg/kg/day nefiracetam singly, or once daily for 1 or 4 consecutive weeks. Serum levels of T, estoradiol (E), luteinizing hormone (LH), follicule stimulating hormone (FSH) and inhibin B were measured every week during the 4-week administration period. After completion of the administration, the testis was histologically examined, and serum and testicular levels of T, E and progesterone (P) were also measured. As results, after the single administration, T level was decreased both in serum and the testis, with an increased serum P level and decreased testicular E level. After the 1-week administration, T level was also decreased in serum and the testis, and E level was increased and decreased in serum and the testis, respectively. However, testicular P level was not changed after the single or 1-week administration. During the 4-week administration, T level in serum was decreased after 1- and 2-week administration, but not later, while E level was continuously increased during the 4-week administration. There was no change in the other hormone levels. Histologically, atrophy of seminiferous tubules containing multinucleated giant cells was observed in the testis. In conclusion, the present results suggest that the decreased T level may be caused by impaired conversion of P to T in Leyding cells, and that the decreased T level may contribute to the testicular toxicity in the early stage.

644 EFFECT OF ETHYL *t*-BUTYL ETHER (ETBE) ON TESTOSTERONE IN MALE RATS.

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Seminiferous tubule degeneration was observed in F344 rats after 13-week inhalation exposure to 1750 or 5000 ppm ETBE, a motor fuel oxygenate. Noting that the testis is also affected by MTBE, which is reported to have increased rat Leydig cell tumors when high doses were given by gavage or inhalation in cancer bioassays, we undertook the current studies to determine whether ETBE alters testosterone (T) levels by mechanisms similar to those studied previously with MTBE. A common mechanism was also suggested by the metabolism of both to t-butyl alcohol (TBA). Leydig cells were isolated from adult Sprague-Dawley rats and exposed to ETBE, MTBE, TBA and acetaldehyde (ACET), another metabolite of ETBE, with/without hCG stimulation for 3 hr. Formaldehyde (FORM), a metabolite of MTBE, was added to some cultures as formalin, controling for 10% methanol also present in this formulation. When potencies were compared, 50 mM concentrations of ETBE, MTBE or TBA inhibited hCG-stimulated T production to 67%, 69% and 57% of control, respectively. As little as 5 mM ACET reduced T to 44% of control, while 5% FORM completely inhibited T production (10% methanol had no effect in this system). ETBE effects on T were also studied in adult male F344 rats gavaged daily with 600, 1200 or 1800 mg/kg in corn oil for 14 days (n=12). 1800 mg/kg doses reduced mean serum T to 66% of corn oil control by necropsy 1 hr after the final dose; however, there was considerable individual variability in response (p>0.05). Suppressed body weight gain in this group may have exacerbated this effect. Testes and accessory sex organ unit weights also suggested a slight dose-related decline, although not statistically significant after this 14-day dosing period. Taken together these results suggest that high concentrations of ETBE (and metabolites) are capable of lowering testosterone if exposure is sufficiently high. The potency of ETBE in these studies was no greater than that seen previously with MTBE, yet their different metabolites may ultimately produce different effects *in vivo*.

645 ORGANOTIN COMPOUNDS ALTER ENDOCRINE FUNCTIONS OF HUMAN PLACENTAL CELLS.

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Tributyltin (TBT) and triphenyltin (TPT) are known to induce an irreversible sexual abnormality of female neogastropod snails which is termed "imposex". However, in humans, no studies have investigated disturbances in sexual development and reproductive function as a result of exposure to organotin compounds. The placenta plays a vital role in the maintenance of a pregnancy by delivering oxygen and nutrients from the maternal circulation to the fetus and by returning fetal metabolites to the mother. The placenta also has many crucial endocrine functions such as human chorionic gonadotropin (hCG) production and the aromatization of androgen substrates to estrogens. Thus these placental functions might be at high risk due to the developmental and reproductive toxicology of environmental contaminants which have endocrine-disrupting effects. In the present study, to extend knowledge on the sexual developmental and reproductive toxicity of organotin compounds in humans, we assessed the possible effect of organotin compounds on aromatase activity and hCG secretion by human choriocarcinoma cells. Both TBT and TPT increased levels of hCG secretion and aromatase activity in a dose- and time-dependent fashion following exposure to non-toxic concentration ranges. In addition, these compounds enhanced 8bromo-cAMP-induced hCG secretion and aromatase activity in JAR cells. TBT caused dose-related increases in steady state mRNA levels of both hCG beta and CYP19 in JAR cells following exposure to non-toxic concentrations of TBT. Our studies suggest that TBT and TPT are potent stimulators of human placental hCG production and aromatase activity *in vitro*; and the placenta represents a potential target organ for these compounds, whose endocrine-disrupting effects might be the result of local changes in hCG and estrogen concentrations in pregnant women. Acknowledgments: This research was supported in part by Industrial Technology Research Grant Program in '01 from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

646 THE ANTIESTROGENIC EFFECTS OF MOTORCYCLE EXHAUST PARTICULATE *IN VITRO* AND *IN VIVO.*

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The effects of motorcycle exhaust particulate (MEP) on estrogenic responses were determined using MCF-7 human breast cancer cells and immature female Wistar rats treated with organic extracts of MEP. Treatments with 0.01 to 50 μg/ml MEP extract for 2 days produced a dose-dependent inhibition of thymidine incorporation in untreated cells and cells treated with 1 nM 17β-estradiol (E2). Treatments with MEP extract for 6 days resulted in dose-dependent decreases of cell growth in untreated and E2-treated MCF-7 cells. Cotreatment with MEP extract and E2 plus α-naphthoflavone, a cytochrome P450 inhibitor and aryl hydrocarbon receptor antagonist, blocked the MEP-mediated decreases of thymidine incorporation and cell growth. MEP extract replaced [3H]E2 from the estrogen receptor in MCF-7 cells in a temperature-, time- and concentration-dependent fashion. Cotreatment with MEP and α-naphthoflavone blocked the inductive effects of MEP on binding to the estrogen receptor and activity of aryl hydrocarbon hydroxylase in MCF-7 cells. The results of E2 metabolism studies showed that treatment with MEP extract increased E2 2-hydroxylation in MCF-7 microsomes. These antiestrogenic effects were further confirmed using a rat uterotrophic bioassay in which treatment with 10 mg/kg MEP extract for 3 days decreased uterine weights of untreated immature female rats and rats induced with 10 μg/kg E2. In parallel studies, treatments of MCF-7 cells and immature female rats with benzo(a)pyrene, a constituent of MEP, produced antagonistic effects on the E2-induced thymidine incorporation, binding to the estrogen receptor, and uterotrophic activity, respectively. The present findings demonstrate that MEP extract has the ability to antagonize the E2-induced responses in MCF-7 cells and female rats. The antiestrogenic effect of MEP may be an important factor to consider in assessing the potential health effects associated with human exposure to the vehicle exhaust.

647 EFFECT OF ATRAZINE ON THE ESTROGEN AND PROGESTERONE INDUCED LUTEINIZING HORMONE SURGE AND GNRH NEURONAL ACTIVITY.

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Atrazine (ATRA), a chlorotriazine herbicide, is widely used throughout agricultural areas in the United States. High doses of ATRA disrupt hypothalamic function, leading to, among other effects, suppression of the estrogen (E2) induced pro-estrous luteinizing hormone (LH) surge in ovariectomized (OVX) rats. To date, the effect of ATRA on the LH surge has only been examined after priming the rat with E2, not E2 and progesterone (PG). Yet, the combination of both gonadal steroids produces a hormonal environment more indicative of endogenous circulating hormones during pro-estrous. Our laboratory examined the ability of ATRA to suppress the LH surge in E2 and PG primed OVX rats. Female Sprague-Dawley rats were bilaterally OVX and jugular catheterized for serial blood sampling. Rats were treated with either 300mg ATRA/kg body weight in a 0.5% carboxymethylcellulose (CMC) vehicle or an equivalent volume of 0.5%CMC vehicle *via* oral gavage between 0900h to 1000h for 5 consecutive days, a treatment regimen that we have previously shown to significantly suppress the E2 induced LH surge in OVX rats. Animals received subcutaneous injections of E2 on days 2-4. On the morning of day 5, PG was administered and serial blood samples were taken hourly from 1200-2000. Serum was analyzed for LH *via* radioimmunoassay. E2 and PG priming produced an LH surge approximately four times greater (23.0±8.1ng/ml;n=11) than E2 priming alone(5.3±2.0ng/ml; n=20). Additionally, ATRA treatment significantly suppressed the E2–PG induced LH surge(3.42±2.6ng/ml;n=10) compared to control. The E2 and PG priming model provided a more robust LH surge that has allowed more sensitivity for dose-response studies with ATRA and comparisons of potency between ATRA and its metabolites. Funded by Syngenta Corporation and EPA STAR Grant (R-828610-01-0).

648 EFFECTS OF POLYBROMINATED DIPHENYLETHER (PBDE) ON REPRODUCTIVE ORGAN AND BRAIN DEVELOPMENT AND GENE EXPRESSION IN RATS.

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PBDE, used as flame retardants, accumulate in the biosphere; levels in human milk have increased during the last decade. We are comparing developmental actions of PBDE99 (2, 2', 4, 4', 5-pentaBDE) and the PCB mixture, Aroclor1254. PBDE99 (1 or 10 mg/kg/day) or Aroclor1254 (10 or 30 mg/kg/day) were injected subcutaneously to time-pregnant Long Evans rats from gestational day 10 to GD 18. The onset of puberty was delayed in female offspring (vaginal opening) by PBDE99 and Aroclor1254 (higher dose), but unaffected in males (preputial separation). Sexually dimorphic behavior and estrous cycle of adult offspring are being analyzed. PBDE99 affected absolute and relative weight of ventral and dorsal prostate (increase), epididymis (decrease), and ovary (increase), with unchanged body weight. Aroclor1254 induced a different effect pattern. Prenatal PBDE99 exposure affected sex hormone target gene mRNA levels determined by Real Time PCR (reference cyclophilin), IGF-I mRNA in ventral prostate and uterus, progesterone receptor mRNA in uterus, androgen receptor and estrogen receptors alpha and beta mRNAs in prostate, with differences between ventral and dorsal prostate. mRNA levels in sexually dimorphic brain regions, and mRNA expression after acute estrogen challenge in gonadectomized offspring, are under investigation. Our date indicate that PBDE99 can interfere with sexual development and exhibits features of endocrine disruptors. (Supported by EU 5th Framework Program, Project PBDE-NTOX).

649 ENDOCRINE ACTIVITY AND DEVELOPMENTAL TOXICITY OF UV FILTERS.

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UV filters used in sunscreens and other cosmetics have been found in the biosphere, in fish and human milk. In order to assess potential effects on reproductive functions, we are investigating endocrine activity in acute *in vitro* and *in vivo* systems, and developmental toxicity in rats. Several UV filters exhibit estrogenic activity on MCF-7 cells. Uterotrophic activity has been demonstrated for 4-methylbenzylidene camphor (4-MBC), octylmethoxy-cinnamate (OMC), benzophenone-3 (Bp-3) (Schlumpf et al., 2001), and recently for 3-benzylidene camphor (3-BC). The activity of 4-MBC was confirmed by Tinwell et al. (2002), 3-BC is also estrogenic in fish (Holbech, 2002). Further, Bp-3 and homosalate act as androgen antagonists in MDA-MB-453-KB2 cells. - Developmental Toxicity: When 4-MBC was administered in chow to Long Evans rats from 10 weeks before mating until adulthood of F1 offspring (1.0, 0.66, 0.33, 0.1 g/ kg chow, yielding daily doses of 70, 47, 24, 7 mg/kg), F0 animals did not exhibit adverse effects after 13 month exposure. Higher doses reduced F1 survival rate and neonatal thymus weight. Male puberty was dose-dependently delayed. Weight changes were noted for testis, epididymis, seminal vesicles, ventral and dorsal prostate. Estrogen target gene mRNA levels, determined by Real Time PCR (reference cyclophilin), were altered in adult F1 uterus (IGF-I mRNA, progesterone receptor (PR) mRNA, androgen receptor (AR) mRNA) and prostate (IGF-I mRNA, AR mRNA). Ventromedial hypothalamus (VMH) and medial preoptic region showed sex- and region-dependent

changes in PR and ER alpha mRNAs from 7mg/kg/day, with loss of sexually dimorphic PR mRNA expression in VMH. Our data indicate that 4-MBC can influence sexual development at peripheral and central levels of neuroendocrine organization. (Supported by Swiss National Research Programme 50, Swiss Environmental Protection Agency (BUWAL), CEFIC, Baugarten Stiftung, Hartmann Muller Stiftung, Olga Mayenfisch Stiftung)

650 ASSESSMENT OF DEMERSAL AND CULTURED FLATFISH SPECIES FOR EXPOSURE AND ADVERSE EFFECTS RESULTING FROM EXPOSURE TO ENVIRONMENTAL ESTROGENS IN THE SOUTHERN CALIFORNIA BIGHT.

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Previous studies with demersal flatfish species in the central Southern California Bight have indicated the uptake of environmental estrogens. Subsequent studies were carried out to determine whether adverse reproductive effects occur in these populations. In addition, sediment collected from the outfall of a local wastewater treatment plant was evaluated with a laboratory exposure to cultured California Halibut (CH) (Paralichthys californicus) and chemical residue analyses for 17-beta estradiol. Initial population metrics indicated no significant differences in hornyhead turbot (HT)(Pleuronichthys verticalis) or English sole (ES) (Parophrys vetulus) collected in this region over the past 10 years. In July of 2002, HT and ES were collected from 8 offshore sites by trawl. Gender ratios, age ratios, length, serum vitellogenin, sperm DNA damage and gonad somatic indices (GSI) were compared. There was a slightly higher percentage of females ES (42% n=59) at the outfall compared to the reference location (37% n=54), and a higher number of sexually mature female ES (25% vs. 0%) and male ES (42% vs. 6%) at the outfall. Although gender ratios were unchanged for HT (33%), there were higher numbers of sexually mature animals at the outfall for both genders. Lengths were greater for male and female ES as well as female HT at the outfall compared to the reference site. GSI for female ES was also significantly higher at the outfall compared to the reference fish. DNA damage of HT sperm was highest at the outfall. Vitellogenin in male CH exposed to sediments was not induced and estradiol concentrations were 0.048, and 0.42 ng/g for reference and outfall sediments. These results indicate estrogenic activity observed in demersal fish of So Cal. is not likely caused by a sediment-only exposure to xenoestrogens.

651 INTERACTION ANALYSIS OF SYNTHETIC XENOESTROGENS AND PHYTOESTROGENS *IN VIVO*.

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The immature rat uterotrophic model was used to investigate interactions between reconstituted mixtures of estrogenic substances as evaluated by the change in slope of the dose-response curve of one chemical in the presence of other chemicals. Conceptually, if these dose-response curves are parallel, then there is zero or no interaction between the chemicals. A 2:1 phytoestrogen mixture of genistein and daidzein (based on general dietary ratios) was experimentally evaluated alone at doses of 0, 60, 120, 240 mg/kg/day or in combination with a synthetic xenoestrogen mixture (o, p-DDT, methoxychlor and beta-hexachlorocyclohexane) in which each chemical was present at 0.01, 0.1, or 1X of its respective NOEL based on their individual dose-response curves. Test materials were given for 3 days *via* gavage and wet and blotted uterine weights measured 24h after the last dose. A parameterized nonlinear Gompertz model was used to determine if the synthetic xenoestrogens altered the slope of the phytoestrogen dose-response curve. The phytoestrogen dose-response curve was not significantly changed by the addition of the synthetic xenoestrogen mixture at 0.01 or 0.1X NOEL levels for either the uterine wet (p=0.449 and 0.763) or blotted (p=0.294 and 0.663) weights, respectively. When the xenoestrogens were present at 1X NOEL levels there was a leftward shift in the phytoestrogen curve, however, with no significant change in slope for either the wet (p=0.677) or blotted weights (p=0.697), thus indicating no evidence of departure from additivity (i.e no interaction) between the phytoestrogen and 1X NOEL level of xenoestrogens. Since environmental levels of synthetic chemicals are generally well below their individual NOEL concentrations, a change in response from that due to phytoestrogen exposure would not be likely. Supported by the American Chemistry Council.

652 SPECIES DIFFERENCES IN THE CYTOCHROME P450 (CYP)-DEPENDENT METABOLISM OF 3, 3'-DIINDOLYLMETHANE (DIM): EFFECTS ON ESTROGENICITY.

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Indole-3-carbinol (I3C) is a glucosinolate hydrolysis product from cruciferous vegetables that has been found to have chemoprotective effects in several animal models. However, I3C given long-term postinitiation modulates liver carcinogenesis similar to the actions of 17β-estradiol so that it promotes hepatic tumors in trout and rat models, but inhibits them in mice. A major acid condensation product of I3C, DIM, is estrogenic in precision trout liver slices and accounts for the majority of the estrogenicity of I3C in trout *in vivo*. In competitive binding studies with trout hepatic estrogen receptor (ER), we found the relative binding affinity of DIM was 17-fold higher than I3C. This evidence, along with the species selectivity of I3C as a hepatic tumor modulator, suggests an estrogenic mechanism of action for hepatocarcinogenesis. In this study, we demonstrate species differences in the relative binding affinity of DIM for the ER after CYP-dependent metabolism. DIM incubated with β-napthoflavone (βNF)-induced rat microsomes in the presence of NADPH generated a metabolite mixture with a binding affinity for the ER 33-fold higher than the parent compound. However, DIM incubated with βNF-induced trout microsomes did not result in metabolites with a different binding affinity from the parent. These data support previous work on the disposition of DIM in trout and rat *in vivo* and suggest that species differences in hepatic tumor promotion are due to the CYP-specific metabolism of DIM to estrogenic metabolites. Supported by NIH grant ES03850.

653 REGULATION OF UTERINE HSP90α, HSP72 AND HSF-1 EXPRESSION IN B6C3F1 MICE BY β-ESTRADIOL AND BISPHENOL A: INVOLVEMENT OF THE ESTROGEN RECEPTOR AND PROTEIN KINASE C.

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We have previously demonstrated that the effects of bisphenol A (BPA), an estrogen-mimic, and β-estradiol (E_2) on heat shock proteins (hsps) in the uterus are mediated through the estrogen receptor (ER). However, the mechanism of the ER involvement in the regulation of uterine hsps remains unknown. The heat-inducible heat shock factor 1 (HSF-1) controls the transcription of hsp genes. HSF-1 is regulated by estrogen, but also by protein kinase C (PKC). PKC may also regulate hsp expression independent of HSF-1. It was therefore of interest to examine the role of PKC and of the ER on the time-related effects of E_2 and BPA on uterine hsp90α and hsp72 expression. Ovariectomized B6C3F1 mice were treated subcutaneously with corn oil (vehicle), 1 μg E_2/kg or 100 mg BPA/kg alone or in combination with 5 mg/kg of the antiestrogen ICI 182, 780 (ICI) or 0.5 mg/kg of the PKC inhibitor GF 109203X (GF), and uteri were collected at 6 or 24 hours post-administration. Both E_2 and BPA altered the expression of hsp90α and hsp72. E_2 appears to increase uterine hsps by regulating the transcription of these genes and/or of HSF-1. BPA may regulate the transcription of hsp90α and hsp72, but not HSF-1. PKC may be involved in the regulation of hsp72 by E_2 and in the regulation of hsp90α by BPA. Collectively, the results of the present study suggest a differential mechanism of regulation of uterine hsp90α and hsp72 levels by E_2 and BPA.

654 ESTROGENIC AND ANTIESTROGENIC ACTIVITIES OF BISPHENOL A AND RELATED BISPHENOLS IN ISHIKAWA CELLS.

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Substantial interest has recently been evoked by the hormone-like effects of bisphenols, in particular bisphenol A (BPA), which is widely used in polymer chemistry. In the present study, we have determined the estrogenic and antiestrogenic activity of BPA, bisphenol B (BPB) and the ring-methylated BPA derivates 3, 3′-dimethyl-BPA (DMBPA) and 3, 3′, 5, 5′-tetramethyl-BPA (TMBPA) in Ishikawa cells. This cell line is derived from a human endometrial adenocarcinoma and responds to estrogens with a marked induction of alkaline phosphatase (AP). Maximal stimulation of AP is achieved using 1 nM 17β-estradiol (E2). Cells were seeded in 96-well plates and incubated for 48 h with bisphenol concentrations ranging from picomolar to micromolar. The AP activity was measured photometrically using p-nitro-

phenylphosphate as substrate and related to the activity obtained with 1 nM E2. All four bisphenols stimulated AP activity at concentrations of 1 to 5 μM, indicating a weak estrogenic activity (0.01-0.03% of E2 activity). However, the maximal AP activity induced by the bisphenols was only 40-60% of that obtained with E2. When micromolar concentrations of the bisphenols were co-incubated with 1 nM E2, a lower induction of AP as compared to E2 alone was noted. To exclude that this putative antiestrogenic effect was due to a reduction of cell viability or cell number, we determined cell viability by the methylthiazoletetrazolium (MTT) assay and cell number by the sulforhodamine B (SRB) test and by electronic cell counting. Incubation with 10-50 μM BPA or BPB alone, but not in the presence of 1 nM E2, gave rise to an elevated cell viability (120-140%) in the MTT test. However, no increase in cell number was observed in the SRB assay or by counting cell number. Furthermore, no direct effect of bisphenols on the activity of commercially available AP from human placenta was noted. Based on these results we conclude that bisphenols alter AP activity due to changes in AP expression and exhibit weak estrogenic and antiestrogenic activity in Ishikawa cells.

655 MCF-7 CELL MITOGENS DIFFERENTIALLY AFFECT MAPK ACTIVATION AND ESTROGEN RECEPTOR-α PHOSPHORYLATION.

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Xenoestrogens are implicated in adverse health effects by modulating the activity of the estrogen receptor (ER) either directly by binding ER or indirectly by affecting signaling pathways that impact ER activity. Estradiol (E2), epidermal growth factor (EGF) and acetaminophen (APAP) each are mitogens in MCF-7 (ER+ human breast cancer) cells, and anti-estrogens inhibit their proliferation, implying ER is involved in the mitogenic pathway(s) they activate. E2 exerts its activity by binding to and enhancing phosphorylation of ER, activating it as a transcription factor; MAPK, PKA and CaM kinase II are implicated in E2-mediated ER phosphorylation. However, EGF reportedly activates ER independent of binding by promoting ER phosphorylation through the MAPK pathway. It is unclear how APAP exerts mitogenic activity, but like EGF it does not bind ER. Therefore this study tests the hypothesis that APAP activates ER in a ligand-independent manner *via* MAPK activation and ER phosphorylation. MCF-7 cells served as an *in vitro* model in which to profile the activity of these 3 mitogens on MAPK activation and ERα phosphorylation, both assessed by immunoblotting. Initial studies indicated E2 enhanced MAPK activity; however additional studies clearly showed this response was an artifact caused by the addition of a small amount of stripped serum when cells were dosed with E2. MAPK is not activated when E2 is given to cells in the absence of additional serum. As previously reported, E2 also increased ERα phosphorylation. In contrast, EGF transiently and robustly induced MAPK activation, but did not increase ERα phosphorylation. Unlike E2 and EGF, APAP had no effect on either MAPK activity or ERα phosphorylation. These findings indicate ER has complex involvement in MCF-7 cell growth, and estrogenic compounds can induce ER-mediated breast cancer cell proliferation through various signaling pathways. Additional studies are needed to determine what kinase(s) are responsible for E2-induced ERα phosphorylation, as it is clear from this study that MAPK is not activated above basal levels by E2.

656 IDENTIFICATION OF ERβ-RESPONSIVE GENES IN THE OVARIES OF ESTROGEN RECEPTOR α KNOCKOUT MICE.

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A number of compounds found in the environment are known to possess estrogen-like biological activity and have been linked to adverse effects on reproduction function in humans and wildlife. Some of these endocrine disruptors preferentially affect estrogen receptor β (ERβ). However, the physiological relevance of ERβ is not known. To gain a better understanding of the role ERβ plays in the reproductive tract, we used estrogen receptor α knockout (αERKO) mice, which only express ERβ, to identify ERβ-responsive genes. Sexually immature (24-day-old) female αERKO mice were injected subcutaneously in the dorsal cervical area with vehicle alone (0.1 ml corn oil), estradiol (E2) (10 μg/kg), ICI 182, 780 (ICI) (20 mg/kg), or E2 plus ICI. At 24 h after treatment, ovaries were collected, and total RNA was isolated from 4 to 5 animals per group. Microarray analysis was used to identify potential ERβ-responsive genes and reverse transcription polymerase chain reaction was used to confirm and quantitate gene expression. Expression levels of *Fas* and *klotho* were significantly decreased 2.5- and 3.6-fold, respectively, in mice treated with E2 compared to control mice and ICI blocked E2-induced down-regulation of these genes. In addition, ICI alone significantly increased *MAPKK4* expression by 1.3-fold compared to control mice, whereas E2 alone or in combination with ICI did not significantly alter *MAPKK4* expression. All three of these genes play a role in apoptosis. Our results suggest that ERβ may act as a repressor of genes involved in regulation of apoptosis in the ovary. Currently we are investigating inhibition of ERβ activity by ERβ-specific compounds in the ovary since these genes may be involved in decreased ovulatory capacity and impaired follicular development observed in ERβ knockout mice.

657 MECHANISM OF ESTROGEN RECEPTOR α/SP1-MEDIATED ACTIVATION OF GC-RICH PROMOTERS BY ESTROGENS AND ANTIESTROGENS.

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Estrogen receptor α (ERα)/Sp1 activation of estrogen-responsive GC-rich gene promoters in breast cancer cells is dependent on activation function 1 (AF1) of ERα; however, contributions of the DNA binding domain (DBD) and AF2 regions of ERα and promoter context on ERα/Sp1 action have not been determined. 17β-Estradiol (E2) induced reporter gene activity in MCF-7 and MDA-MB-231 cells cotransfected with human or mouse ERα (hERα or MOR), but not ERβ and GC-rich constructs containing three tandem Sp1 binding sites ($pSp1_3$) or estrogen-responsive GC-rich promoter inserts from the adenosine deaminase (pADA) or retinoic acid receptor α1 (pRARα1) genes in breast. The antiestrogens 4'-hydroxytamoxifen (4OHT) and ICI 182, 780 (ICI) also activated $pSp1_3$ in cells cotransfected with ERα. However, estrogen and antiestrogen activation of hERα/Sp1 was also dependent on overlapping and different AF1-independent domains (CDEF) of ERα. hERα/Sp1 was activated by E2 and antiestrogens in breast cancer cells transfected with hERα containing mutations in helix 12 (D538A, E542A and D545A) that block interactions with AF2-interacting coactivators and not surprisingly, prototypical steroid receptor coactivators did not enhance ERα/Sp1-mediated transactivation. In contrast, mutation of zinc fingers 1 or 2 resulted in loss of hERα/Sp1 activation by antiestrogens (but not E2) and deletion of the hinge or C-terminal helix 12/helix 13 (aa 538-595) regions resulted in loss of hERα/Sp1 activation by E2 but not antiestrogens. Thus, estrogen/antiestrogen-mediated activation of hERα/Sp1 is dependent not only on AF1 but also selected regions within the CDEF domains that are necessary for E2- or antiestrogen-responsiveness. (Supported by NIH ES09106 and CA76636)

658 ANTI-ESTROGENIC ACTIVITY OF POLYCYCLIC MUSKS IN THE ZEBRAFISH (*DANIO RERIO*).

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Polycyclic musks are an important group of synthetic fragrance ingredients with HHCB (Galaxolide) and AHTN (Tonalid) as the two largest volume products. They are used in perfumes, cosmetics and household cleaning products with a world-wide production volume of about 6000 t/year. They reach the aquatic environment *via* household wastewater and sewage plant effluents. Because of their high lipophilicity, AHTN and HHCB tend to bioaccumulate in the environment. They have been found in surface waters, sediment, fish, and also in human milk, fat and blood. Using a sensitive reporter gene assay we found that both musks showed anti-estrogenic activity towards the human estrogen receptor β and zebrafish estrogen receptor γ. Zebrafish, in which an estrogen responsive luciferase reporter gene has been stably introduced, were used for *in vivo* testing. In this transgenic zebrafish assay both musks showed anti-estrogenic activity. GC-MS analysis of both compounds was used to estimate the total body uptake of musks by the zebrafish.

659 ASSESSMENT OF DE-71, A COMMERCIAL POLYBROMINATED DIPHENYL ETHER (PBDE)MIXTURE, IN THE EDSP MALE PUBERTAL PROTOCOL.

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PBDEs are environmental contaminants that disrupt thyroid hormone homeostasis. To examine the ability of the Endocrine Disruptor Screening Program (EDSP) Tier 1 male pubertal protocol to detect thyroid active agents, we examined thyroid and reproductive endpoints of male Wistar rats following DE-71(DE)exposure. Rats were gavaged with 0, 3, 30 or 60 mg/kg DE in corn oil from postnatal day (PND) 23-53. Thyroid measures were also made on PND 28 to compare a 5 day exposure with this 31 day protocol. T4 was significantly decreased at 30 and 60 mg/kg (74 and 81% below control) on PND 28 and at 3, 30 and 60 mg/kg (20, 80,

and 86%) on PND 53. T3 was decreased only by 30 and 60 mg/kg (25 and 20%) on PND 53. TSH was increased on PND 53 at 30 and 60 mg/kg (64 and 113%). Increased liver to body weight ratios coincided with a induction of UDGPT (uridinediphosphate-glucuronosyltransferase; 2-4 fold), EROD and PROD (ethoxy- and pentoxy-resorufin-O-deethylase) predominantly at the two highest doses. EROD increased 10-14 fold on PND 28 and 53, while PROD increased 12-23 fold by PND 28 and 78-83 fold by PND 53. Of the androgen dependent tissues, seminal vesicle (SV) and ventral prostate (VP) wt were reduced at 60 mg/kg, while testes and epididymal wts were not. Preputial separation (PPS) was significantly delayed at 30 and 60 mg/kg by 2.0 and 2.3 days. Serum testosterone was decreased, but not significantly, at 60 mg/kg. Thus, the 31-day dosing required in the male pubertal protocol will detect thyroid hormone alterations of DE at doses as low as 3 mg/kg, while the 5 day exposure LOEL was 30 mg/kg. This data provides evidence that the 31 day alternative Tier 1 protocol is a sensitive test for thyrotoxic agents that act *via* up-regulation of hepatic metabolism. The delay in PPS and reduction in SV and VP wt may indicate a modification of endogenous androgenic stimulation directly by DE or a secondary effect that occurs in response to a DE-induced change in thyroid hormones. (Abstract does not necessarily reflect EPA policy).

660 THE EFFECTS OF DE-71, A COMMERCIAL POLYBROMINATED DIPHENYL ETHER MIXTURE, ON FEMALE PUBERTAL DEVELOPMENT AND THYROID FUNCTION.

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PBDEs, used as flame-retardants, are persistent environmental contaminants that can alter thyroid homeostasis *via* their action on liver enzyme induction. DE-71 was used to test the sensitivity of the female pubertal protocol to detect thyroid active chemicals. This protocol is being evaluated for the USEPA's Endocrine Disruptor Screening Program as part of a Tier I Screening Battery. Wistar rats were gavaged from postnatal day (PND) 22-41 with DE-71 (0, 3, 30, 60 mg/kg) in corn oil. Females were monitored daily for vaginal opening (VO) as an assessment of pubertal development. To evaluate the temporal effects of DE-71 on serum thyroid hormones and liver microsomal EROD, PROD (ethoxy- and pentoxy-resorufin-O-deethylase) and UDPGT (uridinediphosphate-glucuronosyltransferase) activity, groups of animals were killed after 5 and 20 days of dosing. Serum total thyroxine (T4) was significantly decreased by 30 and 60 mg/kg at both time points. No significant changes were observed in triiodothyronine (T3), while a significant linear trend for increased thyroid stimulating hormone (1.6 X increase in the 60 mg/kg group) was detected in those animals exposed for 20 days. Increased liver to body weight (bw) ratios following exposure to 30 and 60 mg/kg were consistent with induction of hepatic enzymes. UDGPT activity was inducted 2.5-4 X over control. EROD and PROD activity were also induced at 30 and 60mg/kg. EROD increased 5-14 X, while PROD increased 10 and 72 X with 5 and 20-day exposures, respectively. The highest dose of DE-71 caused a small, yet statistically significant delay in the age of vaginal opening (i.e., 1.8 day delay vrs control). No treatment related changes were noted in growth, bw, or any reproductive tissue weights. In summary, these data demonstrate that this protocol will detect chemicals that alter thyroid hormone concentrations by inducing liver metabolic enzymes. This abstract does not necessarily reflect USEPA policy.

661 PERFLUOROOCTANE SULFONATE (PFOS) DISRUPTS THE THYROID STATUS IN LABORATORY RODENTS.

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PFOS is an environmental contaminant ubiquitously found in humans and wildlife. Reproductive and developmental toxicity of this fluorochemical have been indicated in laboratory animals. The current study examines if alterations of the thyroid status are involved in PFOS toxicity. Timed-pregnant Sprague-Dawley rats and CD-1 mice were given PFOS/K daily (1, 2, 3, 5 or 10 mg/kg, GD2-20, for rats; and 1, 5, 10, 15 or 20 mg/kg, GD1-17, for mice) by oral gavage; controls received 0.5% Tween-20 vehicle. Maternal blood was collected at three time intervals during pregnancy, serum T4, T3 and TSH were analyzed by the specific RIA. Some animals were allowed to give birth and thyroid status of the neonates was monitored during postnatal development. PFOS did not interfere with the performance of RIA. During pregnancy, a marked decline of serum T4 concentration, and to a lesser extent T3, was seen in the control rats, with a feedback elevation of serum TSH. Superimposed on these changes, T4 and T3 levels were significantly further reduced in the PFOS-treated dams in all dose groups and at all time points evaluated; while elevations of TSH were also seen in the PFOS-treated dams, the levels did not exceed those of the controls. Similar results were observed in the pregnant mice. Because the thyroid hormones fluctuate during pregnancy, these findings were extended to 90-day old non-pregnant rats who were given similar doses of PFOS daily. After 3 days of PFOS treatment, serum T4 was significantly lowered, while marked reductions of both T4 and T3 were seen after 20 days of exposure; however, an absence of feedback rise in serum TSH was noted in these animals. In the developing rats, hypothyroxinemia was observed without concurrent changes of T3 or TSH. Thus, exposure to PFOS led to disruption of thyroid status in the rats and mice; the underlying mechanisms of this hormonal imbalance as well as its physiological consequences warrant further investigation. (This abstract does not necessarily reflect EPA policy.)

662 ASSESSMENT OF THYROID MODULATING ACTIVITY AND ITS APPLICATION IN PRECLINICAL DRUG DEVELOPMENT: METHODS AND MECHANISMS.

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Chemically diverse compounds can cause thyroid modulation (TM) by a number of direct or indirect mechanisms including alteration of thyroid hormone (TH) synthesis, secretion, distribution and biotransformation, or by modulation of tissue response. This presentation describes a decision tree approach for evaluating TM during preclinical drug development. In early toxicology studies, changes in thyroid gland weights and/or microscopic appearance suggestive of TM can be further investigated by examining serum hormone levels (TSH, T3, rT3 and T4), liver weights, and activities of TH metabolizing enzymes (such as UDP glucuronyl transferase). Alternatively, subsequent toxicology investigations can incorporate additional endpoints for evaluation of TM. Central to the decision tree approach are data from serum TH profiles, which guide the follow-up measurements. Assessment of thyroperoxidase and 5'-deiodinase activity, in light of serum hormones, allows comprehensive evaluation of TH synthesis and secretion. Assessment of serum TH carrier proteins, tissue uptake of TH, and UDP-TH conjugates leads to understanding of TH distribution and biotransformation. Assessment of tissue TH receptor (TR) expression and/or functionality further elucidates tissue TH responsiveness. In addition, routine and specialized thyroid histopathology studies, (e.g., BrdU/PCNA labeling) can provide valuable information for assessing TM. Finally, *in vitro* studies that assess drug activation of TR in transfected cells, TH-binding protein characteristics, and protein or RNA resulting from transcription of TH-responsive genes can clarify mechanism of TM. The decision tree provides a logical and comprehensive approach to addressing thyroid effects early in drug development and is useful in the design and interpretation of subsequent preclinical toxicology studies. Knowledge of species differences in the above endpoints aids in assessment of potential human risk.

663 ANALYSIS OF THYROID HORMONE AND RELATED IODINATED COMPOUNDS BY HPLC-ICP/MS.

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Quantifying the synthetic precursors and metabolic products of thyroid hormone is important toward understanding the effects of xenobiotics on thyroid function. Although various methods have been utilized to assess thyroid hormone status, few allow a comprehensive analysis of the suite of related iodinated compounds without the use of radiolabeled iodine. In this study, we describe the development of a high performance liquid chromatography-inductively coupled plasma/mass spectrometry (HPLC-ICP/MS) method that allows for the detection of MIT, DIT, T2, T3, rT3, and T4 in a single sample. This method has three main steps: sample preparation, chromatographic separation of analytes by HPLC, and detection of iodinated compounds by ICP/MS. Sample preparation uses a proteolytic treatment of tissue homogenates followed by analyte concentration using solid phase extraction (SPE). The eluate from the SPE step is dried and reconstituted in a known volume of buffer and introduced to an HPLC system for chromatographic separation of the analytes. The eluent from the HPLC is introduced directly to an ICP/MS operating in a time-resolve mode monitoring mass 127. The selectivity of the ICP/MS detector allows for quantification of the iodinated species only and does not detect non-iodinated chemicals that are present in a complex sample matrix. This method was validated using analytical standards and detection limits range from 70 to 150 pg as iodine. The method was applied to whole organism samples of *Xenopus laevis* larvae and document the relative concentrations of iodinated compounds in this species at specific developmental stages. This abstract does not necessarily reflect EPA policy.

663a EFFECT OF *IN UTERO* EXPOSURE TO COPLANAR PCBS ON THE THYROID HORMONE LEVELS AND LIVER MICOROSOMAL T4-UDP-GT IN THE RAT.

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Sponsor: R. Nagata.

Both 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and coplanar PCBs are widely distributed, environmentally persistent organic compounds. It has repeatedly been demonstrated that these two compounds reduce circulating thyroxin (T4) levels. In this study, the effects of *in utero* exposure to two coplanar PCB congeners, PCB 126 (3, 3', 4, 4', 5-pentachlorobiphenyl) and PCB 169 (3, 3', 4, 4', 5, 5'-hexachlorobyphenyl) on the thyroid hormone status are evaluated and discussed. Sprague-Dawley rats were given, *via* gavage, PCB 126 (3 μg/kg/day), PCB 169 (30 μg/kg/day), or a corn oil compound, on days 7-21 of gestation. Later we collected specimens at a specific schedule and determined the plasma T4, thyroid stimulating hormone (TSH) levels and the liver microsomal T4-uridine diphosphate glucuronosyltransferase (UDP-GT) activities. After PCB 126 was administered to the pregnant rats, the plasma T4 levels were determined to be decreased on PND (postnatal day) 7, 42 and 105 for the male offspring and on PND 7, 21, and 105 for the female offspring. The plasma TSH values were decreased on PND 7 for the male offspring, but were unchanged for the female offspring. The liver microsomal T4-UDP GT activities were increased on PND 7 through 42 for both male and female offspring. When PCB 169 was administered parentally, the plasma T4 levels were decreased on PND 7 through 42 for the male offspring, and on PND 7 through 105 for the female offspring. The plasma TSH levels were unchanged for both male and female offspring. The liver microsomal T4-UDP GT activities were increased on PND 7 through 42 for the male offspring and on PND 7 through 105 for the female offspring. We concluded that *in utero* coplanar PCB exposure, with the specific compounds used, resulted in decreased plasma T4 levels and increased liver microsomal T4-UDP GT. The observed decrease in the T4 values may be partially attributable to the increased T4 glucuronidation by T4-UDP GT.

664 A FRAMEWORK FOR EVALUATING RELATIVE POTENCY DATA IN THE DEVELOPMENT OF TOXICITY EQUIVALENCY FACTORS (TEFS).

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The human health risk assessment for mixtures of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) has utilized a toxicity equivalency (TEQ) scheme, centered on 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototypical dioxin. This scheme necessitates the development of toxicity equivalency factors (TEFs) for individual PCDD, PCDF, and PCB congeners. The importance of the TEF values is underscored by the fact that >85% of the background TEQ dose in humans is comprised of congeners other than TCDD. Yet the current international (WHO) TEFs remain order-of-magnitude estimates, largely due to the broad range of relative potencies (REPs) from which they are derived. The WHO TEF Workgroup addressed this issue by applying an *ad hoc* REP weighting scheme. However, we have observed considerable inconsistencies in the selection of TEFs from among the 28 PCDD/F and PCB congeners that were included in the WHO analysis. We propose a framework for a systematic evaluation of REP data, which could be used to support a REP weighting scheme based on data quality. Such a framework could enhance the development of probability density functions (PDFs) for the TEFs. This approach would retain a large degree of the heterogeneity of the REP data, but would also rank these data based on reliability and relevance. Relevance is based on the eventual use of TEFs in the human health risk assessment with cancer as the endpoint. Rating schemes are proposed for 16 and 10 methodological criteria that were identified as key elements of the *in vivo* and *in vitro* REP studies, respectively. We also propose criteria for converting cumulative scores into a simplified quality score (1 to 5), in accordance with Klimisch et al. (1997; Regul. Toxicol. Pharmacology 25(1):1-5.), which has been widely applied to the evaluation of other toxicology data. The use of this framework for evaluating REP data could advance the uniform development of TEFs for a growing list of chemical families.

665 A SCREENING-LEVEL RISK ASSESSMENT APPROACH FOR EVALUATING INTERACTIONS IN CHEMICAL MIXTURES.

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The conventional risk assessment approach for chemical mixtures involves the calculation of hazard index (HI) without accounting for the possible impact of interactions between components. Some interactions can modify the tissue dose of components, resulting in a change in the health risk during mixed exposures compared to single exposures. Given that metabolic interference is a common mechanism by which chemicals interact, this study developed an approach for screening and identifying mixtures that are of health concern due to the impact of metabolic inhibition. The methodology involved: (i) the establishment of the tissue dose corresponding to the threshold limit value (TLV) of each of the mixture components, (ii) the calculation of the maximal tissue dose of each component that can possibly result during mixed exposures, and (iii) the calculation of tissue dose-based HI by dividing the maximal tissue dose of components by the values corresponding to their TLVs. Whenever the resulting tissue dose-based HI values exceed 1, it indicates a mixture exposure scenario of health risk concern. The proposed screening level risk assessment method was applied to mixtures of varying concentrations of toluene, m-xylene and ethylbenzene (1 to 90 ppm). The maximal value of tissue dose (i.e., area under the blood concentration vs time curve) for each of these chemicals was calculated using physiologically-based pharmacokinetic models in which the hepatic extraction ratio value was set to 0. For inhalation exposures of 1 to 90 ppm, the corresponding maximal tissue doses were 0.06 and 2.35 mg/L for toluene, 0.11 and 10.3 mg/L for m-xylene, and 0.12 to 10.9 mg/L for ethyl benzene. These maximal tissue doses were then used along with the tissue dose associated with the TLV of each compound to calculate HI and thereby identify those mixtures that could be of concern, due to metabolic interactions. The approach developed in this study, for the first time, facilitates the calculation of HI of mixtures by accounting for the maximal influence of metabolic interactions on tissue dose.

666 STATISTICAL ANALYSIS OF AN INTERACTION THRESHOLD IN CHEMICAL MIXTURES ALONG A FIXED-RATIO RAY.

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Humans are exposed to a variety of chemicals on a daily basis, thus mixture toxicology is an important and relevant issue. Risk assessors often assume additivity at low concentrations of mixture exposures. Recent studies on complex mixtures have revealed interaction thresholds in chemical mixtures through physiologically based pharmacokinetic (PBPK) modeling. We propose the use of empirical models to detect the presence of an interaction threshold along a fixed-ratio ray of the chemicals under study. Our approach is illustrated using a cytotoxicity study involving three metals (arsenic, chromium, lead) and Syrian Hamster Embryonic (SHE) cells. The fixed-ratio ray under study was chosen based on the LC50's of each metal estimated from individual metal studies. This corresponds to a fixed ratio of (5.4:3.6:100.0) As:Cr:Pb, or approximately (0.05:0.033:0.917) As:Cr:Pb. Total concentrations are taken along this fixed-ratio ray by specific dilution of the reference concentration, 1X, which represents the combination of 5.4 μM As, 3.6 μM Cr, and 100 μM Pb. Initial tests for overall departure from additivity along the ray were significant. Therefore, the interaction threshold model is used to detect the presence of a concentration where significant interactions start to occur. An interaction threshold was found to exist at approximately 7.9 mM total concentration. However, the model parameter was not significant. This could be due to the design of the experiment. Since design points were considered according to dilutions, there are "gaps" in the design that could influence the parameter estimates. Based on this initial model, Ds-optimal designs are presented that would minimize the variance of the interaction threshold parameter. (Supported by ATSDR U61/ATU 881475 and NIEHS T32-ES07334-01A1)

667 DO 'ESTROGEN EQUIVALENTS' MAKE SENSE FOR RISK ASSESSMENT?

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Toxicity Equivalence Factors (TEFs) have been used to assess Ah-receptor-mediated toxicity and have been proposed for other modes of action, including toxicity mediated through estrogenic mechanisms. Applying an equivalence factor approach depends on the validity of a number of assumptions, including that 1) chemicals act through the same biochemical pathway, 2) have parallel dose-response curves, 3) exhibit dose-additive toxicity at sub-maximal doses in mixtures, 4) manifest identical toxic effects over the dose range of concern, and 5) that potency can be related to a reference chemical. Two assumptions necessary for applying an equivalence factor approach - dose additivity in mixtures and an unambiguous potency estimate relative to a reference chemical - were evaluated based on data published in more than 40 reports on binary combinations of putative estrogenic chemicals and in numerous other publications that reported estrogenic potencies at molecular and

physiological endpoints for various chemicals. Neither assumption appears to be supportable on the basis of the data analyzed. Although application of published criteria for evaluating interaction studies reveal fundamental inadequacies in many studies that purport to test combined estrogenic action, the assumption of dose additive combined toxicity does not appear to be supportable. Wide variations in estrogenic potency measurements preclude the derivation of single estrogenic potency estimates relative to a reference chemical. The inability to find support for these two fundamental assumptions argues strongly that an estrogenic equivalence approach is inappropriate for risk assessment of environmental estrogens. Alternative approaches will be required if mode-of-action assessments are to be made for mixtures of xenoestrogens.

668 CRITICAL REVIEW OF PRINCIPLES, PRACTICE AND TOXICOLOGY OF CHEMICAL MIXTURES: IMPLICATIONS FOR RISK ASSESSMENT.

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A critical analysis of mixture theory and practice was conducted by examining reference texts, regulatory documents and journal articles investigating mixtures of one or more chlorinated chemicals. The objective was a judgement about the suitability of proposed regulatory risk assessment procedures given the current understanding of mixture toxicology. Recent guidance documents by ATSDR and EPA recommend using three different types of data for health risk assessment of chemical mixtures. When data on a particular mixture is unavailable, data on a similar mixture may be extrapolated to the scenario of concern. For some mixtures, the presence of only a few prominent constituents may be used to conclude similarity while for other mixtures, similarity may be assumed from a common toxicological effect or a presumed similar toxic mechanism. However, clear criteria are yet to be developed for determining when two mixtures are sufficiently similar to use one as a toxicological surrogate for the other. In particular, there is no generally accepted classification scheme for modes/mechanisms of toxic action. This is a prerequisite for judging whether two mixtures exert effects *via* a common toxicological pathway. Furthermore, it is currently not possible to reasonably predict toxicity interactions mechanistically because no methodology exists for classifying the mechanisms by which interactions occur. Current approaches do not account for the dose dependence of mechanisms of toxicity interaction because data from interaction studies that test a full dose-response range are unavailable (i.e., doses above and below the observed effect levels of individual chemical constituents. We argue the need to develop a mode/mechanism of toxicity classification scheme as well as a mechanism of toxicity interaction classification scheme in order to advance mixtures research in a way that will be useful for mixtures risk assessment and policy development.

669 TOXICITY ASSESSMENT OF COMPLEX MIXTURES REMAINS A GOAL.

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One of the initial steps in remediating contaminated environments is to assess the human and ecological health risk associated with exposure to contaminants in a specific medium. This abstract summarizes the results of a five-year study investigating the toxicity of simple and complex mixtures. A series of model compounds and simple mixtures including polycyclic aromatic hydrocarbon (PAHs), pentachlorophenol (PCP), and halogenated aliphatic hydrocarbons (HAH) were analyzed. Mixture toxicity was studied using microbial genotoxicity assays and cytotoxicity assays using renal and neural cells. The majority of simple mixtures studied induced additive responses. A limited number of samples were identified where binary mixtures induced inhibitory effects. For example, benzo(a)pyrene (BAP) alone induced 30% renal cell death, whereas an equimolar dose of chrysene and BAP only produced 1.6% cellular death. In none of the mixtures tested did the mixture toxicity results deviate from the predicted results by an order of magnitude. Thus, the results from testing simple mixtures indicate that the results did not deviate significantly from additivity. Complex mixture results were more difficult to interpret. The toxicity of complex mixtures could not be accurately predicted based on chemical analysis. This could be due to chemical interactions or due to the presence of unidentified chemicals, such as alkyl PAHs or high molecular weight PAHs, that were not included in the risk assessment procedure. Even though the results from these *in vitro* studies indicate that additive assumptions will generally be accurate for simple mixtures, the risk associated with complex mixtures is more difficult to predict. Before the results of toxicity testing can be used to adjust risk assessment calculations, it is important to understand the mechanism of observed chemical interactions in animals chronically exposed to low doses of chemical mixtures. This research was supported by ATSDR Grant No. ATU684505.

670 ASSESSMENT OF WEIGHT-OF-EVIDENCE METHOD USING MIXTURES OF BENZENE, LEAD, METHYL MERCURY, AND TCE.

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A prospective *in vivo* study was conducted to evaluate a weight-of-evidence (WOE) method for assessing the joint toxicity of chemical mixtures. This methodology was used to predict interactions in liver, kidney, spleen, lymphocytes and hemoglobin. Groups of 5 rats were gavaged for 17 days with individual chemicals or their mixtures at mg/kg doses of benzene (200 or 400), lead [Pb] (50 or 100), methyl mercury [Hg] (0.25 or 0.75), and TCE (500 or 1, 500). Interactions were analyzed using both linear and quadratic models. For the kidney weight, less than additive statistically significant ($p=0.05$) interactions were observed for Hg and TCE that were consistent with the WOE methodology predictions. As predicted for the kidney, more than additive effects of Pb and benzene and less than additive effects of Pb and TCE based on response surface analysis (RSA) were observed, but the interactions were statistically insignificant. Contrary to prediction, no statistically significant interactions were observed in the kidney between Hg and Pb or between benzene and Hg. For the liver weight, the predicted less than additive interactions of Pb and TCE were observed with marginal significance ($p=0.08$). For the spleen weight, synergism was predicted for benzene and TCE, but the observations were statistically insignificant. Less than additive effects of Pb and TCE were observed to be significant ($p=0.001$). For the red blood cells, synergism between Hg and benzene, predicted based on the WOE method, was observed with marginal significance ($p=0.092$). For lymphocytes, antagonism was predicted for the mixtures of benzene with Pb or Hg, but were not significant. Only one of the RSA coefficients (TCE/Hg) was statistically significant ($p<0.05$), and it was found to be consistent with the WOE prediction. The WOE method appeared to be generally consistent with significant and marginally significant interactions.

671 HEPATOTOXICITY OF CHLOROFORM, ALLYL ALCOHOL, AND TRICHLOROETHYLENE TERNARY MIXTURE IS LESS THAN ADDITIVE.

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Unusual toxicity from exposure to complex mixtures is a concern in public health. The role of interactions was studied in the hepatotoxicity of a ternary mixture (TM) of chloroform ($CHCl_3$), trichloroethylene (TCE), and allyl alcohol(AA). Male S-D rats (250-300 g) received single ip injection of 5-fold dose range of $CHCl_3$ (74 to 370 mg/kg), TCE (250 to 1250 mg/kg) in corn oil and 7-fold dose range of AA (5 to 35 mg/kg) in distilled water simultaneously. Liver injury was assessed by plasma ALT activity, and tissue repair by ^{3}H-thymidine incorporation into hepatonuclear DNA. Parent compounds and two major metabolites of TCE [trichloroacetic acid (TCA) and trichloroethanol (TCOH)] were quantified by GC. Blood and liver $CHCl_3$ and AA levels after TM administration were similar to $CHCl_3$ or AA alone. However, the TCE levels in blood and liver were substantially lower after TM administration. Decreased TCE levels were consistent with decreased production of metabolites and its elevated urinary excretion. The antagonistic interaction resulted in less than additive joint hepatotoxicity. However, tissue repair was unaffected and showed a dose-response leading to regression of injury explaining the lack of dose-response for injury. Although the liver injury was lower and progression was compensated by timely tissue repair, 50% mortality occurred at the highest mixture concentration. This could be due to CNS toxicity. Studies are underway to investigate the cause of death. Since the mortality occurred only at highest combination and there was no interaction at lower combinations, the present study supports the additivity assumption used in risk assessment of chemical mixtures for short-term exposures to defined mixtures. In addition, these data underscore the critical role of tissue repair in the outcome of liver injury. However, the role of interactions following multiple exposures to complex mixtures in toxicity outcome remains unknown (supported by ATSDR U61/ATD 681482).

672 EVALUATION OF MODE OF ACTION IN ASSESSMENT OF CANCER RISK ASSOCIATED WITH EXPOSURE TO 1, 4-DICHLOROBENZENE.

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1, 4-Dichlorobenzene (1, 4-DCB) has been shown to produce renal tubular cell adenomas in male F344 rats and hepatocellular adenomas and carcinomas in male and female B6C3F1 mice following chronic oral exposure. Inhalation studies found

no tumors in rats or mice, but were inadequate as cancer bioassays due to less than lifetime exposure duration and failure to achieve the maximum tolerated dose. Several lines of evidence indicate that the renal tumors in male rats are a consequence of 2 -globulin nephropathy, a male-rat specific condition that is not predictive of carcinogenicity in humans. The mode of action by which 1, 4-DCB produces mouse liver tumors is uncertain. Genotoxicity data for 1, 4-DCB are primarily negative. Evidence for a non-genotoxic mechanism is mixed. Several studies have found that 1, 4-DCB produces a mitogenic response in mouse liver. A sustained proliferative response to damage produced by 1, 4-DCB in the liver is a plausible mode of action for induction of liver tumors by this compound. In the cancer bioassay, liver tumors were found only at dose levels at which hepatotoxicity was also observed. However, the evidence for this mode of action is incomplete. Several studies observed no liver damage at doses that produce proliferative responses. Other studies found that the mitogenic effect is not sustained with long-term exposure, and that the effect is similar in rats, which do not develop liver tumors, and mice, which do. Hyperplasia was not among the hepatic effects associated with 1, 4-DCB exposure in the mouse cancer bioassay. Under EPA's proposed cancer guidelines, a chemical such as 1, 4-DCB with a plausible, but not fully established, nonlinear mode of action can be assessed using both linear and nonlinear dose-response approaches. The oral slope factor derived using the default linear dose-response methodology was compared with the results of the non-linear margin of exposure analysis. The results show how information regarding mode of action can influence the risk assessment of chemical carcinogens.

673 A THRESHOLD LINKAGE BETWEEN CHLOROFORM-INDUCED CELLULAR DAMAGE AND CYTOLETHALITY PROVIDES A BETTER FIT TO HEPATIC LABELING INDEX DATA THAN A LINEAR LINKAGE.

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Chloroform is carcinogenic to rat kidney and mouse liver and kidney in a manner dependent on dose, route of administration, gender, and animal model. Extensive experimental evidence indicates that tumor induction is secondary to events associated with toxicity and regenerative cell proliferation rather than to direct genotoxicity. In this study, a mode of action-based model was used to evaluate alternative dose-response linkages between chloroform-induced cellular damage and cytolethality. An established PBPK model for chloroform was extended as follows. Cellular damage is created at a rate proportional to the rate of chloroform metabolism. A saturable repair process removes the damage. The cellular death rate is a function of the amount of damage. A mechanism is described whereby the cell division rate increases to replace cells lost due to cytolethality, allowing for simulation of the labeling index (LI). LI data from a chloroform inhalation $C \times t$ (concentration $\times$ time) study in female B6C3F1 mice were simulated (Constan et al., Toxicol. Sciences. 66, 201-208, 2002). Two alternative linkages between damage and death rate were evaluated: threshold and low-dose linear. After formal optimization of parameter values, the log-likelihood ratios of the two models were compared. A X^2 statistic test showed that the threshold model provided a better fit to the data than the linear model at the $\alpha = 0.01$ significance level (df = 1). This result shows that dose-response data for chloroform-induced cytolethality in female B6C3F1 mice are preferentially described by a threshold model. Thresholds are generally thought to exist for chemically-induced cytotoxicity. The model described here thus appears relevant in the development of a mode of action-based human cancer risk assessment for chloroform.

674 NONMONOTONIC DOSE-RESPONSE RELATIONSHIPS: MECHANISTIC BASIS, KINETIC MODELING, AND IMPLICATIONS FOR RISK ASSESSMENT.

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Dose-response curves for first-line interactions of a chemical with a biochemical target molecule are usually monotonic. However, for reactions of a complex biological system to a toxicant, nonmonotonic dose-effect relationships can be observed. This phenomenon has been accepted for a number of pharmacological examples, but is met with skepticism in toxicology, because it could mean that low dose of a high-dose toxicant may have beneficial effects. We present four examples to demonstrate that non-monotonic dose-response relationships can result from superimposition of monotonic dose responses of component biological reactions. Examples include (i) a membrane receptor model with receptor subtypes of different ligand affinity and opposing downstream effects (adenosine receptors A1 vs. A2), (ii) androgen receptor-mediated gene expression driven by homodimers, but not heterodimers formed with an exogenous ligand, (iii) repair of background DNA damage by enzymatic activity induced by adducts formed by a xenobiotic, (iv) rate of cell division modulated by cell-cycle delay at low-level DNA damage vs. cell-cycle acceleration due to regenerative hyperplasia at cytotoxic dose levels. Quantitative analyses based on biological models and physiological kinetics will be shown, with particular emphasis on the critical factors that determine the degree of nonmonotonicity. Statistical analysis indicates that continuous data are better suited than quantal data for the investigation of the low-dose part of the curve and that the higher the background effect level, the smaller the sample size required to detect significant nonmonotonicity. It is important to note that weakly nonmonotonic dose-response curves can be misinterpreted as being thresholded. Our analysis should promote a scientific discussion of a concept termed "hormesis", and encourage reconsideration of default procedures for low-dose extrapolation in toxicological risk assessment.

675 A RISK ASSESSMENT FOR INHALED ARSENIC BASED ON URINARY ARSENIC CONCENTRATION USING A PHARMACOKINETIC MODEL.

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Several epidemiological studies of copper smelter workers have demonstrated an association between inhalation exposure to arsenic in dust and increased lung cancer mortality. However, the nature of the dose-response relationship for the carcinogenicity of inhaled arsenic remains a matter of controversy. In particular, the dose-response from a study of workers at a copper smelter in Tacoma, Washington, was reported to be nonlinear, with a potency that decreased with increasing arsenic exposure. We considered it possible that this apparent nonlinearity was due to the impact of differences in particle size distribution and personal protective equipment use on arsenic bioavailability at high versus low dust levels In order to avoid the uncertainties associated with predicting the relationship between airborne dust burden and the bioavailable dose of arsenic, we have evaluated the dose-response relationship between lung cancer mortality and urinary arsenic concentrations in three published studies of workers at the Tacoma, Washington, smelter. In contrast with the nonlinear dose-response relationship reported for lung cancer and airborne arsenic, the dose-response relationship for lung cancer and urine concentration was found to be linear. A physiologically based pharmacokinetic (PBPK) model of arsenic was then used to estimate the urinary arsenic concentration associated with a given airborne arsenic concentration and particle size distribution. Risk estimates for environmental exposure were in the same range as the current EPA inhalation risk estimates in IRIS. However, the use of the PBPK approach makes it possible to incorporate empirical data on the bioavailability of arsenic for specific exposures of concern. As an example, use of data on the relative bioavailability of arsenic in fly ash as compared to copper smelter dust resulted in risk estimates for inhalation of arsenic in fly ash dust several fold lower than those obtained with the default EPA inhalation slope factor.

676 ESTIMATION OF METHYLMERCURY EXPOSURES IN US WOMEN OF CHILD-BEARING AGE USING MARKOV CHAIN MONTE CARLO ANALYSIS WITH A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL.

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A Bayesian approach known as Markov Chain Monte Carlo (MCMC) analysis was applied, together with a physiologically based pharmacokinetic (PBPK) model of methylmercury (MeHg), to evaluate the variability of MeHg exposure in the US population. The analysis made use of the newly available National Health and Nutrition Survey (NHANES) blood and hair mercury concentration data for women of childbearing age (16-49 years). Bayesian analysis was performed to estimate the population variability in MeHg exposure (daily ingestion rate) implied by the variation in blood and hair concentrations of mercury in the NHANES database. The measured variability in the NHANES blood and hair data represents the result of a process that includes inter-individual variation in exposure to MeHg and interindividual variation in the pharmacokinetics (distribution, clearance) of MeHg. The PBPK model includes a number of pharmacokinetic parameters (e.g., tissue volumes, partition coefficients, rate constants for metabolism and elimination) that can be varied from individual to individual within the subpopulations of interest. Using MCMC, it was possible to combine prior distributions of the PBPK model parameters with the NHANES blood and hair data, as well as with kinetic data from controlled human exposures, to MeHg, to derive posterior distributions that refine the estimates of both the population exposure distribution and the pharmacokinetic parameters. In general, the results of the MCMC analysis indicate that

a small fraction, on the order of ten percent, of the US population of women of child-bearing age may have mercury exposures greater than the EPA Reference Dose for MeHg of 0.1 micrograms per kilogram per day, and that a few percent may have exposures greater than the ATSDR MRL of 0.3 micrograms per kilogram per day. However, the predicted exposure distribution was sensitive to some assumptions and decisions made with regard to the formulation of the MCMC analysis.

677 FURTHER DEVELOPMENT OF A PBPK MODEL FOR GASOLINE USING A CHEMICAL LUMPING APPROACH.

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Gasoline consists of a few toxicologically significant components and a large number of other chemicals in a complex mixture. We have developed a method for assessing the pharmacokinetics (PKs) of selected target components (benzene, toluene, ethyl benzene, o-xylene (BTEX) and n-hexane) in gasoline by conducting gas uptake PK studies and physiologically based PK (PBPK) modeling of whole gasoline in rats. The PBPK model tracks a lumped chemical group representing all non target components, and includes competitive metabolic inhibition between all target compounds and the lumped chemicals. Gas uptake studies were performed with male F344 rats in a closed chamber, fitted with a metal ring and oxygen and CO_2 probes. NaOH scrubber solution did not adequately control CO_2 levels, increasing the rat's breathing rate, but solid Baralyme adsorbents did. Chamber air samples were analyzed every 10-20 minutes by gas chromatography/flame ionization detection and all non target chemicals were co-integrated. A 4-compartment PBPK model for gasoline was constructed using the BTEX, n-hexane, and the lumped chemical data. Estimates of kinetic parameters for target chemicals were refined by studies with either the single chemical or all five chemicals together. When o-xylene was present at high levels, alveolar ventilation decreased, consistent with respiratory irritation. A six-chemical model with the lumped chemical group was used to estimate lumped chemical parameters for a winter blend of gasoline with methyl t-butyl ether and a summer blend without oxygenate. Our model adequately simulated the data for all chemicals. The preliminary model indicated that total metabolism of components would be reduced by 4%-27% for a six hour exposure to 300 ppm gasoline. [Supported in part by a Cooperative Agreement from ATSDR (U61/ATU 881475) and the NIEHS Quantitative Toxicology Training Grant T32 ES07321].

678 IDENTIFICATION OF GASOLINE-RELATED AIR POLLUTANTS WITH HIGH EXPOSURE POTENTIAL AND/OR TOXICOLOGICAL CONCERN.

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A baseline assessment of cancer and chronic respiratory health risks in California that can be attributed to air pollution resulting from the use of gasoline is being conducted. The assessment focuses on the period from 1996 to 1998, when California Reformulated Gasoline 2 (CaRFG2) was first introduced. This presentation reports on the results of the hazard identification component of the assessment. Because adequate health effects data on gasoline-related mixtures (e.g., gasoline engine exhaust) were unavailable, the assessment focused on the individual components of gasoline-related air pollution. Speciation profiles of gasoline-related sources generated by the California Air Resources Board were used to identify gaseous organic pollutants. Literature reports on the atmospheric chemistry of these emitted substances were reviewed to identify additional pollutants likely to be present in ambient air as breakdown products. Criteria air pollutants emitted from gasoline-related sources were also identified. The list of more than 300 gasoline-related substances was then screened for exposure potential and toxicological concern. The non-methane organic gas-phase pollutants with the highest primary emissions, a surrogate for exposure potential, were identified. Some of these high emitters were also recognized as carcinogens or respiratory toxicants. Toxicology data were unavailable for the majority of the gasoline-related substances. Using the limited toxicology database, gasoline-related substances having the potential to cause cancer or chronic respiratory toxicity were identified. The results of the hazard identification indicated that 49 gasoline-related substances had high exposure potential and/or toxicological concern. Of these, 14 substances were identified as carcinogens, 17 as recognized chronic respiratory toxicants, and 17 as suspected chronic respiratory toxicants.

679 APPLICATION OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL TO THE CALCULATION OF A REFERENCE CONCENTRATION (RfC) FOR XYLENES.

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Physiologically-based pharmacokinetic (PBPK) models for inhalation exposure to xylene have been developed for both rats and humans. These models consist of five tissue compartments, representing the lung, adipose, liver/metabolism, slowly-perfused tissues, and richly-perfused tissues, and predict concentration in the tissue components, arterial blood, and venous blood. Based on a duration-adjusted NOAEL for neurological effects identified by a subchronic study in rats, several approaches for calculation of a human equivalent concentration (HEC), to be used in the calculation of an RfC, are being considered. The default approach for calculation of a HEC for a category 3 gas, based on USEPA's 1994 RfC guidelines, would be to calculate the HEC using the ratio of the blood-gas partition coefficients for the animal species and humans to adjust the duration-adjusted NOAEL. An alternate approach involves application of the PBPK model to calculate the HEC. The duration-adjusted NOAEL from the rat experimental data was used as the exposure concentration for the PBPK model, and pooled venous blood concentration was predicted; venous blood concentration was used as the dose metric (because validation experiments in rats and humans were most often performed using measurements of venous blood). The concentration in the venous blood at steady state (which occurred at ~20 hours for the rat) was used in the human model to calculate an airborne concentration of xylene that would result in the same venous blood concentration following continuous exposure. Calculation of RfCs using the two approaches result in similar numerical values. A discussion of the areas of uncertainty using each approach, and other possible approaches to reducing uncertainty in the RfC, is provided. (This document does not necessarily reflect EPA policy)

680 A CADMIUM PHARMACOKINETICS/PHARMACODYNAMICS (PKPD) MODEL FOR USE IN RISK ASSESSMENT.

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Risks of kidney toxicity in humans exposed to cadmium from the ingestion pathway can be estimated using a PKPD model. The model was used to interconvert external and internal cadmium dose estimates from epidemiological studies, allowing direct comparison of dose-response relationships across studies that might otherwise be incomparable. Based on estimates from 15 epidemiological studies of diverse exposures (occupational, general environmental, environmental contamination), the median kidney cadmium level associated with a 0.1 probability (K10) of low molecular weight proteinuria was predicted to be 108 μg Cd/g renal cortex (95% confidence limits: 70-240). The model predicted that the lower confidence limit on the K10 (K10L, 70 μg Cd/g cortex) would be reached at age 55 years (the age at which kidney cadmium levels are predicted to peak) with a constant chronic intake of 1.1 μg/kg/day in females or 2.5 μg/kg/day in males. A lower cadmium intake in females is predicted to result in a similar kidney cadmium level as in males because females were assumed to absorb a larger fraction (10% vs 5%) of an ingested cadmium dose (Choudhury et al., 2001). The K10L exceeded the peak kidney cadmium level (at age 55 years) predicted to result from the average dietary intake of cadmium in US non-smokers (28 μg Cd/g cortex in female, 14 μg Cd/g cortex in males), and also exceeded the 95th percentile kidney cadmium levels predicted to result from dietary intakes in the US (43 μg Cd/g cortex in females or 23 μg/g cortex in males). That these values are lower than the K10L, suggests that, for most of the US population, risks of kidney toxicity from dietary intake of cadmium are negligible. (Views expressed in this report are those of the authors and do not necessarily represent the views or policies of the USEPA.)

681 THE CONCEPT OF BENCHMARK INTERNAL CONCENTRATION (BMIC) IN RISK ASSESSMENT.

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The concept of the benchmark internal concentration (BMIC) is presented as an appropriate alternative to derive risk-screening levels for chemicals which express toxicity that can be related to internal concentrations of the parent compound or a metabolite in body tissues or fluids. In certain cases, internal concentration of an agent may be more directly related to hazard and risk than measured or estimated external dose or exposure concentration. The authors illustrate this concept using data from toxicological studies of a non-metabolizable, poorly-eliminated compound that expresses cumulative toxicity, perfluorooctanesulfonate (PFOS). PFOS

has been found widely distributed in serum and liver samples from human and wildlife populations. Although the major producer (3M) has largely phased-out production, exposure to PFOS may have come from a variety of sources including its use as a surfactant, or through degradation of related chemistries that have been used in wide variety of commercial and consumer applications. This fact makes estimating external exposure for risk assessment problematic. Serum and liver PFOS concentrations represent an integration of exposures over time and can be directly related to toxicity. Most toxicology studies of PFOS have included evaluation of serum and liver tissue PFOS concentrations. The relationship of effect to external dose versus serum or liver PFOS concentration in toxicology studies suggest that toxicity is more a function of cumulative body burden than magnitude of external dose. In this illustration, benchmark doses from a variety of PFOS toxicology studies were calculated based on external dose-response, and corresponding BMICs were calculated based on the relationship of serum or liver PFOS concentration to effect. The BMIC provides a risk-based means of assessing margin of exposure based on population monitoring data.

682 ABSORPTION, DISTRIBUTION AND CLEARANCE OF 2, 6-DI-*TERT*-BUTYL-4-NITROPHENOL (DBNP), A SUBMARINE ATMOSPHERIC CONTAMINANT.

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The nitrophenol, 2, 6-di-tert-butyl-4-nitrophenol (DBNP), is a submarine atmospheric contaminant. DBNP is produced when atmospheric oil mist from synthetic turbine lubricants containing the antioxidant additive 2, 6-di-tert-butylphenol (DNP) is nitrated during passage through the air filtration system. Submarine personnel can be exposed to DBNP for periods up to 6 months 24 hours a day during missions. While dermal or respiratory exposure may provide little human risk due to the minimal solubility of DBNP, there is a possible risk from the oral ingestion of the compound. For this reason, the present study measured the absorption, distribution and clearance of radiolabeled DBNP in rats following a single oral gavage exposure (in a 99.2% canola oil:0.8% DMSO vehicle). Sets of 16 rats were given a single oral dose of vehicle, 15, or 40 mg/kg DBNP. Two rats per dose were sacrificed daily from 1-11 days following the single exposure. Nine different tissue samples, as well as blood, urine, and feces, were collected for subsequent scintillation counting. It was clearly demonstrated that these doses of DBNP are readily absorbed from the gastrointestinal tract and differentially distributed to a wide range of tissues based upon their lipid content. The clearance of DBNP following oral ingestion involves a complex pattern where approximately 30% is excreted as the parent compound and the remaining 70% being absorbed from the GI tract into the blood. It is then metabolized to a glucuronide conjugate and eliminated through the urine and bile. DBNP has generally been eliminated from the body within 72-96 hours post exposure and is primarily cleared through the feces within 4 days. Because DBNP is deposed in various organ compartments for up to 96 hr, the possibility of significant organ accumulation in repeatedly exposed personnel must be considered in risk assessment.

683 EVALUATION OF THE GENOTOXIC POTENTIAL OF STYRENE.

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Styrene is a widely used monomer in the plastics industry. There has been considerable attention and scientific debate regarding the potential genotoxic effects in workers occupationally exposed to styrene. The genotoxicity of styrene in *in vitro* and *in vivo* assays was studied. Clastogenic effects in the peripheral blood lymphocytes of styrene-exposed workers were also evaluated. Styrene, *via* metabolism to styrene-7, 8-oxide (SO), is clastogenic *in vitro* and may have clastogenic activity *in vivo* at near lethal, exposures administered by i.p. injection. The results of *in vivo* studies demonstrated that inhalation exposure to styrene results in the formation of SO-associated DNA adducts; however, there was no indication of a genotoxic effect. The results of the human lymphocyte studies were difficult to interpret given that: the statistical significance of the observed effects was often marginal; dose- and/or temporal response relationships often were missing; confounding variables could not be excluded; and, concomitant exposures to other chemicals may also have occurred. No definitive causal relationships could be drawn from the study of lymphocytes from styrene-exposed workers. Overall, the cytogenetic studies in occupationally exposed persons were inconclusive. Analysis of the blood lymphocytes of styrene-exposed workers did, however, reveal the presence of DNA- and/or protein-adducts that occur from exposure to styrene and/or SO. The formation of DNA adducts per se does not lead to a conclusion of mutagenic activity. Only adducts that are made permanent, and which disrupt normal DNA replication, transcription, and translation processes can produce "mutagenic" effects. The significance, if any, of SO-specific adducts has yet to be determined.

684 STYRENE TOXICITY IN HEPG2 AND HEP3B CELLS.

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Environmental, occupational and recreational exposures to carcinogens contribute to cancer risk in humans. Styrene is one of the most important organic chemicals. It is mainly produced to prepare solid polystyrene foam, expanded polystyrene foam and styrene-butadiene rubber. It has been shown to be genotoxic after metabolic activation and to induce cytogenetic effects in many experimental systems. The toxic effect of styrene on HepG2 (wild-type p53) and Hep3B (deleted p53) cells was analysed. Both cell lines are considered to be metabolically competent to activate different classes of mutagens into biologically active metabolites. We exposed HepG2 and Hep3B cell lines at subtoxic concentrations (up to 0.5mM) of styrene to analyse the expression of genes and proteins involved in apoptosis and cell cycle regulation. We performed a cDNA macroarray on HepG2 cells and found an overexpression of TGFβ2, TGFβ receptor III and c-jun proto-oncogene. We also analysed the protein levels of Bax (pro-apoptosis) and Bclx-L (anti-apoptosis) in Hep3B and HepG2 cell lines treated with styrene. The endogenous level of Bclx-L increased according with the increasing concentration of the chemical. On the contrary, Bax protein level did not change in treated and untreated cells. Cell cycle analysis revealed no significant changes in cells treated with styrene at 0.5mM for 48 hours. Our data suggest that the activity of styrene on cell proliferation/cell death should be monitored as an early endpoint of exposure.

685 USING MECHANISTIC DATA FOR ASSESSING CANCER MODE OF ACTION: 1, 3-DICHLOROPROPENE AS A CASE STUDY.

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There are increasing opportunities for mechanistic toxicology data to be a driving force in the outcome of cancer risk assessments. This advancement is clearly shown by proposed changes in the US Environmental Protection Agency (EPA) cancer risk assessment guidelines published in 1999. However, despite the availability of updated EPA guidelines, published human health risk assessments have been slow to move from default dose-response approaches. We conducted a critical analysis of the mechanistic data for 1, 3-dichloropropene, the active ingredient of the pesticide Telone II, as a case study to identify key uncertainties that might be responsible for differences in the outcomes of recent cancer risk assessments for this chemical. Key uncertainties included (1) determining the relative contribution of alternative metabolic pathways at environmentally-relevant doses, (2) identifying secondary responses that link the putative key precursor event of GSH depletion to increased tumor formation, (3) relating available short-term mechanistic data to chronic effects such as tumorigenesis, and (4) resolving questions about the *in vivo* genotoxic potential of 1, 3-dichloropropene in the face of mixed results in genotoxicity assays. Resolution of these data gaps is needed to meet USEPA criteria for moving away from the default dose-response approach of assuming linearity in the quantitative dose-response analysis. Ongoing experiments designed to resolve these key data gaps are described. The issues and data gaps identified here in the context of 1, 3-dichloropropene are similar to those of many other chemicals. Thus, the results of this analysis are useful in communicating to mechanistic toxicologists the types of data that are needed to present a persuasive argument for moving away from default assumptions of low-dose linearity in conducting cancer risk assessments.

686 ESTIMATION OF THE CARCINOGENIC POTENTIAL OF POLYCYCLIC AROMATIC HYDROCARBONS.

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Numerous polycyclic aromatic hydrocarbons (PAHs) are known to cause cancer in experimental animals. A subset of these compounds (chrysene, 5-methylchrysene, dibenzo[a, h]pyrene, dibenzo[a, i]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[a]pyrene) have produced tumors in the neonatal mouse model. In the published studies, dating from 1982 to 1994, mice developed lung and liver tumors following intraperitoneal injection in the early days of life. The route and time of administration used in these studies has presented a challenge for the estimation of carcinogenic potency for these PAHs by

the oral exposure route. Our approach has been to apply a dose correction for variable dosing over time based upon the model of Doll and Armitage (1954) and further developed by Crouch (1983). Estimation of potency also accommodated data sets for which the response was 100%, a special case in which the whole-life quantal multistage model fails. Where tumors developed at multiple sites, a combined distribution representing cancer potency for all sites affected by treatment with the carcinogen was derived through Monte Carlo analysis. In order to calibrate the extrapolation to the oral route, results from studies of the carcinogenicity of benzo[a]pyrene were used as an "internal standard, " since this PAH has been examined both in oral and intraperitoneal injection studies. The resulting oral potency estimates in animals for the PAHs under consideration here range from 0.83 to 140 $(mg/kg\text{-}day)^{-1}$.

687 DEVELOPMENT OF CANCER POTENCY ESTIMATES FOR CALIFORNIA'S PROPOSITION 65.

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The Office of Environmental Health Hazard Assessment (OEHHA) develops cancer potency estimates for carcinogens listed under California's Safe Drinking Water and Toxic Enforcement Act of 1986, better known as Proposition 65. Cancer potencies - defined as the slope of the dose response curve (daily dose versus lifetime cancer risk) in the low dose region - are used to estimate risk-specific intake levels, which facilitate compliance with the law. For carcinogens that induce cancers at multiple sites, Monte Carlo analysis is used to sum cancer potencies calculated for different treatment related cancer sites, according to their probability distributions. The upper 95 percent confidence bound from the combined distribution of the sum of the potencies is taken as the multisite cancer potency. The potency derivation takes into account body size differences between humans and experimental animals. Recent cancer potency assessments for the following carcinogens are posted on the OEHHA website (www.oehha.ca.gov). Cancer potencies in units of (mg/kg-d)-1 are: 0.63 for benzofuran, 1.0 for carboxymethyl-N-nitrosourea, 3.7 for 3, 3'-dimethoxybenzidine dihydrochloride, 12 for 3, 3'-dimethylbenzidine dihydrochloride, 49 for NNK, 3.9 for 5-morpholinomethyl-3[(5-nitrofurfurylidene)-amino]-2-oxazolidinone, 0.17 for carbazole, 0.0047 for chloroethane, 0.0023 for di(2-ethylhexyl)phthalate (DEHP), 0.095 for isobutyl nitrite, 0.047 for lead (oral), 0.030 for lead acetate (oral), 0.012 for lead phosphate (oral), 0.017 for lead subacetate (oral), 1.5 for MeIQ, 1.7 for MeIQx, 0.0044 for methyl carbamate, 12 for methylhydrazine (oral), 7.8 for methylhydrazine (inhalation), 25 for 2-methylaziridine, 6.4 for MX, 0.025 for nalidixic acid, 0.016 for o-phenylenediamine dihydrochloride, 0.21 for p-chloro-o-toluidine, 0.14 for phenyl glycidyl ether, 0.68 for phenylhydrazine, 0.00060 for polygeenan, 12 for tetranitromethane, 0.029 for trimethyl phosphate, and 0.0063 for 2, 6-xylidine.

688 USE OF BENCHMARK CONCENTRATION METHODOLOGY IN RISK ASSESSMENT FOR AIR TOXICS.

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In risk assessment for non-cancer toxicity a NOAEL, or failing that a LOAEL, has generally been identified by inspection of toxicity data. Uncertainty factors are then applied to derive health protective levels. The LOAEL/NOAEL procedure suffers from reliance on a small portion of the available data, inability to quantify uncertainty, and susceptibility to experimental design factors. The benchmark dose (BMD) methodology was developed to help address these failings. The BMD methodology uses likelihood-based curve fitting to estimate a BMD or concentration (BMC), i.e. the dose to achieve a chosen incidence criterion (e.g. 5% or 10% incidence of the effect of interest), or its lower confidence bound. It avoids the statistical problems of the LOAEL/NOAEL methodology, but requires appropriate definition of the benchmark. The California Air Toxics Hot Spots program used this methodology to develop twelve chronic Reference Exposure Levels (cRELS), allowing comparison with the earlier approach in practical cases. The benchmark used (BMC05) was the lower 95% confidence bound on the 5% effect concentration. This reflects uncertainty and variability in the source data, and lies within or close to the range of those data. The relationship to the previously used criteria needs to be established in order to select uncertainty factors. In four cases, a NOAEL was also available, and the BMC05 was found to be close to the NOAEL (NOAEL/BMC05: chloropicrin 2.38, methanol 3.28, MDI 0.8, phosphoric acid 0.78). In the eight other cases, no NOAEL could be determined, but the overall ratio of the LOAEL to the BMC05 for all twelve compounds was 6.8, which is close to the acute LOAEL/NOAEL ratio observed by Alexeeff et al. (1997). In view of this concordance, similar uncertainty factors were used in deriving a cREL from a BMC05 as would apply to a NOAEL based on the same data. Use of the BMC05 resulted in a more reliable estimate, with defined confidence bounds, especially in cases with data quality problems, e.g. lack of an observed NOAEL.

689 NEW APPROACHES FOR DERIVING A REFERENCE CONCENTRATION FOR METHYL ETHYL KETONE.

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MEK is listed on IRIS with a reference concentration (RfC) of 1 mg/m^3 based on developmental effects (decreased fetal body weight and increased incidence of misaligned sternebrae) reported in CD-1 mice exposed 7 h/day on gestation days 6-15 (Schwetz et al., 1991). This derivation used a NOAEL/LOAEL approach. A review of the available toxicity data for methyl ethyl ketone (MEK) uncovered no new data for inhalation exposures. Current EPA guidance provides for a number of alternative approaches to derive an RfC. A benchmark dose approach to derivation of an RfC for MEK was explored using the same data for developmental effects in CD-1 mice, as well as data for increased incidence of extra ribs in Sprague-Dawley rats as reported by an inhalation developmental study (Deacon et al., 1981). Modeling of these data and calculation of benchmark concentrations (BMC) and 95% confidence limits on the BMC (BMCL) identified points of departure for each endpoint. The points of departure for each endpoint fall between the NOAEL and LOAEL observed in the respective developmental studies; thus, all are equally plausible. The available pharmacokinetic, pharmacodynamic, and mechanism of action data do not provide sufficient evidence to argue convincingly for either peak exposure level or area under the curve as the most appropriate surrogate metric for internal effective dose; consequently, duration adjustment was also explored in order to time-weight the exposures used in the critical studies for each endpoint. In addition, an adjustment can be made to account for the difference in the blood:air absorption coefficient between rats and humans based on literature reports. This exercise identifies and explores a number of alternative approaches to derive an RfC for MEK, which primarily reflects new approaches to RfC derivation, rather than new data. The impact of each of these decisions on the RfC will be presented.

690 UPDATED PHENOL REFERENCE DOSE: CONSIDERATIONS IN APPLYING IMMUNOTOXICITY DATA AND THE ROLE OF ENDOGENOUS PRODUCTION.

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Phenol is an industrially-important chemical and is produced in bulk in the USA. The toxicity data for phenol have been reassessed. A reference dose (RfD) of 0.3 mg/kg-day has been derived based on a benchmark dose from a developmental toxicity study (Argus Research Laboratories, 1997). This RfD replaces the previous RfD of 0.6 mg/kg-day, which was based on the NOAEL from an earlier developmental toxicity study (Argus Research Laboratories, 1997). This RfD replaces the previous RfD of 0.6 mg/kg-day, which was based on the NOAEL from an earlier developmental toxicity study (NTP, 1983a), on the USEPA Integrated Risk Information System (IRIS) in 1989. Updating the assessment required delving into key scientific issues that have not been fully developed in the risk assessment community. For example, several newer studies suggested that phenol is immunotoxic. The updated phenol assessment provides a systematic examination of these data as an example of the level of analysis required to determine the adversity of immunotoxicity endpoints. This issue was resolved through the discussion of an uncertainty factor to address immunotoxicity. The assessment also presents an approach, and relevant discussion of alternatives, for deriving risk values for chemicals that are produced endogenously. The issue of endogenous production was addressed by applying the RfD to ingested phenol in addition to the normal daily endogenous level of production. Resolution of these issues as presented in the reassessment of phenol provides key learnings that are relevant for many other chemicals of environmental interest. Key words: RfD, phenol, endogenous production

691 SHORT-TERM INHALATION TOXICITY BENCHMARK FOR NICKEL OXIDE.

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EPA requires that short-term or acute health effects be considered from inhalation of compounds as part of the multi-pathway risk assessments that are conducted for hazardous waste combustion units. EPA has recommended a hierarchy of sources for acute toxicity benchmarks that are intended to protect the general public from discomfort or mild adverse health effects over one-hour exposure periods. EPA has

recommended the California EPA reference exposure level (REL) for nickel and nickel compounds of 6 ug/m3 as the acute criterion for evaluating potential short-term inhalation health effects associated with nickel emissions. California EPA's REL for nickel compounds is based on toxicity studies conducted on nickel sulfate. Although the nickel REL is being applied to all nickel compounds, there is considerable evidence indicating that different nickel compounds have different levels of toxicity when inhaled. Nickel emissions from hazardous waste combustion units are primarily in the form of oxides of nickel (NiO, NiO2, and Ni2O3). A literature analysis revealed that an alternative acute toxicity benchmark for nickel oxide is appropriate for use in evaluating potential acute toxicity associated with inhalation of nickel emitted from hazardous waste combustors, particularly if site-specific data support that nickel emissions are primarily in the form of nickel oxides. Based on several toxicity studies, an alternative acute toxicity benchmark was developed for nickel oxide.

692 ACUTE AND CANCER RISK ASSESSMENT VALUES FOR *TERT*-BUTYL ACETATE.

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Tert-butyl acetate (TBAc) is an industrial chemical with potential uses as a degreaser and in architectural coatings. Limited toxicity data exist for TBAc. However, acute inhalation exposure data are available for TBAc. Additionally, although no TBAc carcinogenicity data are available, TBAc has been demonstrated to be substantially metabolized to *tert*-butanol (TBA) in rats. TBA genetic toxicity data are mixed, but information from a positive genotoxicity study for TBA suggests that TBA may cause oxidative DNA damage. TBA has been shown to induce tumors in both rats and mice, and OEHHA has calculated an oral cancer slope factor (CSF) for TBA of 3×10^{-3} $(mg/kg\text{-}day)^{-1}$. This raises a concern that exposure to TBAc may result in a cancer risk because of the metabolic conversion to TBA. It has been proposed that the TBA carcinogenicity data may not be relevant to human cancer risk assessment because 1) the male rat kidney tumors are the result of TBA-induced α2u nephropathy, a pathological effect specific for male rats, and 2) the female mouse thyroid follicular cell tumors may be due to an effect on thyroid hormone levels, which might have a threshold above the level of expected human exposures. However, in our opinion these data are insufficient to allow the determination that the TBA carcinogenicity data are not relevant to human cancer risk assessment. Therefore, TBAc should be considered to pose a potential cancer risk to humans. An acute one-hour Reference Exposure Level (REL) of 1 mg/m^3 (0.21 ppm) can be calculated from the extrapolated No Observed Adverse Effect Level (NOAEL) of 50 mg/m^3 (11 ppm) using a Haber's Law exposure time correction. A CSF of 0.002 $(mg/kg\text{-}day)^{-1}$ can be derived for TBAc, assuming 100% metabolism of TBAc to TBA and a molar conversion factor of 0.64 (TBA mw 74.12 / TBAc mw 116.16). An inhalation unit risk value for TBAc of 4×10^{-7} $(\mu g/m^3)^{-1}$ can then be derived from the CSF value for TBAc by assuming a human breathing rate of 20 m^3/day, 70% fractional absorption, and an average human body weight of 70 kg.

693 CYCLIC ACID ANHYDRIDES AS OCCUPATIONAL SENSITISERS - A NORDIC EXPERT GROUP CRITERIA DOCUMENT.

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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) is a Nordic collaboration mainly financed by the Nordic Council of Ministers and the Swedish National Institute for Working Life. The main task is to produce criteria documents to be used by the regulatory authorities of the Nordic countries as the scientific basis for setting occupational exposure limits (OELs) for chemical substances. The actual setting of an OEL is a national concern. The NEG and the Dutch Expert Committee on Occupational Standards (DECOS) have jointly produced a criteria document on cyclic acid anhydrides (CAA) (Keskinen H, accepted for publication in Arbete och Halsa). CAAs are known as potent occupational sensitizers. Since the first published case of asthma due to phthalic anhydride (PA) in 1939, occupational asthma and rhinitis due to several CAAs have been reported. Contact urticaria is more usual than allergic contact exzema. In addition to their sensitizing properties CAAs, are also irritants of the skin and mucous membranes. IgE-mediated allergy due to several CAAs has been demonstrated in exposed workers as well as in animal studies. Apart from the allergic reactions, rhino-conjunctivitis due to irritation without sensitization has been reported frequently. The sensitising potency of CAAs varies greatly. According to the dose-response studies available, trimellitic (TMA), hexahydrophtalic (HHPA), and methyltetrahydrophthalic anhydride (MTHPA) seem to be more potent sensitizers than PA and tetrachlorophthalic anhydride (TCPA). In conclusion, there is a need to re-evaluate the present OELs in the Nordic countries and to establish OELs for the more recent anhydrides.

694 PROVISIONAL TOXICITY VALUES FOR 0-NITROTOLUENE FOR THE HEALTH EFFECTS ASSESSMENT SUMMARY TABLE (HEAST).

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EPA is updating toxicity values for chemicals listed in the 1997 HEAST. The previous assessment for o-nitrotoluene (o-NT) included a subchronic RfD of 1E-1 mg/kg-day and a chronic RfD of 1E-2 mg/kg-day, based on a LOAEL of 200 mg/kg for splenic lesions in a 6-month rat gavage study. Subchronic and chronic NTP feeding bioassays in rodents provide a basis for deriving new toxicity values for o-NT. In the subchronic assay, a LOAEL of 44 mg/kg-day was identified for increased levels of methemoglobin in female rats after 3 weeks; this LOAEL was considered minimal because other erythrocytic effects (anemia, splenic lesions) were limited to the higher doses. A provisional subchronic RfD of 4E-2 mg/kg-day was derived from the minimum LOAEL. A significant finding in the subchronic assay was the occurrence of malignant mesotheliomas in the epididymal tunica vaginalis in rats exposed at 353 mg/kg-day for 13 weeks. Similar mesotheliomas and a hepatic cholangiocarcinoma were observed in rats fed 292 mg/kg-day for 13 or 26 weeks in another assay. No provisional chronic RfD was derived because frank effects were observed at the lowest tested dose in the chronic feeding assay (25 mg/kg-day). In the chronic assays, clear evidence for multiple-organ carcinogenicity was observed: subcutaneous skin fibroma/fibrosarcoma in male and female rats, mammary fibroadenoma in female rats, hemangiosarcoma in male and female mice, and hepatocellular adenoma/carcinoma in female mice. o-NT is a genotoxic carcinogen following bioactivation; direct evidence for mutagenicity in cell regulatory genes (p53, K-ras, and -catenin) was shown in mice exposed for 2 years. Dose-response modeling was performed based on the most prominent tumor types. The provisional oral slope factor of 2.3E-1 (mg/kg-day)-1 was based on the incidence of skin tumors in male rats, the tumor type associated with the highest numerical risk. (This abstract does not necessarily reflect EPA policy.)

695 COMPARISON OF THE NUTRITIONAL REQUIREMENT AND RISK ASSESSMENT FOR ESSENTIAL TRACE ELEMENTS (ETES) BY THE INSTITUTE OF MEDICINE (IOM) AND THE USEPA: ZINC A CASE STUDY.

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Recently, the IOM has reviewed the nutritional requirement and toxicity data for the ETEs. In addition to the Recommended Dietary Allowance (RDA), a Tolerable Upper Intake Level (UL) has been derived and published for the ETEs. The UL is somewhat analogous to the USEPA's Reference Dose (RfD), but unlike the RfD the UL relies on a case-by-case consideration for derivation and does not have published paradigm for data review and uncertainty factor (UF)application. The ETEs occupy a unique place among chemicals regulated by the USEPA as both nutritional deficiency and adequacy and toxicity data are available (and in many cases human data) that must be considered for review. Data on human requiremnts, exposure and bioavailability must be integrated with the toxicity data. The RDA makes recommendations based on sex, age and physiological status; whereas the RfD is calculated as a single lifetime exposure value for individuals. This creates difficulties in the risk assessment process. The RfD for zinc is 3E-1 mg/kg-day (20 mg/day) based on LOAEL of 60 mg/day (1.0 mg/kg-day) from a critical effect of 47% decrease in erythrocyte superoxide dismutase in adult females (60 kg) after 10 weeks, and an UF (LOAEL to NOAEL extrapolation) of 3. The zinc RDA for adults is 11 mg/day for males and 8 mg/day for females (11 mg/day for pregnancy; 12 mg/day for lactation). The UL adults is 40 mg/day and is 23 mg/day for children 9-13 years of age and 34 mg/day for adolescents 14-18 years of age. The current RfD exceeds the RDA for infants and children when adjusted for body weight. Thus, the current USEPA RfD recommendations are contrary to IOM recommended optimum zinc intake levels in these populations. Additionally, the IOM derived ULs are twice the USEPA's RfD. We recommend the creation of an RfD category for ETEs without UFs and/or the use of modifying factors in lieu of UFs to rectify differences. The opinions expressed in this abstract are those of the authors and not necessarily that of the USEPA.

696 CROSS SPECIES MODE OF ACTION INFORMATION ASSESSMENT FOR BISPHENOL A.

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The goal of the project was to identify the mode of action (MOA) information for Bisphenol A (BPA) across animal species and to explore how to use this information for evaluation of ecological and human health risk. BPA developmental and reproductive effects and MOA data were identified from the literature for only 17 species

representing 7 animal classes. For invertebrates, some species showed similar effects after estrogen (E) or BPA exposure but the significance of the findings is unclear since the role of E or E analogs in invertebrate development is not well understood. Within the vertebrates, *in vitro* binding data indicate that BPA can bind to the ER of reptiles, amphibians, fish, birds, and mammals at a much lower affinity than ethinyl estradiol. For amphibians, some developmental effects data were consistent with BPA acting as an E agonist and *in vitro* data suggest effects on thyroid hormone bioavailability. In birds, *in ovo* BPA treatment led to some effects consistent with an E agonist MOA. In fish, BPA effects data were consistent with a number of MOAs including E agonism and mechanistic data support BPA causing an increased E activity. Mammalian effects data, limited to rat and mouse, are consistent with a number of MOAs and most mechanistic studies support an E agonist MOA. In conclusion, BPA exposure can elicit effects in vertebrate and invertebrate species at sublethal concentrations and the majority of evidence supports BPA acting to increase E activity with mechanistic studies supporting E agonism. Limitations of the study were the small number of species with data, most mechanistic studies focused on E agonism, and the lack of knowledge of the role of hormones in invertebrate development. Studies to investigate if BPA affects alternative or multiple MOAs in different species, at different doses or under different conditions are needed. Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

697 ANALYSIS OF NESTED DATA FROM DEVELOPMENTAL STUDIES: A RE-EVALUATION OF AZINPHOSMETHYL DEVELOPMENT STUDIES.

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In a developmental study, the maternal animal, but not the conceptus, is the individual treated during gestation, and study results usually have the characteristic of nested data. As suggested in EPA's guidelines for developmental toxicity, such data "generally are calculated as incidence per litter or as number and percent of litters with particular end points". In practice, however, some developmental responses in pups, such as survival index and lactation index, are calculated as number of live offspring (at lactation day 4 or 21) divided by number of live offspring delivered. This latter approach is based on an assumption that the response is from independently exposed individuals (pups). These two approaches of data analysis might result in a different conclusion in dose-response analysis, as shown in an evaluation of azinphosmethyl developmental studies. We present our results from the two approaches as well as EPA benchmark dose (nested models) analysis for the effect of azinphosmethyl on pup viability in two developmental studies. Based on the litter-based approach, the NOAEL for pup viability is 15 ppm azinphosmethyl while it is 5 ppm based on the pup-based approach; the BMD analysis also supports to use 15 ppm as the NOAEL. Our results suggest that a litter-based data analysis is more appropriate in an evaluation of development toxicity results.

698 HOW DIETARY IODINE AFFECTS THE DOSE-RESPONSE FOR PERCHLORATE INHIBITION OF IODIDE UPTAKE BY THE THYROID: A MODELING ANALYSIS OF CLINICAL EXPOSURE DATA.

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Perchlorate is a competitive inhibitor of iodide uptake from the serum into the thyroid. The site of this inhibition is the sodium-iodide symporter. Perchlorate inhibition of thyroidal radioiodine uptake in humans has been evaluated in three clinical studies (Lawrence et al., Thyroid 10: 659-663, 2000; Lawrence et al., Thyroid 11: 295, 2001; Greer et al., Environment Health Perspect. 110: 927-937, 2002). In each of these studies, the uptake measured during perchlorate exposure was compared to the baseline uptake, with each subject serving as his/her own control. Greer et al. (2002) evaluated the dose-response for perchlorate inhibition of uptake on day 14 of exposure (E14) at a dose of 0.007, 0.02, 0.1 or 0.5 mg/kg-day and extrapolated a no-effect level of 0.005 to 0.006 mg/kg-day using a linear-log dose model. The work presented herein addresses the influence of the ad libitum dietary iodine intake in that study. Goodman and Greer (unpublished data) found that the 24-hr urinary iodine excretion (IE), a surrogate for daily iodine intake, varied several-fold within subjects between baseline and E14 and an order of magnitude among subjects. [All quantitative analysis of iodine was expertly performed by M. Previti and S. Pino in the laboratory of L. Braverman at the Boston Medical Center.] I report here the development of a method to adjust the E14:baseline relative uptake (RU) for the relative change in IE between baseline and E14 (RIE) and to evaluate how the RIE-adjusted RU depends upon the absolute baseline IE. The results indicate that subjects in the lowest baseline IE category (49 to 137 microgram) tended to have higher RIE-adjusted RU values at a given perchlorate dose. Thus, the data indicate that in comparison to higher IE levels, an IE level between 49 and 137 microgram is associated with lesser sensitivity to perchlorate inhibition of thyroidal iodide uptake. These results suggest that persons at the low end of the US population's range of dietary iodine intake are not at increased risk for perchlorate inhibition of thyroidal iodide uptake.

699 WEIGHT-OF-EVIDENCE CHARACTERIZATION OF THE ENDOCRINE-MODULATING EFFECTS OF ATRAZINE: IMPLICATIONS FOR HUMAN HEALTH RISK ASSESSMENT.

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Atrazine, a systemic triazine herbicide that blocks plant photosynthesis, is one of the two most widely-used agricultural pesticides in the US, primarily for weed control of corn, sorghum, sugarcane, and wheat, as well as on range, orchard, and turf grasses. Atrazine, and/or its primary metabolite, diaminochlorotriazine (DACT), have been suspected of having the potential to modulate hormonal activity by a variety of mechanisms, including mimicking the effects of estrogen or progesterone exposure; altering serum levels of reproductive hormones in normal, pregnant, and developing animals; inducing histomorphologic changes in reproductive organs and tissues; and disrupting the neuroendocrine feedback systems, specifically those involving the hypothalamus-pituitary-gonadal axis. Due to widespread population exposure, the endocrine-active effects have been extensively studied, and atrazine has a relatively large data base of both *in vitro* and *in vivo* specialized hormonal studies. Key studies are briefly reviewed and their strengths and limitations noted. The weight-of-evidence suggests that although there are no human data implicating atrazine and/or DACT as endocrine modulators, it is not unreasonable to expect that these compounds might cause adverse hormonal effects in humans at high exposures. However, concerns about the animal data are numerous, and include the validity of using a rodent model (Sprague-Dawley rat) whose reproductive patterns differ markedly from those in humans to characterize potential human health hazards; the intrinsic variability among strains/species of test animals with regard to sensitivity to atrazine; temporal and diurnal (circadian) variation in some chemically-induced responses; high-to-low dose extrapolation of observed effects; the use of new test protocols; and the lack of a rodent control data base for many of the developmental and other end points under study. [The opinions expressed are those of the authors and do not necessarily reflect USEPA policy.]

699a CANCER RISK ASSESSMENT OF ACRYLAMIDE IN FOODS.

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The University of Stockholm, Sweden reported in April 2002 analyses demonstrating high concentrations of acrylamide in certain fried, baked and deep-fried foods. Acrylamide is classified as a Group 2A carcinogen by the International Agency for Research on Cancer and as a Category 2 carcinogen and Category 2 mutagen by the European Union. The Norwegian Food Control Authority has estimated the intake of acrylamide from food in Norway based on the content of acrylamide detected in Norwegian products, in addition to Swedish analytical data for those food items not covered by the Norwegian sampling. The mean dose of acrylamide was estimated to 0.36 and 0.33 mg/kg bodyweight/day for males and females, respectively. Quantitative estimations of lifetime cancer risk, given the assumption that there does not exist a dose threshold for this genotoxic carcinogen, have been performed based on long-term studies where rats was given acrylamide in drinking water. In the risk calculations a linear extrapolation was performed using the tumorigenic dose indicators T25, that has been used in several risk assessments of chemicals in the EU, and LED10, that the USEPA has proposed to use in its estimation of cancer risk. After adjustment using an animal to human scaling factor, the lifetime cancer risk was calculated to 0.5x10-3 for males (corresponds to 5 cases of cancer per 10, 000 individuals). For those 2.5% males with the highest intake, the lifetime risk was calculated to 1.7x10-3. For females the numbers were slightly lower. It should be emphasised that such a point estimate is uncertain, both because of uncertainties in the estimation of the intake and uncertainty in the assessment methodology. The given estimate is conservative, which means that the real figures may be lower. If it is presumed that approximately 30 per cent of all cancer cases are related to food consumption, the intake of acrylamide in Norway represents approximately 1% of all cases associated with food intake.

700 FEASIBILITY OF LONG-TERM CONTINUOUS INTRAVENOUS INFUSION IN UNRESTRAINED MARMOSET MONKEYS.

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The marmoset monkey (Callithrix jacchus) has become an important nonhuman primate model to be used as the second species in the safety evaluation of new drugs. We demonstrated previously the feasibility of prolonged infusions in this species by using a suitable port-catheter technique. Owing to the increased demand for the marmoset in toxicology, the development of a continuous infusion model in the unrestrained animal became a requirement. The work conducted investigated the feasibility of long-term and continuous intravenous infusion (from 24 hours to 4 weeks) in the common marmoset. Current models for this administration technique still rely on a tethering system. Only the validation of a tether less-jacket infusion model for the unrestrained marmoset was considered to be best practice, as the marmoset exhibits a very complex and rich behavioural and mobility repertoire that would not be compatible with any tethering device. A light weighted (< 10% of nominal body weight incl. 5 mL respectively 5 mg for the fluid reservoir) backpack-minipump system was successfully tested and proved reliable over 7 and 14 continuous days, with a room temperature calibrated minipump delivering 5mL daily or weekly with an accuracy of 98%. The used system allowed free choice of the dosing volume to be administered hourly, as the minipump was either exchanged daily or weekly. The implantable port-catheter systems were successfully used for up to 90 days. Ongoing validation trials now will need to prove the usability of the developed delivery device (backpack system) for longer than 4 weeks. In conclusion, long-term continuous infusion proved feasible in the marmoset model and now extends the range of administration techniques available for toxicity studies in this small nonhuman primate.

701 A PHARMACOKINETIC STUDY OF CJC-1008, A NOVEL OPIOID, IN MONKEYS USING DUAL ISOTOPE LABELING DEMONSTRATES A LONG ELIMINATION HALF-LIFE AND DISTRIBUTION IN PERIPHERAL TISSUES.

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CJC-1008 was designed by applying the Drug Affinity Complex (DAC) technology to the short acting peptide opioid agonist Dynorphin A (1-13) (Dyn A). This chemical modification with maleimidoproprionic acid (MPA) allows CJC-1008 to covalently bind to circulating plasma proteins, after parenteral administration, thus prolonging its half-life and maintaining it within the periphery. In order to study the pharmacokinetic (PK) and tissue distribution profile of CJC-1008, the compound was labeled with [^{14}C] on the MPA and with [^{3}H] on tyrosine at position 1 (Tyr^{1}) of the Dyn A molecule. Cynomolgus monkeys were dosed IV with the formulated [^{14}C/^{3}H]-CJC-1008 at a target level of 8 mg/kg. Quantifiable levels of [^{14}C] and [^{3}H] were observed in both plasma and blood up to 96 hours post dose. The elimination half-life ($t_{1/2el}$) was similar in blood and plasma for both isotopes, ranging between 49 and 79 hours. Radioactivity levels in tissues peaked quickly within 2-24 hours with the highest radioactivity concentrations observed in peripheral tissues including kidneys, liver, lungs, testes, ovaries, adrenal glands, prostate gland, and spleen for both isotopes, and in the pancreas and salivary glands for [^{3}H]. The highest percentage of administered dose was observed in the kidneys, liver, lungs, heart and bone for both isotopes, and in the brain (cortex) for [^{3}H] only, likely due to free Tyr^{1}. The rate of radioactivity elimination from tissues was faster than elimination from plasma. Recovery of radioactivity in excreta at 96 hours post dose was 52-67% and 11-19% of the dose for the [^{14}C] and [^{3}H] isotopes, respectively. In conclusion, the dual isotope labeling has provided a method to analyze the distribution of the protein-conjugated CJC-1008, showing rapid distribution of the intact molecule to peripheral tissues without crossing the blood-brain-barrier at toxicologically-relevant levels, and a long plasma $t_{1/2el}$ of 49-79 hours.

702 ORAL BIOAVAILABILITY OF DICHLOROACETATE IN HUMAN SUBJECTS.

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Dichloroacetate (DCA) is a drinking water disinfection by-product and a known rodent carcinogen. DCA has also been used therapeutically to treat a variety of metabolic disorders in humans, including diabetes, hypercholesterolemia and congenital or acquired lactic acidosis. An important question in the assessment of human risk to DCA is accurate exposure information from consumption of drinking water. Previous studies in rodents suggest the oral bioavailability of DCA becomes negligible at doses between 0.05 and 1 mg/kg depending on exposure history and capacity to metabolize DCA. The bioavailability of DCA in humans at lower dose rates (< 10 mg/kg) has not been studied. Estimation of DCA bioavailability is problematic as repeated exposures to DCA reduce metabolism and overall clearance, preventing the use of a crossover experimental design. Bioavailability estimation at actual exposure rates from drinking water is also difficult because of analytical detection limitations. We used two approaches to estimate DCA bioavailability: 1) in-vitro measurements of liver metabolism using human liver cytosol and PBPK modeling for simulation of blood profiles after an oral dose and 2) direct measurement in human volunteers using a semi-simultaneous experimental design. In human studies, volunteers were given a 2 mg/kg oral dose of 12C-labeled DCA followed 10 min later by a 0.25 mg/kg intravenous dose of 13C-labeled DCA which serves as the reference dose. To reduce the influence of prior DCA exposure, volunteers were provided purified drinking water as an alternative to municipal drinking water for 2 weeks prior to bioavailability measurements. *In vitro* metabolism / PBPK modeling exercises suggested oral bioavailability would be 22 % after a 2 mg/kg dose and approach zero at dose rates comparable to those found in drinking water. These predictions will be compared to values obtained from the ongoing human studies. Supported by EPA grant R828044.

703 TOLERABILITY AND REPEAT-DOSE PHARMACOKINETICS (PK) OF ACETAMINOPHEN (APAP) AT 4, 6, AND 8 G /DAY IN HEALTHY ADULTS.

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The objective of the study was to assess tolerability and PK of 1, 1.5 and 2 g APAP dosed every 6 h for 3 days (1, 1.5 and 2 times the currently recommended daily dose). The study had a double-blinded, placebo-controlled, three-regimen design. Subjects were housed for the duration of the study and were divided into two groups: (I) 6 adults on placebo and 12 on APAP at 4 g and 6g/day and (II) 6 adults on placebo and 12 on APAP at 4 g and 8 g/day. Safety was monitored daily during the baseline, treatment, and washout phases between dose levels. Blood samples were collected during the first and last dose of each dose regimen. PK results for 4, 6 and 8 g/day of APAP showed that plasma levels did not accumulate with repeat doses. Also, steady-state APAP levels were linearly related to these repeat doses up to 8 g/day, indicating apparent first-order kinetics. Mean peak levels for the last dose were 18±4, 24±6, and 31±7 μg/mL, and elimination half-lives were 2.8±0.3, 2.5±0.2, and 2.3±0.4 h, respectively. Multiple doses up to 8g/day over 3 days were well tolerated and aminotransferase values stayed within normal limits during the conduct of the study.

704 THE USE OF A VALIDATED METHOD FOR THE ANALYSIS OF THUJONE IN PRELIMINARY TOXICOKINETIC RODENT PLASMA AND BRAIN SAMPLES.

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Thujone is a ketone component of essential oils from a variety of plants and is found as a component of flavorings and fragrances. A headspace/gas chromatography/mass spectrometry method was developed to analyze plasma and brain specimens for the purpose of characterizing the disposition of alpha-thujone in rodents. Samples for validating the method were obtained by conducting a preliminary toxicokinetic study with male and female F344 rats and B6C3F1 mice given a single intravenous or gavage administration of alpha-thujone or alpha/beta-thujone and collecting samples at specified time points (one animal/species/sex/dosage level). Analytical standards were prepared (range 5 to 600 ng/mL for plasma, or ng/g for brain). A linear regression equation weighted 1/x was calculated relating the response (y) to the concentration (x) of the standards. The concentration of each sample was calculated using its individual response and the regression equation. Following a single intravenous dosage (3.2 mg/kg, rats; 6.4 mg/kg, mice), the maximum plasma and brain concentrations were approximately 1000 ng/mL and greater than 600 ng/g, respectively. Following a single intravenous dosage of alpha-thujone (1.6 mg/kg, rats; 3.2 mg/kg, mice), the maximum plasma and brain concentrations were approximately 350 ng/mL and 500 ng/g, respectively. After a single gavage dosage (75 mg/kg), the maximum plasma and brain concentrations were sex and species dependent. In rats, maximum plasma concentrations were approximately 800 (M) and 3000 (F) ng/mL and maximum brain concentrations approximated >1400 (M) and >5000 (F) ng/g. Mice concentrations were approximately one-half to one-third less than the rat concentrations after intravenous and gavage administration. The validation of an analytical method for alpha-thujone together

with the preliminary toxicokinetic study results will be used to design a definitive toxicokinetic study in order to determine toxicokinetic parameters after thujone administration.

705 ABSORPTION, ELIMINATION AND METABOLISM OF 1-PHENOXY-2-PROPANOL IN RATS?

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This study was conducted to determine the absorption, elimination and metabolism of 1-phenoxy-2-propanol in Fischer 344 rats following oral administration. Adult male F344 rats were administered a single oral dose of 10 or 100 mg/kg radiolabeled 1-phenoxy-2-propanol in 0.5% methylcellulose. Urine was collected at 0-12, 12-24, and 24-48 hr and feces at 0-24, and 24-48 hr post-dosing in dry-ice cooled traps and radioactivity was determined. Urine samples were pooled by timepoint and dose level and analyzed for metabolites using LC/MS/MS. Both of the administered doses were rapidly absorbed from the GI tract and excreted. The major route of elimination of 1-phenoxy-2-propanol was through urine, accounting for 88±12% of the low and 94±1% of the high dose. Most of the urinary excretion of 1-phenoxy-2-propanol derived radioactivity occurred within 12 hr after dosing; 81±9% of the low and 90±1% of the high dose. Total fecal elimination was <10%. Rats eliminated entire administered dose within 48 hr after dosing, with total recovery of the administered dose ranged from 100-104%. Metabolites tentatively identified in urine were conjugates of phenol (sulfate, glutathione) as well as conjugates of parent compound (glucuronide, sulfate) and a hydroxylated metabolite of parent. There was no free parent or phenol detected in non-acid-hydrolyzed urine. In acid-hydrolyzed urine, 61% of the dose was identified as phenol and 13% as 1-phenoxy-2-propanol. Although the parent compound was stable to acid hydrolysis, some of the phenol in acid hydrolyzed urine may have come from degradation of acid-labile metabolite(s) as well as hydrolysis of phenol conjugates.

706 CORTICOSTERONE IN DRINKING WATER ALTERED THE TIME AND PLASMA CONCENTRATION CURVE OF A SINGLE ORAL DOSE OF CORTICOSTERONE AND LEVELS OF PLASMA SODIUM, ALBUMIN, GLOBULIN, AND TOTAL PROTEIN.

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Effects of chronic exposure to corticosterone (CORT) in drinking water on CORT kinetics, blood chemistry, and brain catecholamines and esterases were studied in adult male Long Evans rats. Groups of rats (n=8) were given either water, 2.5% ethanol, or 400 mcg/ml CORT in 2.5% ethanol for 28 days. On Day 28, rats were gavaged either with corn oil or CORT 20 mg/kg (n=4). Blood samples were collected at 0, 15, 30, 60, 120, 240, 480, and 720 min for CORT levels. Day 14 after gavage blood samples were collected for clinical pathology and brains were dissected into hippocampus (H), cerebral cortex (CC), caudate putamen (CP), and pons (P) for levels of catecholamines and neurotoxic esterase (NTE), carboxylesterase (CbE), and acetylcholinesterase (AChE)activities. Plasma CORT levels of CORT-drinking rats (58+/-14 ng/ml) were lower than the water (433+/-40 ng/ml) and the ethanol group (387+/-25 ng/ml). Plasma CORT was at similar peak levels in 15 min in all rats gavaged with CORT. The change from basal to peak CORT in the CORT-drinking rats was greater than for naive and vehicle rats, with no return to baseline by 720 min. Plasma sodium levels were lower in CORT-drinking rats (137.8+/-0.8 mEq/dL) than water-drinking rats (141.9+/-0.8 mEq/dL), suggesting effect of the hormone on the adrenal gland. Plasma albumin, globulin, and total protein were higher in CORT-drinking rats, an additional effect of CORT treatment. Brain NTE, CbE and AChE were not different among the groups except in the CC (NTE lower in CORT-drinking rats). Brain catecholamines levels were similar except the norepinephrine in H was higher in CORT-gavaged rats (23.7+/-5.4 nmol/g tissue) than in the corn oil group (13.5+/-5.5 nmol/g tissue). The ethanol-drinking, CORT-gavaged rats had lower levels of CP norepinephrine than the ethanol-drinking, corn oil-gavaged rats. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government).

707 COMPARATIVE DISPOSITION OF N, N-DIMETHYL-P-TOLUIDINE (DMPT) IN MALE F344 RATS AND B6C3F$_1$ MICE.

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DMPT is a high-production-volume chemical used to accelerate polymerization in the manufacture of bone cements and dental materials, is found in industrial glues and artificial fingernail preparations, and is used as an intermediate in dye and pesticide synthesis. Human exposure to DMPT is possible in prosthesis users, people with dental plates, and occupational settings. The reported LD_{50} values for DMPT in rodents are as follows: oral, rat, 1650 mg/kg; intraperitoneal (IP), mouse, 212 mg/kg; 4-h inhalation (LC_{50}), rat, 1400 mg/m^3; and dermal, rat, >2000 mg/kg. We are investigating the disposition of [^{14}C]DMPT in male F344 rats and B6C3F$_1$ mice after a single dose. The objectives are to evaluate rates and routes of excretion, tissue distribution, and metabolism of [^{14}C]DMPT. Rats and mice received oral doses of [^{14}C]DMPT, then were housed individually in metabolism cages; urine, feces, volatile organics (VOCs), and CO_2 were collected for up to 72 h. At sacrifice the tissue distribution of carbon-14 was determined. By 6 h after dosing, all mice that received 250 mg DMPT/kg showed decreased activity and labored breathing. Following the death of one mouse at ~24 h, the remaining mice were euthanized. Mice excreted little urine and feces during the study. In contrast, rats that received 250 mg DMPT/kg exhibited no overt signs of toxicity and remained healthy for the duration of the 72-h study. Rats excreted ~82% of the dose by 72 h, with ~72% excreted in urine, 3% in feces, 6-7% as VOCs, and <1% as $^{14}CO_2$. By 24 h after dosing >60% of the dose was recovered. Based on results in rats at 250 mg/kg, orally administered DMPT is well absorbed. This appears to be the case in mice as well since the 250 mg/kg oral dose, which is close to the IP LD_{50} in this species, was acutely toxic. We continue to evaluate the metabolism and disposition of DMPT in rats and mice at lower oral doses to better understand the species-specific toxicity of DMPT to aid the National Toxicology Program in designing toxicity studies and assessing potential human health risk from DMPT exposure.

708 IODINE KINETIC BEHAVIOR IN TISSUES OF PND10 MALE AND FEMALE PUPS FOLLOWING ORAL DOSING.

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The iodide ion is the essential element for synthesis of thyroid hormones (T_3 and T_4). Thyroid hormones are important for normal brain development in both fetuses and in neonates of rodents and humans. The objective of this study was to understand kinetic behavior of iodide in Sprague-Dawley PND10 pups and to determine if there were gender differences. PND10 male and female pups weighing 20 g were orally dosed with 1μg/kg ^{125}I and returned to their dams. Pups (n=6) received 0.3 μCi ^{125}I mixed with non-radiolabeled iodide and were sacrificed at 0.5, 2, 4, 8, 12, 24, and 72 h post dosing. Thyroid, blood, skin, gastrointestinal (GI) tract and GI contents (GC) were collected to study tissue distribution of iodide in males and females. The only statistically significant gender differences in iodide levels were in thyroid at all time points; skin at 2h; and GI tract at 72h post dosing. Levels of bound iodide in thyroid of male and female pups at 0.5, 2, 4, 8, 12, 24, and 72 h were 3.9, 58.5, 100.6, 172.8, 334.9, 429.6, 515.9 and 2.6, 38.8, 97.0, 194.9, 296.8, 387.9, 684.6 ng/g, respectively. Free iodide levels in serum of males and females at the same time points were 0.36, 0.57, 0.56, 0.50, 0.45, 0.35, 0.14 and 0.35, 0.53, 0.55, 0.50, 0.44, 0.33, 0.13 ng/mL, respectively. Iodide levels in thyroid for both male and female pups were increasing until the end of experimental period (72h) while concentrations in serum were decreasing after 4h post dosing. Iodide levels in skin of male pup at the same time points were 0.29, 1.12, 1.14, 1.21, 1.24, 1.06 and 0.99 ng/g. Iodide levels of skin in both males and females were increased until 12 h post dosing and started to decrease until the end of study. Iodide concentrations in thyroid, skin, GC and GI tract were higher than serum levels, indicating sequestration of iodide in these tissues. This information will be used to develop a physiologically based pharmacokinetic model for postnatal pups.

709 PHARMACOKINETICS OF GENISTEIN FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION TO ADULT FEMALE SPRAGUE-DAWLEY RATS.

A. Upmeier, B. A. Elswick, H. D. Parkinson, W. L. Krol and S. J. Borghoff. *Division of Biological Sciences, CIIT Centers for Health Research, Research Triangle Park, NC.*

Genistein is a phytoestrogen present in soy-based food. It has been associated with potential health benefits in the chemoprevention of cancer and for relief of menopausal symptoms. Genistein is also present in virtually all rodent diets and has been reported to elicit estrogenic effects in developing rodents. Understanding the metabolism and distribution of genistein in rats is important for evaluating both the risks and benefits from exposure to this compound. Most kinetic studies only report total genistein levels (after hydrolysis of all metabolic conjugates) or only the nonconjugated parent compound in plasma. The objective of this study was to evaluate the elimination of both genistein and its conjugated metabolites from blood of female Sprague-Dawley rats following intravenous (i.v.) and oral administration of 4 or 40 mg genistein/kg b.w. Blood samples from cannulated (jugular vein) rats were collected at various time points. Genistein, genistein-7-O-glucuronide (C-7), genistein-4`-O-glucuronide, and 2 sulfate conjugates were quantitated in plasma by LC-MS/MS. Fitting the genistein plasma concentration-time

curves following i.v. administration to a 2-compartment model, the initial half-life (alpha-phase) for genistein was estimated to be ~ 6 min for the 40 mg/kg b.w. dose and ~ 5 min for the 4 mg/kg b.w. dose. The terminal half-life (beta-phase) for genistein following the high dose was estimated to be 30 min. Following oral administration, genistein and its conjugated metabolites were detectable in plasma as early as 5 min, indicating rapid uptake from the gastrointestinal tract. The level of conjugated genistein exceeded the level of parent compound at all time points by an average factor of 10, with C-7-glucuronide being the major metabolite (~ 63 % of all metabolites). In this study, the metabolism and distribution of genistein is described by time profiles distinguishing parent from conjugated forms. This study was funded by the American Chemistry Council.

710 TRANSPLACENTAL TRANSFER OF GENISTEIN AND CONJUGATED METABOLITES IN SPRAGUE-DAWLEY RATS.

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Genistein is a phytoestrogen present in soy and virtually all rodent diets. It has been reported to elicit estrogenic effects in developing rodents. Understanding the placental transfer of genistein in rats as a route of fetal exposure is important for evaluating the dose of genistein associated with a biological effect in the developing fetus. The objective of this study was to determine whether genistein and selected metabolites transfer across the placenta to the fetuses of pregnant Sprague-Dawley rats (gestation day 19) administered 40 mg/kg genistein. At 1 hr after administration, rats were asphyxiated with CO_2, and blood was removed by cardiac puncture. Blood from each fetus in the litter was taken from the jugular vein and pooled by gender. The concentration of genistein and several of its conjugated metabolites (genistein-7-O-glucuronide (C-7), genistein-4-O-glucuronide (C-4), and sulfate conjugates (S1 and S2) were quantitated in plasma by LC-MS/MS. C-7 was the major metabolite in the maternal plasma, whereas S2 and C-7 were the major metabolites in fetal plasma. S1 was detected in maternal, but not fetal, plasma. There were no differences in concentrations of genistein or conjugated metabolites present in male and female fetal plasma. The fetal:maternal plasma ratios for genistein, C-7, C-4, and S2 were approximately 0.25, 0.04, 0.05, and 0.55, respectively. These data suggest that the glucuronide conjugates may not be transported across the placenta as readily as genistein or the sulfate conjugate S2. Conjugation may also take place in the fetal liver and contribute to the concentration measured in fetal plasma. Since genistein, but not its conjugates, interacts with the estrogen receptor, the level of genistein that gets transferred to the developing fetus and remains unconjugated is critical in understanding the exposure of this compound to the developing fetus. (This study was funded by the American Chemistry Council.)

711 DISPOSITION OF JUGLONE IN MALE F344 RATS.

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Juglone, 5-hydroxy-1, 4-napthoquinone, has been extracted from black walnut hulls. Black walnut extract (BWE) is marketed as a natural product folk medicine to treat various ailments, eg. constipation, warts, eczema, herpes, psoriasis, fungal infections, and internal parasites. As part of the National Toxicology Program studies on BWE, the disposition of juglone was studied in male F344 rats with oral, iv, and dermal administration of ^{14}C-juglone. Juglone-derived radioactivity is excreted primarily in the feces (63-69%) and urine (18-24%) 24 hours after a single oral dose (0.1 or 1.0 mg/kg). Only trace amounts are found in respired air. After iv administration (0.1 mg/kg), juglone binds with blood components (13% of dose in blood after 24 h) and is excreted in urine (33%) and feces (23%); bile collection after iv dosing yielded 23.5% of the dose after 6 h. Twenty-four hours after 4 mg/kg dermal administration 85% of the dose remained at the dose site. Based on the iv and oral excretion data it is estimated that 40-50% of the oral dose is absorbed. Tissue levels are highest in the kidney and forestomach after oral dosing. The tissue to blood ratio is 2 to 27 in the kidney after all dosing routes while it is less than one for tissues other than the gastrointestinal tract. The high tissue concentrations of radioactivity are at the site of administration (SOA) for the three routes, forestomach, blood, and skin, as expected for a reactive chemical. Kidney is the only non-SOA tissue with high concentration of radioactivity. There is precedent for thioethers formed from quinone/GSH adducts to react with protein in kidney. This in turn is postulated to be responsible for the nephrotoxicity of some quinones or chemicals with quinones as metabolic intermediates.

712 DISPOSITION OF 5-NITROACENAPHTHENE IN F-344 RATS.

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Acenaphthene, a polycyclic aromatic hydrocarbon, occurs naturally in the environment by carbonization or pyrolysis of organic materials. With excess NO2 in the atmosphere, acenaphthene becomes nitrated, thus, producing 3- and 5-nitroacenaphthene (3- and 5-NAN). 5-NAN is the predominant compound formed and is present in environmental samples such as air particulates, cigarette tar, diesel exhaust, and in occupational settings such as the tire industry and power generators. 5-nitroacenaphthene is a suspected carcinogen and/or mutagen. Whether this compound is accumulated in tissues or undergoes rapid biological disposition and eliminated through urine or feces is not yet known. The objective of this study is to understand the disposition of pure 5-NAN in F-344 rats. This compound was administered at a dose of 50mg/kg *via* oral gavage and the animals were sacrificed at specific time intervals ranging from 0 to 15 hours. We used HPLC to quantify concentrations of 5-NAN in biological tissues and fluids. The extraction procedure involved homogenization of different tissues and liquid-liquid extractions using water, methanol, and chloroform (3:10:5). After centrifugation of the extracts, the organic phases were concentrated under N2 and reconstituted with acetonitrile. The results of the time course study show that 5-NAN is detected in the brain at a concentration of 11ng/g but not in the testes or adipose tissue at 1 h. This suggests that 5-NAN crosses the blood-brain barrier but not the testicular-blood barrier. 5-NAN is not sequestered in adipose tissue prior to the 2 h time point. There was a biphasic distribution of 5-NAN in the liver at 0.5, 2, and 6 h time points with concentrations of 3.5ng/g, 0.88ng/g, and 48ng/g, respectively. A similar biphasic distribution was seen in the small intestine at 0.5, 2, and 6 h time points with concentrations of 5.7ng/g, 63ng/g, and 16ng/g, respectively. In this study, we were able to show that trace concentrations of 5-NAN are detectable and quantifiable in biological tissues. We believe that 5-NAN is sequestered in tissues and can cause organ-specific toxicity in F-344 rats.

713 SULFURYL FLUORIDE: PHARMACOKINETICS AND METABOLISM IN F344 RATS.

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This study was conducted to determine the pharmacokinetics and metabolism of inhaled sulfuryl fluoride in the rat. Rats were exposed to 30 or 300 ppm sulfuryl fluoride for 4-hr. Blood, urine and feces were collected from rats exposed to 35S-labeled sulfuryl fluoride during and after the exposures and samples were analyzed for radioactivity, 35S-labeled fluorosulfate and sulfate, and fluoride (urine and feces only). Selected tissues were collected 7-days post-exposure and analyzed for radioactivity. Blood, brain and kidney were collected from rats exposed to unlabeled sulfuryl fluoride during and after the exposures and these tissues were analyzed for fluoride ion. Sulfuryl fluoride was rapidly absorbed, achieving maximum concentrations of 35S in both plasma and red blood cells (RBC) near the end of the exposure period. The 35S was rapidly excreted, mostly *via* the urine. Seven days post-exposure, small amounts of 35S were evenly distributed among the tissues, possibly a result of incorporation of 35S into amino acids. The highest concentration of 35S was detected in portal of entry tissues. The lungs had the highest levels of 35S 7 days post-exposure and the nasal turbinates also had detectable 35S. Radioactivity associated with the RBC remained elevated 7 days post-exposure and highly perfused tissues such as the spleen and kidneys had higher levels of 35S than other non-respiratory tissues. Radioactivity was cleared from plasma and RBC with initial half-lives of 2.5 hr after the 30 ppm exposure and 1-2.5 hr after the 300 ppm exposure. The terminal half-life of radioactivity was 2.5-fold longer in RBC than plasma. Although not directly assayed, there was no evidence of parent 35S-sulfuryl fluoride in the blood based on the radiochemical profiles. The identification of fluorosulfate and sulfate in blood and urine suggests that sulfuryl fluoride is first hydrolyzed to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride.

714 INCREASED ORAL BIOAVAILABILITY OF PYRIDOSTIGMINE BROMIDE FOLLOWING DERMAL CO-EXPOSURE TO DEET AND/OR PERMETHRIN IN RATS.

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Since their return from Persian Gulf War (PGW), many veterans have complained of symptoms including muscle and joint pain, ataxia, chronic fatigue, headache, and difficulty with concentration. The causes of the symptoms remain unknown. Because these veterans were exposed to a combination of chemicals including pyridostigmine bromide (PB), DEET, and permethrin, we investigated the pharmacokinetics of these agents, alone and in combination, in rats. Male Sprague-Dawley rats (200-250 gm, 5 per treatment group) were treated with a single oral dose of PB (13 mg/kg) alone and in combination with DEET (400 mg/kg, dermal) and/or permethrin (1.3 mg/kg, dermal). Plasma concentrations of PB, DEET, and permethrin were simultaneously determined by a method previously developed in our lab

(J. Chromatogr. B. Biomed. Sciences. Appl. 749:171-178, 2000). When administered alone, PB was moderately rapidly absorbed, with a C_{max} of approximately 7 μg/mL at a T_{max} of 2 hr. Elimination was biphasic, with an initial phase half-life of approximately 3 hr and a terminal phase half-life of approximately 25 hr. The calculated AUC∞ was approximately 80 μg·hr/mL. Co-administration of DEET or permethrin increased the Cmax to over 10 μg/mL and increased the AUC∞ to 140 μg·hr/mL without significantly altering the elimination half-lives. Co-administration of both DEET and permethrin increased the C_{max} to over 10 μg/mL, increased the AUC∞ to 140 μg·hr/mL, and reduced the terminal elimination half-life to 13 hr. These results suggest that DEET and/or permethrin, given dermally, can significantly increase the bioavailability of oral doses of PB. Therefore, administered doses of PB should be adjusted for those individuals who are being co-administered DEET and/or permethrin. (Supported in part by a grant from the US Army Medical Research and Materiel Command (DAMD) 17-99-1-9020 to MBA)

715 DISPOSITION OF DECAMETHYLCYCLOPENTASILOXANE IN F344 RATS FOLLOWING A SINGLE ORAL DOSE.

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The disposition of ^{14}C-decamethylcyclopentasiloxane (^{14}C-D5) was evaluated in male and female Fischer 344 rats following a single oral administration of 1000 mg of ^{14}C-D5 in corn oil/kg of body weight. Animals (N=4/sex) were housed in glass metabolism cages for collection of urine, feces and expired volatiles. At 168 hr postdose, animals were sacrificed and selected tissues and remaining carcasses were collected. All samples were analyzed for radioactivity content. Feces and expired volatiles were also analyzed for parent D5 concentration. A separate group of animals (N=6/sex) was used to determine radioactivity and parent D5 concentration in blood at 15 min, 1, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hr post dosing. Whole-body autoradiography (WBA) was used for qualitative *in vivo* assessment of tissue distribution of radioactivity. Rats in the WBA groups were sacrificed at 3, 12, 24, 48, 96 and 168 hr post-dose. The majority of administered radioactivity, regardless of sex, was eliminated in the feces (81.43% for males and 80.03 % for females). Total absorption (radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass) in males and females was 21.82 and 19.62%, respectively. Both sexes showed similar patterns of disposition (Urine: 4.70 and 4.37%; Expired volatiles: 13.36 and 10.37%; Expired CO_2: 0.92 and 0.97%; Tissues: 0.38 and 0.49%; Carcass: 2.46 and 3.43% for males and females, respectively). Qualitative assessment of tissue distribution (WBA) showed that the radioactivity was systemically available and distributed to major organs such as bone marrow, liver, kidney and fat. All of the radioactivity in the expired volatiles was attributed to parent D5. Metabolic profile evaluation of urine and feces showed that the entire radioactivity in the urine consisted of metabolites, whereas in the feces the majority was parent D5 with a trace metabolite. In summary, approximately 20% of ^{14}C-D5 was absorbed after single oral administration in corn oil in Fischer 344 rats.

716 PHARMACOKINETICS OF PERFLUOROOCTANOIC ACID IN MALE AND FEMALE RATS.

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Perfluorooctanoic acid (PFOA) is a perfluorinated fatty acid analogue used in surfactant and emulsifier applications. In the current study, the plasma pharmacokinetics of PFOA in male and female Sprague-Dawley rats were investigated. PFOA was administered by oral gavage at doses of 0.1, 1, 5, and 25 mg/kg. In order to estimate oral bioavailability, PFOA was also administered at a dose of 1 mg/kg by i.v. injection. PFOA in serial plasma samples was quantitated by LC/MS/MS. Following oral administration, maximum plasma concentrations of PFOA were observed at approximately 10.5 and 1.25 hours in males and females, respectively. In male rats, elimination kinetics of PFOA were monophasic and independent of the administered dose, with an average elimination half life of approximately 171 hours. In female rats, plasma kinetics appeared biphasic at higher dose levels, with elimination half lives of ranging from approximately 3-15 hours, depending on the dose level. Areas under the plasma concentration vs time curves (AUC) for oral and i.v. 1 mg/kg doses were similar in both sexes, indicating that oral bioavailability of PFOA was approximately 100%. To further characterize sex differences in persistence of PFOA, male and female rats were administered 0.1 mg/kg PFOA by oral gavage, and serial plasma samples were analyzed until plasma PFOA concentration fell below detection limits of the analytical method. PFOA was detectable for at least 84 days in male rats, with an apparent plasma half life of approximately 288 hours. In females, plasma PFOA had dropped below detection limits within 36 hrs, with a half life similar to that observed in previous experiments. These studies confirm the marked sex differences in elimination of PFOA in rats and serve to more fully characterize plasma pharmacokinetics of PFOA at multiple dose levels. This research was sponsored by the Association of Plastics Manufacturers in Europe (APME).

717 COMPARATIVE TISSUE DISTRIBUTION AND URINARY EXCRETION OF INORGANIC ARSENIC (iAs) AND ITS METHYLATED METABOLITES IN MICE FOLLOWING ORAL ADMINISTRATION OF ARSENATE (AsV) AND ARSENITE (AsIII).

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Valence state is a critical determinant in the disposition and metabolism of pentavalent compared to trivalent inorganic arsenic. In this analysis, the time-course tissue distribution of iAs and its methylated metabolites, monomethyl arsenic (MMA) and dimethyl arsenic (DMA), were compared in blood, liver, lung, kidney and urine of female B6C3F1 mice given equivalent oral doses (0, 10 or 100 micromol/kg) of sodium arsenate or sodium arsenite. The concentration of iAs in both blood and liver were very similar at earlier time points (1 to 8 hours post dosing); however by 24 hours levels of iAs in liver of AsIII-dosed mice were 5-fold higher, most likely due to binding of AsIII. MMA and DMA levels in blood were generally higher following AsIII compared to AsV administration; this is most likely a consequence of more rapid initial absorption at early time points and release from bound sites with subsequent metabolism at later time points. Kidney concentrations for iAs, MMA and DMA were generally higher (up to three-fold) following AsV compared to AsIII administration reflecting the more rapid urinary excretion of total As following AsV administration. Levels of MMA measured in kidney following AsV administration are not reflected in amounts excreted in urine suggesting the possibility of sequestration. DMA is preferentially accumulated in lung following either AsV or AsIII administration; two-fold higher levels are achieved following AsV administration, but levels are maintained higher for a longer time period following AsIII administration. These data demonstrate distinct organ-specific differences in the distribution of metabolites following administration of comparable doses of AsV and AsIII that will be important to consider when investigating mechanisms of arsenic-induced toxicity. (This abstract does not necessarily reflect EPA policy.)

718 TOXICOKINETICS OF AZT WHEN ADMINISTERED IN COMBINATION WITH TRIMETHOPRIM AND SULFAMETHOXAZOLE TO MICE.

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Trimethoprim (TMP) and sulfamethoxasole (SMX) are administered in combination for prophylactic *Pneumocysti carinii* pneumonia infection; the most common complication of AIDS, and a major recurrent cause of illness in HIV-infected patients. Since these medications are likely to be dosed together with AZT to many HIV-infected patients, it is important to assess the potential for adverse interactions. The objective of the present study was to evaluate the pharmacokinetics of AZT when administered in combination with TMP/SMX. In an AZT toxicokinetic study, mice were administered a single iv or oral dose of AZT (15, 25, 50 or 100 mg/kg). In combination studies, mice were administered a single iv (100 mg/kg) or oral (25, 50, or 100 mg/kg) dose of AZT and an oral dose of 250, 500 or 1000 mg/kg TMP/SMX. In repeated dose studies, mice were administered oral doses of 25, 50 or 100 mg/kg AZT and oral doses of 250, 500 or 1000 mg/kg TMP/SMX twice daily for 7 days. A single blood sample was collected from each animal at a predetermined time point from 5 min to 24 h (n = 3 per time point) following the last dose. Blood samples were processed for plasma and analyzed for AZT by HPLC. Pharmacokinetic parameters were estimated after constructing plasma concentration-time curves for each of the dose regimens. After iv dosing of AZT alone, AUC increased greater than expected at 100 mg/kg compared with lower doses. No substantial differences were noted in iv AZT pharmacokinetics when coadministered with oral TMP/SMX. After oral dosing of AZT alone, AUC and Cmax increased greater than expected across all doses. Coadministration with 1000 mg/kg TMP/SMX reduced Cmax with little change in AUC estimates compared with respective oral doses of AZT alone. Repeated dosing of AZT+TMP/SMX resulted in greater than proportional increases in AUC only at 1000 mg/kg TMP/SMX, but not at lower doses. Thus, the highest TMP/SMX dose resulted in changes in AZT pharmacokinetics when administered in combination with oral AZT after single and repeated dosing.

719 DISTRIBUTION OF PCB 84 ATROPISOMERS IN FEMALE C57BL/6 MICE.

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Nineteen of the 209 possible PCB congeners exist as pairs of stable rotational isomers that are enantiomeric to each other. A racemic mixture of PCB atropisomers is present in technical PCB mixtures, thus raising concerns about enantioselective dis-

tribution, metabolism, and disposition of these congeners. We investigated the distribution and enantiomeric fractions (EF) of PCB 84 in untreated female C57BL/6 mice. PCB 84 was injected intraperitoneally with 600 µmol/kg body weight, and the EFs were determined by chiral gas chromatography in liver, brain, lung, heart, spleen and kidney after three or six days. The EFs in brain, liver, lung and heart were significantly different from the racemic PCB 84 standard at days three and six, with an enrichment of (+)-PCB 84 in all four tissues. A significant enrichment of (+)-PCB 84 in the kidney was observed for day six. No significant difference was observed for the spleen on both time points. Tissue EFs for the brain showed the highest EF, whereas the EFs in the spleen were almost identical with the PCB 84 standard. The EFs did not change significantly between day three and six, suggesting that the differential distribution of (+)-PCB 84 may primarily occur during the initial distribution phase. (Supported by ES 07380)

720 CHLOROTRIAZINE KINETICS IN FEMALE RATS FOLLOWING A SINGLE ORAL GAVAGE DOSE OF ATRAZINE OR ITS CHLORINATED METABOLITES.

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATRA) is a selective triazine herbicide commonly used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, and other crops. Diaminochlorotriazine (DACT) is the major metabolite seen in *in vivo* studies following oral ATRA administration. We have developed an analytical method to identify and quantitate ATRA and all chlorinated metabolites in plasma, and then used this method to assess plasma time courses of ATRA and its three chlorinated metabolites, DACT, des-ethyl (DE-ATRA), and des-isopropyl (DI-ATRA) after oral dosing. A single oral gavage dose was administered at 90 mg ATRA/kg in a 1% methyl- cellulose suspension. The majority of total area under the curve (AUC) for chlorotriazines in plasma was DACT (>95% total AUC) followed in order by DI-ATRA, DE-ATRA and ATRA. Equimolar oral dosing studies were completed with DACT, DE-ATRA and DI-ATRA. Maximum absorption of DACT was observed 8 hours post dosing, followed by approximate first-order elimination with a plasma half-life of 12 hours and a rate of elimination equal to 0.0578. DI-ATRA reached higher initial plasma concentrations than did DE-ATRA following oral doses of each compound. With each compound, absorption appeared to be multi-phasic, probably representing solubilization of the slurried dosing solutions after gavage. Due to rapid metabolic clearance from plasma, the blood AUC observed after oral dosing with DE-ATRA, DI-ATRA or ATRA itself, is dominated by DACT. Work supported by STAR grant (R-828610-01-0).

721 USE OF WHOLE-ORGANISM CHEMICAL RESIDUE ANALYSIS AND LASER SCANNING CONFOCAL MICROSCOPY TO DESCRIBE THE DISTRIBUTION OF PBTS IN FISH EARLY LIFE STAGES.

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Fish early life stages (ELS) are more sensitive than juveniles or adults to many persistent bioaccumulative toxicants (PBTs). To better understand the mechanisms by which these chemicals produce toxicity during fish ELS, dose-response relationships need to be determined in relation to the distribution of chemicals in sensitive tissues. In this study, whole-organism residue analysis was combined with *in situ* imaging using a multi-photon laser scanning confocal microscope (LSCM) to describe the tissue distribution of PBTs during fish ELS. The method was tested in Japanese medaka (*Oryzias latipes*)using pentamethyl-difluoro-boro-indacene (BODIPY), a model chemical having high fluorescence efficiency and a log K_{ow} value near 5.0, and the polyaromatic hydrocarbon benzo[a]pyrene (BaP). The tissue distribution of fluorescent signal was compared to the whole-embryo chemical concentration at time points during the embryo and early larval stages. The fluorescent signal of BODIPY was initially very strong in yolk lipid droplets with moderate signal in the yolk. During development the fluorescent signal became diffusely distributed throughout the embryo with strong signal remaining in the yolk and yolk lipid droplets. As embryonic development progressed, fluorescence became evident in the gall bladder, bile ducts, and gut. The distribution of BaP signal was similar to that of BODIPY at 1 d post-fertilization (dpf); however, by 5 dpf much of the signal was gone from the lipid droplets, and strong signal was observed in the bile ducts, gall bladder and gut. Analysis of tissue extracts indicated that BaP was metabolized during early development. This metabolism is thought to account for the difference in distribution of the two chemicals as determined with the LSCM. The LSCM is a useful tool for describing the tissue distribution of PBTs during fish ELS and can be employed to understand how metabolism affects this distribution. This abstract does not necessarily reflect EPA policy.

722 MACROPHAGE ACTIVATOR GENE EXPRESSION PROFILE DETERMINED USING cDNA MICROARRAYS.

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Macrophage activation contributes to the adverse effects produced by a number of hepatotoxic compounds. We used cDNA microarray technology to compare transcriptional profiles elicited by two macrophage activators, lipopolysaccharide (LPS) and zymosan A, to those produced by approximately 100 prototypical hepatotoxicants to determine a shared signature profile for predicting macrophage activation-associated hepatotoxicity. Male rats, three per group, were administered each compound at a toxic dose determined by review of the literature and livers were harvested at a 24 hour time point. RNA was extracted, purified, amplified, fluorescently labeled, and hybridized to cDNA microarrays containing probes for 3434 rat genes. A database was constructed from hybridized chip images and the data were normalized and ratioed to vehicle control data. Multiple approaches to gene selection were compared and most of the resultant gene sets yield similar results by clustering analysis. Transcriptional profiles of the macrophage-specific toxin gadolinium and the lymphocyte activator concanavalin A co-cluster with LPS and zymosan, as do several other compounds (carbon tetrachloride, thioacetamide, dimethylnitrosamine, a-naphthyl isothiocyanate (ANIT), galactosamine and allyl alcohol) that are reported to act at least partially *via* macrophage activation. A number of NSAIDs share the same pattern, possibly as a consequence of endotoxin exposure secondary to their adverse effects on the gastrointestinal system. A few compounds such as coumarin whose toxicities have not previously been linked to macrophage activation also clustered with LPS and zymosan.

723 AROCLOR 1254 INDUCED TRANSCRIPT PROFILES IN RAT.

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Aroclor 1254 (polychlorinated biphenyl) is known to be a strong inducer of xenobiotic metabolizing enzymes such as various cytochrome P450 isoenzymes and a few phase II activities. Liver homogenate from Aroclor 1254 treated rats is widely used as metabolic activation system for xenobiotics in *in vitro* assay systems for genotoxicity testing. In contrast to the widespread use of this exogenous metabolizing system, relatively little is known about the enzyme induction profile of Aroclor 1254 in the liver and other organs. In the present study we have chosen a DNA microarray approach to evaluate the Aroclor 1254 induced mRNA levels in different rat organs. A group of male rats received a single intraperitoneal injection of 500 mg/kg Aroclor 1254. The control animals received only the vehicle. Five days later liver, jejunum and lung samples were taken and the RNA analyzed on Rat UG34A GeneChip expression probe arrays. The effects in the different tissues are compared and discussed, in particular the mechanism of induction and metabolic competence of the liver.

724 A COMPARISON OF ARRAY HYBRIDIZATION, MRNA DIFFERENTIAL DISPLAY, AND REAL-TIME PCR IN THE EVALUATION OF THE EFFECTS OF TROGLITAZONE ON GENE EXPRESSION IN PRIMARY HUMAN HEPATOCYTES.

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Troglitazone, a 2, 4-thiazolidinedione antidiabetic, was found to be associated with fatal liver toxicity, leading to its withdrawal from the US market in March 2002. The mechanism responsible for the idiosyncratic hepatotoxicity in humans remains unknown, but is believed to be related to the interaction of the parent compound or its metabolites with the liver parenchymal cells (hepatocytes). Freshly isolated human hepatocytes represent a relevant experimental model for the evaluation of troglitazone toxicity for two important reasons: 1. The cells retain human-specific drug metabolizing enzyme activities; and 2. The cells are the *in vivo* target cells for troglitazone hepatotoxicity. In our laboratory, we have initiated the application of toxicogenomics to advance our understanding of the hepatotoxicity of troglitazone. Our initial goal is to establish the validity of the cDNA human microarray studies by comparing gene expression data using gene arrays with two additional platforms, mRNA differential display and real-time PCR. Using the PHASE-1 human microarray that contains cDNAs for 600 genes involved in various toxic response pathways we show that median cytochrome P450 3A4 mRNA levels increased 2.5-3.9 fold in human hepatocytes from three different donors treated with 25 uM troglitazone for 24hr. This is consistent with the finding that troglitazone is an inducer of hepatic P450 3A4. These results were confirmed using two additional gene

expression platforms: Real-time PCR and mRNA differential display. Further, mRNA differential display also clearly demonstrated the induction of not only CYP3A4, but also unique genes by troglitazone. Our results suggest that toxicogenomics studies using human hepatocytes and gene arrays as the experimental platform, can be used to further our understanding of the mechanism of the toxicity of troglitazone.

725 A SEARCH FOR MOLECULAR TARGETS OF LEAD: A NOVEL PROTEOMIC APPROACH.

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Proteomics provides a powerful approach for detecting protein:protein interactions involved in cellular function or dysfunction. We have modified a bacterial two-hybrid system as a proteomic approach to identify potential protein targets of the neurotoxin lead. Lead is known to interfere with neurotransmitter release. Low levels lead toxicity produces subtle chronic neurological dysfunction, including biochemical and electrophysiological effects. The SNARE complex of proteins is required for neurotransmitter release *via* vesicular membrane fusion and exocytosis. Numerous proteins are involved in the formation and disassembly of active SNARE complexes during membrane fusion. We hypothesize that neurological dysfunction is the result of lead 's effects on the SNARE complex and it 's associated partner proteins. To identify the molecular targets of lead we used two approaches. In an "anchored screen", the SNARE complex was used to focus the search for associated proteins. In an "unanchored screen", numerous proteins encoded by human brain cDNA libraries were screened against each other, in order to identify any protein complex effected by the presence of lead. In both screens a primary antibiotic selection to identify potential protein complexes was followed with a secondary blue/white screen to confirm protein:protein interactions and to exclude false positives. A tertiary toxicology screen in the presence of 10^{-12}M, 10^{-9}M and 10^{-6}M lead identified protein:protein interactions affected by the presence lead, see Table 1. The molecular targets of lead included the SNARE complex as well as associated proteins. The small subset of protein complexes detected illustrates the power of such a simple proteomic screen to identify potential targets of any compound, drug or toxin, for further research. Table 1-Protein:protein interactions profiles for 1°, 2°and 3°screens|

Type of screen	Theoretical #of program complexes	Primary selection	Secondary screen	Tertiary toxicology screen
Unanchored	$\sim 10^{6}$	293	59	6
Anchored	5.6×10^{6}	50	11	1

726 CLUSTER ANALYSIS OF GENE EXPRESSION IN PRIMARY HUMAN HEPATOCYTES AND HEPG2 CELLS TREATED WITH HEPATOTOXICANTS AND HEPATOCARCINOGENS.

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Macroarray and microarray data are currently being widely used to develop signature chemical profiles that have the potential to be used for the prediction of adverse properties in new drugs and chemicals. Another significant outcome may be the identification of new pathways by which toxicants and carcinogens affect the target cell. In this study, primary human hepatocytes from 3 male donors and HepG2 cells were treated for 18 hours with three genotoxic compounds, aflatoxin B_1, 2-acetylaminofluorine and dimethylnitrosamine as well as two non-genotoxic drugs, acetaminophen and methapyrilene. ^{33}P-radiolabeled cDNA from each sample was hybridized to seven individual Gene Filters® screening > 35, 000 distinct human genes. The data were analyzed to identify 1) patterns consistent with genotoxic or non-genotoxic agents and specific chemicals, 2) individual genes with altered expression after exposure to one or more toxicants, 3) subsets of genes that are consistently expressed or have variable expression in cultured primary human hepatocytes and 4) differential gene expression between cultured primary hepatocytes and HepG2 cells.

727 IDENTIFYING POST-TRANSLATIONAL MODIFICATIONS INDUCED BY CHEMICAL TREATMENT USING MALDI-MS AND HPLC-ESI-MS/MS.

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Identification of novel post-translational modifications (PTM) of proteins remains a major challenge. Natural PTM induced by chemicals are usually reversible and alter protein function, whereas "unnatural" PTM (covalent adduct formation) may be irreversible and result in loss of protein function. To identify chemically-induced PTM we use comparative analysis of proteolytic digest MALDI-MS spectra of control and treated proteins to target differences due to PTM without initial assumption as to type or residue localization. Differences between modified and unmodified digest spectra highlight peptides of interest for MS/MS analysis. HPLC-ESI-MS/MS is then used to fragment peptides, and *de novo* sequencing to determine the type and location of the PTM. This approach presents a broad strategy for identifying reasonably abundant PTM in an unbiased manner, and is useful for identification of novel toxicant induced PTM. Successful characterization of a multiply modified histone protein and chemically adducted cytochrome c represent examples of this strategy. The PTM of histones plays an important role in regulating chromatin structure and function. Hyperacetylated histone 4 from LLC-PK1 cells was separated on a triton-acid-urea gel into 5 bands representing different levels of acetylation. Bands were digested in-gel and then analyzed by MALDI-MS and HPLC-ESI-MS/MS to identify the sites of methylation and acetylation. Benzoquinone (BQ) is a model non-redox-cycling quinone capable of alkylating proteins. Cytochrome c, a key protein involved in apoptosis, is adducted with BQ *in vitro*. A novel di-quinone species is the major adduct and is detected at two positions on the protein. The adduct targets lysine residues on cytochrome c, in contrast to peptide studies where single BQ and N-acetylcysteine-BQ adducts are seen preferentially adducting to cysteine residues. This result emphasizes the importance of sequence context in directing sites of adduction. (GM39338, ES07784, DK54991).

728 THE COMPARATIVE TOXICOGENOMICS DATABASE (CTD).

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Over 70, 000 existing toxic chemicals are described by the Environmental Protection Agency (http://www.epa.gov/nheerl/research/ecosystems.html); however, the toxic potential of these chemicals and the molecular mechanisms underlying their action are not well understood. To promote understanding about the impact of environmental agents on genes and proteins, the Mount Desert Island Biological Laboratory (http://www.mdibl.org/) is developing the Comparative Toxicogenomics Database (CTD). CTD will be the first community-supported, publicly available resource to: 1) provide annotated associations between genes, references, and toxic agents; 2) include nucleotide and protein sequences from diverse species with a focus on aquatic and mammalian organisms; 3) offer a range of analytical tools for customized comparative studies; and 4) provide information to investigators on available molecular reagents. This collection of features will facilitate cross-species comparisons of toxicologically significant genes and proteins. The goal is to promote understanding of molecular evolution, the significance of conserved sequences, the genetic basis of variable sensitivity, and the complex interactions between the environment and human health. The planned scope and functions of CTD are described in this poster presentation (ctd@mdibl.org).

729 SOURCES OF VARIABILITY IN TRANSCRIPT PROFILING EXPERIMENTS.

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A critical question in toxicology is how individual animals respond to any given stimulus and how to generalize interpretations made from a small sample number. Frequently the assumption is made that the greatest source of variability is due to the treatment effect. However with a small biological response to the treatment other sources of variability become significant. In the area of safety evaluation this bears not only on the interpretation of toxicity studies (if inter-animal variability is observed) but also in extrapolation of animal responses to heterogeneous human populations. For toxicogenomics, this question has enormous bearing on whether samples from individual animals may be pooled prior to transcript profile analysis (TxPA) without affecting the overall results. An ancillary question is whether variability is greater at the level of the individual animal or the TxPA procedure. To this end we have examined four sources of variability: inter-animal, inter-scientist, tissue preparation and RNA preparation. Replicate samples from vehicle-treated animals were assessed using Affymetrix DNA arrays, and the variability in subsets of genes estimated using ANOVA. In the case of rat liver samples, the greatest contribution to variability was due to differences in gene expression seen between individual animals. Most genes had consistent expression patterns in all the samples but a few genes had biphasic expression patterns, i.e. were expressed at levels 5-fold or lower in some animals compared to age-matched animals housed in adjacent cages. It is possible that such expression differences could correlate with important biological responses, and may provide a means to identify factors contributing to apparent outlier responses seen in some in-life studies.

730 ADDUCTION AND INHIBITION OF HUMAN GSTP1-1 BY ACROLEIN: A PARALLEL PROTEOMICS APPROACH.

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α, β-Unsaturated aldehydes and ketones are reactive electrophiles that are formed from numerous endogenous and environmental chemicals. These compounds can react with nucleophilic centers in proteins to form adducts and possibly inhibit their activity. We are using mass spectrometry-based proteomic approaches to map and quantify modifications on human GSTP1-1 to examine the relationship between adduct formation and the inhibition of enzyme activity. We are developing this system for use in parallel analysis of protein adduction and its effects on protein functions and protein-protein interactions. N-terminally hexahistidine-tagged human GSTP1-1 (His-GSTP1-1) was expressed in BL21(DE3) cells. The His-GSTP1-1 was purified from the bacterial lysate on a Ni-coated 96-well plate resulting in approximately 8.0 ± 1.1 μg of pure His-GSTP1-1 protein per well. The activity of His-GSTP1-1 was measured by the conjugation of 1-chloro-2, 4-dinitrobenzene with glutathione spectrophotometrically and correlated with the amount of protein per well. The resulting His-GSTP1-1 plate was then incubated with 0.25 μM, 2.5 μM and 25 μM acrolein for 1 hr at 37°C. This resulted in a 47%, 92% and 100% inhibition of activity, respectively, as compared to control. The His-GSTP1-1 was then digested in the wells and analyzed by LC-MS-MS analysis of tryptic and chymotryptic peptides. Tandem mass spectral data confirmed the formation of a Michael addition adduct at cysteine 47. This adduct increased in a dose-dependent manner and increased adduct levels correlated with decreases in enzyme activity. This system will enable studies of other adducts, adduct stoichiometry, and site specificity as well as the effects of adduction on protein-protein interactions.(Supported by NIH Grants ES10056 and ES06694).

731 DEVELOPMENT OF A RAT LIVER GENE EXPRESSION REAL-TIME PCR DATABASE FOR TOXICOGENOMICS USING RAT TOXICOLOGY CARDS.

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Real-time PCR is a sensitive, accurate and specific method for determining differential gene expression following exposure to toxic agents. Two rat toxicology Cards were developed for the ABI Prism 7700 Sequence Detection System. Each Card contains 24 sets (quadruplicates) of primers and TaqMan MGB probes for targets involved in pathways relevant to toxic response such as: DNA repair, metabolism, oxidative stress, carcinogenesis, apoptosis, detoxification and cell proliferation. Three male Sprague-Dawley rats (2.5 months old) were dosed with one of 52 compounds or vehicle control. The compounds used for real-time PCR database generation were hepatotoxins, nephrotoxins, apoptosis inducers, carcinogen/mutagens and teratogens. At 24 hours post-exposure, the rats were sacrificed and total RNA was isolated from exposed and control rat livers. The RNA was reverse transcribed and the RT-PCR data was evaluated using the ABI Prism 7700 Sequence Detection System. The Cards produced relevant, highly reproducible differential gene expression patterns. Linear regression analysis of gene expression data showed a range of correlations (0.9-0.2) between real-time PCR differential gene expression data and the cDNA rat microarray data from the PHASE-1 TOXbank Rat Database. The degree of correlation for many genes was excellent to good (0.9-0.5) for these two expression measurement methods.

732 REFINEMENT OF A HIGH-THROUGHPUT 2D-PAGE TECHNIQUE FOR THE EVALUATION OF UBIQUITIN-CONJUGATED PROTEIN STATUS INDUCED BY DEVELOPMENTAL TOXICANTS.

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The tremendous contributions made by the genomics revolution have allowed toxicologists to reexamine mechanisms of toxicity associated with environmental chemicals. However, limitations exist with the extrapolation of genomic data without a thorough understanding of the complex issues linked with post-transcriptional and post-translational modifications attributable to the proteome, such as ubiquitination. The standard method for quantitative proteomic analysis relies on a combination of high-resolution (isoelectric focusing, IEF) two-dimensional gel electrophoresis (2D-PAGE) and mass spectrophotometric (MS) identification of selected protein spots. Due to the laborious and often irreproducible nature of established 2D-PAGE techniques, we have attempted to refine various steps enabling a high-throughput and sensitive analysis of proteomic function. Samples were prepared from mouse embryonal fibroblasts (MEF). Using a commercial 2D-PAGE system, we optimized all steps involving sample isolation, purification, quantity, IEF, PAGE, staining and detection. These modifications resulted in significant reduction or removal of various artifacts, such as sample overload, streaking, and spot loss or gain that otherwise render 2D-PAGE highly problematic. SYPRO Ruby fluorescence staining and laser scanning with PD Quest software significantly enhanced spot identification and detection versus a traditional silver-stain procedure. In parallel analyses we used the refined system to compare ubiquitin-conjugated protein profiles in cells treated with methyl mercury (MeHg) or lactacystin, a potent inhibitor of proteosomal degradation, which induces the accumulation of ubiquitin-protein conjugates. Using western-blot detection, we were able to identify both conserved as well as unique patterns of spots associated with ubiquitinated protein targets in the various treatments. Our refined 2D-PAGE technique in combination with MS analysis will enhance our understanding of MeHg-associated toxicity. Supported by NIH Grants ES10613-01, ES09601-02, ES07033

733 GENE EXPRESSION BIOMARKERS THAT ACCURATELY PREDICT KIDNEY TUBULAR NECROSIS.

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Phase-1 TOXBank(TM) gene expression data for kidney samples from rats treated with 44 compounds (single i.p. injection) were analyzed to identify patterns of gene expression that could accurately predict or classify treatments that produced tubular necrosis at 72 hours after treatment. The data used were produced using Phase-1 Rat CT microarrays of some 700 toxicology-responsive genes. Several groups of genes were identified whose expression could accurately predict or classify tubular necrosis using predictive models such as the K-nearest neighbor model. Using randomized training and test sets of data predictive accuracy of >90% was observed for gene sets using expression data measured at 24 h. Accurate prediction of dose levels producing toxicity was observed for two compounds that produced tubular necrosis at a high dose but not at a low dose. Significant, but lower, predictive accuracy was observed for gene sets using expression data from samples obtained 6 and 72 hr after dosing. There appeared to be a fair level of redundancy in the predictive genes. Several different small subsets of genes (3-10 genes) had almost as high predictive accuracy as larger sets of genes. In addition to using multiple randomized training and test sets predictive accuracy was confirmed by using randomly classified data, which removed the predictive accuracy, and by accurately predicting tubular necrosis in samples that were not present in the original database. These results clearly demonstrate the ability of gene expression data to accurately predict or classify a specific conventional toxic endpoint.

734 ACUTE SARIN EXPOSURE-INDUCES EARLY AND PERSISTENT ALTERED GENE EXPRESSION IN THE NERVOUS SYSTEM: A MICROARRAY STUDY-BASED MODEL IN RATS.

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Sarin induced gene expression patterns for an early time point (15 minutes:0.5 X LD50) and late time point (3-months:1 X LD50) have been studied in the nervous system of male Dawley-Sprague rats using affeymetrix: Rat neurobiology U34 chips. We have identified a total number of 65 and 38 genes showing statistically significant alteration from control levels at 15 minutes and 3-months, respectively. At the early time point, there were more altered genes, classified as ion channels, cytoskeletal and cell adhesion molecules, as well as neuropeptides and their receptors. They were followed by genes of cholinergic signalling, calcium channels and binding proteins, transporters, chemokines, GABAergic, glutamergic, aspartate, catecholaminergic, nitric oxide, purinergic, and serotonergic signalling molecules. At the late time point, there were more altered genes, classified as calcium channels and binding proteins, cytoskeletal and cell adhesion molecules, and GABAnergic sinalling molecules than any other groups. A total number of 6 genes were identified to be showing persistent altered expression in both time points. Selected genes from each of these time points were further validated using RT-PCR approaches. Some of the genes, identified in the present study were shown to be involved in the organophosphate-induced pathology by our and other groups. Principle component analysis of the expression data of the time points was used for comparative analysis of the gene expression, which indicated that the changes in gene expression is a function of dose and time of termination after the treatment. Our model also predicts that besides dose and duration of post-treatment period, aging and possibly other factors may be playing important roles in the regulation of pathways, leading to patho-physiological developments.Supported in part by the US Army contact No: DAMD-17-98-C-8027.

735 GENE EXPRESSION PROFILES: EVALUATION OF ANALYTICAL AND BIOLOGICAL NOISE IN CELL LINES.

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Quadruplicate oligonucleotide microarrays were used to measure relative gene expression levels in 6 Epstein Barr virus transformed lymphoblast cell lines and 3 lymphoid tumor lines, in duplicate cultures, compared to a pooled control RNA reference sample. Clustering of expression profiles should be improved if analytical and biological noise can be omitted from data analysis. Analytical variability is being examined by comparing expression values on each chip using analysis at 95% or 99% CL, and identifying outliers based on 3 of 4 versus 4 of 4 replicate hybridizations. Experiments were designed to identify genes: 1) uniquely expressed in a cell line; 2) that differ among replicate cell culture samples (that may represent biological noise); 3) that change expression level over several weeks in culture. We evaluated expression of ~18, 000 genes in 9 cell lines. At the least stringent analysis level (95% CL; 3 or 4 of 4 replicates), we observed a total of 2208 genes as expression outliers relative to the pooled reference sample. Cell lines had an average of 310±183 expression outliers. An average of 53.3%±15.5% of expression outliers appeared in both biological replicates for a given cell line. Preliminary analysis of biological replicates allowed identification of a large number of genes (1451) whose expression level varied in culture (biological noise). Of these, 306 genes disagreed between replicates in more than one cell line. Removing genes identified as biological noisy left 756 "reliable" expression outliers. Examining the reliable genes in lymphoblast cell lines, we observed an average of 11±4 expression outliers unique to each cell line; in tumor cell lines an average of 44±27. These expression profiles may represent genetic traits of each cell line. Preliminary evaluation of data at more stringent levels (99% and 4/4 replicates) reduced total outliers by 75% and we are determining how this improves the reliable v noise classification in replicate cultures, and if it can improve the hierarchical clustering of cell lines.

736 MECHANISM OF RAT RENAL TOXICITY PRODUCED BY A KINASE INHIBITOR: A TOXICOGENOMIC APPROACH.

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Kinase inhibitors are important cellular targets in a number of therapeutic areas. Due to the large number of intracellular kinases involved in physiological processes, specificity of a selected kinase inhibitor is crucial for exerting its pharmacological action without undue toxic effects. Compound X is a kinase inhibitor that was being developed for the treatment of asthma and rheumatoid arthritis. In preclinical studies, treatment-related effects were observed in the rat kidney (tubular degeneration) and liver (hepatocellular hypertrophy) in rats. The objective of the present study was two fold: 1) utilize toxicogenomics to evaluate gene expression alterations in the kidney to help elucidate the potential mechanism(s) of toxicity and, 2) determine the overall utility of toxicogenomics in drug safety. Male rats were dosed orally at 0, 10 or 200 mg/kg/day for 1 or 14 days. Kidney and liver target organs were collected at necropsy for toxicogenomic analysis and histopathology. Toxicogenomics experiments were performed using the Affymetrix rat RgU34A chip. Treatment-related tubular degeneration of the kidney was observed at 200 mg/kg/day and a mild hepatocellular hypertrophy. Principal component analysis of individual kidney tissue showed that the affected rats clustered separately from the controls. Specific mRNA transcript involved in intermediary metabolism, redox regulation, fatty acid metabolism, signal transduction, and transcription were altered. These alterations were associated with treatment-related histopathologic changes in the kidney. Results from this study indicate that toxicogenomic evaluation may provide useful information in regards to molecular mechanisms of drug-induced toxicity.

737 PROTEOMIC PROFILING OF SUMO-1 AND SUMO-2/3 PROTEIN CONJUGATION IN HEK 293 CELLS FOLLOWING EXPOSURE TO 4-HYDROXYNONENAL.

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Stress conditions such as heat shock, UV, alkylating agents and H_2O_2 have been shown to result in the modification of a variety of protein targets *via* the production of reactive electrophiles. These modifications can directly impact protein function or can alter posttranslational modifications and protein turnover, thus leading to a disruption of cellular regulatory processes. Recent studies have shown that stresses affect posttranslational modification by the small ubiquitin related modifier (SUMO) proteins. Sumo affects protein-protein interactions, localization and ubiquitination. Previous work in this lab has demonstrated that protein damage by the alkylating agent 4-hydroxynonenal results in a dose-dependent increase in the formation of both SUMO-1 and SUMO-2/3 conjugates and that SUMO-1 and SUMO-2/3 have unique protein targets. The purpose of the current studies is to identify proteins targeted by SUMO-1 and SUMO-2/3 and to localize the SUMO and SUMO-conjugates before and after treatment. HEK 293 cells were treated with 4-hydroxynonenal at doses of 10and 250 μM or equal volumes of vehicle (0.1% ethanol). After 60 minutes the medium was removed for determination of LDH leakage and the cells were lysed and separated into cytoplamsic and nuclear fractions. The fractions were then immunoprecipitated with anti-SUMO-1 or anti-SUMO-2/3 antibodies and processed for analysis by western blot and LC-MS-MS. Ten proteins containing the general consensus sequence for sumoylation have been identified in digests of immunoprecipitates of the treated cell fractions. This represents a list of potential targets of SUMO-1 and SUMO-2/3 conjugation not previously identified. Western blot analysis indicates that the sumoylated proteins are preferentially located in the nuclear fractions, especially for SUMO-1. Whole cell localization studies utilizing confocal microscopy are in progress. (Supported in part by NIH grants ES06694 and ES10056.)

738 ANALYSIS OF GENE EXPRESSION PATTERNS IN LARGEMOUTH BASS THROUGHOUT THEIR REPRODUCTIVE CYCLE AND WITH EXPOSURE TO p, p' DDE USING GENE ARRAYS.

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The goal of this study was to determine changes in the specific expression profile of 132 genes in livers of female and male largemouth bass (*Micropterus salmoides*) throughout their reproductive cycle and as a consequence of exposure to the endocrine disrupting chemical, 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (p, p-DDE). A custom built, targeted array containing many genes that are estrogen responsive, was used for the experiment. Fish were housed in outdoor holding pens and were sampled throughout the year. For experiments testing normal seasonal changes, largemouth bass were sampled before, during, and after vitellogenesis. As expected, gene expression patterns in the livers of male and female bass are different, especially for genes that are involved in oogenesis. Genes that change in females include those that encode vitellogenin and the zona radiata proteins, among others. The up-regulation of these genes coincided with the peak of plasma estradiol and peak plasma vitellogenin protein in these animals. Female and male LMB were also exposed to p, p-DDE in a laboratory setting. In males, exposure to DDE resulted in an increase in some estrogen-responsive genes including the vitellogenins and choriogenins. In females, the results were reversed; there was a down-regulation of these estrogen responsive genes. Other genes were also down-regulated. The results from these experiments suggest that gene arrays can be used in order to gain a better understanding of critical windows in reproduction in largemouth bass, and to assess sensitive life stages for exposure to various pollutants.

739 DIFFERENTIAL EXPRESSION OF PROTEINS DURING EARLY MOUSE LIVER CARCINOGENESIS INDUCED BY NON-GENOTOXIC MODEL CARCINOGENS OXAZEPAM AND WYETH-14, 643.

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The effects of two dietary non-genotoxic carcinogens in mouse liver, the anti-anxiety agent, oxazepam (Ox) and hypolipidemic agent, Wyeth 14, 643 (Wy), were studied by proteomics with the hypothesis that early common and unique protein expression changes might be observed prior the the onset of malignancy. Livers from untreated B6C3F1 mice were compared to those of mice receiving dietary 2500 ppm of Ox and 500 ppm of Wy for either two weeks or six months. Livers were homogenized and fractionated into cytosol, microsomes and nuclei prior to two dimensional gel electrophoresis and protein identification by mass spectrometry. A total of 146 proteins were identified of which 66 were cytosolic, 59 microsomal and 21 nuclear proteins. Peroxisome bifunctional enzyme was greatly induced by Wy (10-20 fold increase)at 2 week and 6 months and was also increased by Ox (3-4 fold increase). Biochemical markers of oxidative stress were indicated by increases in GSH-peroxidase, superoxide dismutase, epoxide hydrolase, peroxiredoxin-1 and GST-zeta 1 and were increased 2-4 fold in Ox and Wy treated mice. Several proteins were induced specific to Wy treatment at 6 months including fu-

marylacetoacetate hydrolase (7 fold), aconitase (11 fold), farnesyl pyrophosphate synthase (3 fold) and ENO1 (5 fold), the latter often being inducible during hypoxia. Ox treatment produced increases in a homolog to human PTD012 at 2.5 fold at 2 weeks and 4 fold at 6 months compared to unchanged levels in Wy. Histopathology data demonstrated that although no tumors were detected at 6 months, mice exposed to Ox exhibited centrilobular hypertrophy whereas Wy treated mice showed a generalized hepatocellular hypertrophy. While there are many similarities in protein expression after Wy and Ox exposure, proteomics may assist in biomarker discovery of early biochemical changes produced by non-genotoxic agents that ultimately lead carcinogenesis.

740 GENE MODULATION PATTERNS ASSOCIATED WITH AROMATIC GAMMA-DIKETONE NEUROTOXICITY.

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The neurotoxic gamma-diketone metabolites of n-hexane and 1, 2-diethylbenzene, namely 2, 5-hexanedione (2, 5-HD) and the more potent 1, 2-diacetylbenzene (1, 2-DAB), respectively, form colored protein adduct polymers and induce neurofilament(NF)-filled giant axonal swellings [Kim et al., Toxicol Appl Pharmacology 177:121-31, 2001; 183:55-65, 2002]. We have used 1, 2-DAB and 1, 3-DAB, a non-protein-reactive, non-chromogenic and non-neurotoxic isomer, to assess gene modulation and attempt to isolate the neurotoxic component. Forty-eight hours after a single i.p. dose of 20 mg/kg body weight of either isomer (n=3) dissolved in 2% acetone in saline (vehicle, n=4), animals were anesthetized, the spinal cord removed by saline extrusion, and RNA isolated by the Trizol method. cDNAs were hybridized using a single label (Cy5) to mouse cDNA spotted arrays containing ~13, 000 sequence-verified clones. Signal intensity was background-corrected, the data normalized, and significance assessed in pair-wise comparisons among the three groups using t-tests with a common standard deviation. False discovery rate was used to correct for numbers of genes examined. Approximately 400 genes showed significant differential modulation in vehicle- and DAB-treated data sets. Gene expression profiles for DAB isomers and vehicle were compared to identify differentially expressed genes selective for the neurotoxic isomer. These included genes coding for structural proteins (tubulin), myelin (myelin basic protein, proteolipid protein) and a glycolytic enzyme (glyceraldehyde-3-phosphate dehydrogenase). These proteins differ from those identified to date as direct protein targets of 1, 2-DAB, such as the NF proteins NF-H & NF-M. Future studies will compare the gene expression profiles for 1, 2-DAB and 2, 5-HD to determine whether a common gamma-diketone neurotoxic signature can be identified. Supported by NIEHS Superfund Basic Research Center #ES10338, a NIEHS Neurotoxicogenomics and Child Health Center Cooperative Agreement #ES11384, and the Oregon Workers' Benefit Fund.

741 INDENTIFICATION OF MOLECULAR TARGETS OF HEPATOTOXICANT THIOACETAMIDE USING 2-DIMENSIONAL ELECTROPHORESIS AND MALDI-MS.

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Thioacetamide (TA) is used as a model hepatotoxicant to study toxicant-induced liver injury and regeneration. The specific molecular targets of TA are largely unknown. Molecular targets of TA were resolved by 2-dimensional electrophoresis and identified by MALDI mass spectrometry. Total homogenates of livers of male S-D rats treated with a lethal dose (600 mg/kg, ip) were subjected to isoelectric focusing on either tube gels (pH 4-8) or IPG strips (pH 3-10) and the second dimension was run on 20 x 25 cm polyacrylamide gels. The gels were stained by Coomassie brilliant blue and densitometric analysis was performed by PDQuest software (BioRad). The data indicate that TA modifies more than 50 predominantly microsomal proteins. Charge modified proteins by TA include endoplasmic reticulum proteins, cytochrome b5, calreticulin, cellular chaperons such as grp75 and 94, hsp90, and hsc70. TA rendered oxygen-regulated protein (ORP150) undetectable and adducted protein disulfide isomerase and estrogen sulfotranferase. Interestingly, TA down regulated expression of carbamoyl phosphate synthase, a mitochondrial protein even though most of the molecular targets of TA are microsomal. The identification of other proteins adducted by TA using MALDI-MS is currently underway. These data indicate that the molecular targets of TA include proteins in endoplasmic reticulum where TA is bioactivated to its reactive metabolite (TA-sulfone). Modification of carbamyl phosphate synthase indicates that TA is capable of adducting proteins in mitochondria of liver cells, a novel finding suggesting the penetration of TA reactive metabolites into mitochondria. The outcome of this study will lead eventually to identification of TA-adducted proteins and shed light on the mechanism of TA-induced liver injury (Supported by ES-9870).

742 DBZACH: AN INTEGRATIVE TOXICOGENOMIC SUPPORTIVE RELATIONAL DATABASE SYSTEM.

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The dbZach toxicogenomic system couples dbZach (http://dbzach.fst.msu.edu), a Minimum Information About Microarray Experiments (MIAME) supportive toxicogenomic relational database, with Java-based data mining and visualization tools. The dbZach database indexes large microarray datasets and currently consists of six functional subsystems (i.e. Clones, Genes, Microarray, and Protocols, Sample Annotation, Toxicology) with three more in development (Real-Time Polymerase Chain Reaction (Real-Time PCR), Affymetrix, Pathway, and Promoter). The Microarray, Clones and Genes subsystems relate features spotted on our internally developed arrays to gene annotation information such as name, abbreviations, chromosomal locations, and function. The Sample Annotation, Toxicology, and Microarray subsystems relate annotative information about experimental samples and their sources to microarray data, in addition to integrating data from pathology, biochemical assays, clinical chemistry, pathway maps, and real-time PCR, thus facilitating an integrative analysis solution for toxicogenomic studies. dbZach is currently populated with human and mouse data with the incorporation of rat data in the next year. The Java-based data mining and visualization tools facilitate microarray data mining, quality control monitoring, and correlation analyses between microarray and real-time PCR data. The dbZach system is built on Oracle 9i, Java2SE, and Java2EE technologies within a Linux-Windows mixed environment. The dbZach system represents a comprehensive solution to the informatic impediments that toxicologists face regarding microarray data management and the integration of other relevant toxicological, pathological, and chemoinformatic data. Supported by NIH grants ES 04911-12, ES 011271 and ES 011777.

743 TOXICANT CLASS SEPARATION USING GENE EXPRESSION PROFILES DETERMINED BY cDNA MICROARRAY.

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Traditional drug development programs soon will be overwhelmed using only established patho-histological evaluations of toxicity. Toxicogenomics-monitoring gene expression changes in response to compounds and comparing them to prototypical toxicant induced gene expression signatures-shows promise for detecting toxicity prior to pathological manifestation. A microarray determined gene expression database of over 100 paradigm compounds was constructed using a maximal tolerated dose at a single 24 hour timepoint in 3 male rats per treatment. Three types of hepatotoxicity were examined: peroxisome proliferation, macrophage activation and oxidative stress. Each type of toxicity was easily distinguished from control based on gene expression, and by a variety of statistical and multivariate analyses, gene sets were identified selective for each toxicity. Training set compound selection was straightforward for peroxisome proliferators (PPAR agonists) and macrophage activators (LPS and zymosan). The training set for oxidative stress was chosen from compounds known to be oxidative stressors at high dose; many genes selected using this training set contain the anti-oxidant/electrophilic response element in their promoter. Many peroxisome proliferators and macrophage activators are oxidative stressors. Even so, a subset of 100 genes could be selected which clearly separate controls, peroxisome proliferators, macrophage activators and oxidative stressors. This allows a two-dimensional representation of multi-dimensional space where proprietary compounds can be compared to these paradigm compounds and their liabilities extrapolated from well characterized nearest neighbor compounds. The long-term goal is to determine additional toxicity specific gene expression patterns and perform similar (or improved) analyses to prioritize drug candidates before they move into traditional development.

744 SUPPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND TUMOR NECROSIS FACTOR-A EXPRESSION BY BISPHENOL A *VIA* NUCLEAR FACTOR-KB INACTIVATION IN MACROPHAGES.

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Bisphenol A [BPA, 2, 2-bis(4-hydroxyphenyl)propane] is reported to have estrogenic activity; however, its influence on cytokine production or immune system function remains unclear. In this study, we investigated the effects of BPA on the

production of nitric oxide (NO) and tumor necrosis factor-a (TNF-a), and on the level of inducible nitric oxide synthase (iNOS) and TNF-a gene expression in mouse macrophages. BPA alone did not affect NO or TNF-a production. In contrast, BPA inhibited lipopolysaccharide (LPS)-induced NO and TNF-a production, and the levels of iNOS and TNF-a mRNA in a dose-dependent manner. Treatment with ICI 182.780, an estrogen-receptor antagonist, inhibited the suppressive effects of BPA. Transient expression and electrophoretic mobility shift assays with NF-kB binding sites revealed that BPA reduced the levels of the LPS-induced NF-kB transcription factor complex. These results demonstrate that BPA may affect the regulation of the immune system function by reducing NO and TNF-a production *via* the inhibition of NF-kB transactivation mediated through the estradiol receptor.

745 USE OF THE MCF-7 FOCUS ASSAY TO CHARACTERIZE PCB ESTROGENIC MODULATION.

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Characterization of human risks resulting from exposure to xenobiotics that modify natural endocrine function continues to be a major public health issue. Detection and assessment of PCB and other xenobiotic estrogenicity has been proposed using various methods. These methods are weighted towards sensitivity, efficiency and physiological relevancy. We have developed an efficient, relevant *in vitro* assay which measures specific estrogen dependent induction of cell proliferation in estrogen responsive human breast cancer cell cultures which results in tissue restructuring analogous to that observed *in vivo*. This system is the MCF-7 focus assay, in which confluent cultures of MCF-7 human breast cells respond to 17β -estradiol (E2) treatment by post-confluent cell proliferation and formation of multicellular nodules or foci with an EC50 of 0.01 nM. The characterization of estrogenic and antiestrogenic activity of various polychlorinated biphenyls (PCBs) and their hydroxylated metabolites, obtained through this assay, is reported here. One hundred PCB congeners and hydroxyated metabolites were assessed for estrogen modulation using the MCF-7 focus assay. Of the PCBs tested, 13 demonstrated estrogenic activity with the most potent being 1/200, 000th that of E2 while 10 hydroxyated PCBs were estrogenic with the most potent being 1/10, 000th that of E2. Orthochlorinated PCBs and most frequently their para-hydroxylated metabolites showed the highest structure specific activity. These results complement the results from other short term *in vitro* assays to demonstrate thorough characterization of estrogenic activity. These results indicate that the MCF-7 focus assay is a useful, efficient, and physiologically relevant *in vitro* estrogen responsive assay which allows quantitative detection of xenoestrogens and the study of their mechanisms of action. Further refinement and automation of this assay system will allow its extended applicability. Supported by NIH grant P42 ES04913

746 MEASUREMENT OF FREE CONCENTRATION WITH ND-SPME IN AN *IN VITRO* ASSAY FOR ESTROGENIC ACTIVITY.

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It is generally assumed that only freely dissolved molecules can pass cell membranes and can therefore be available to cells and receptors. Cells of *in vitro* assays are usually maintained in medium containing some percentage of serum, including serum proteins that can bind test compounds. The resultant dose-response curves of these assays are usually based on the nominal (i.e. added) concentration of test compound. However, various authors have shown that the response of test compounds in *in vitro* assays decreases as more protein is present in the assay medium. Some suggested that calculating the free concentration of the test compound would give a better, independent measure for the dose. Calculating free concentrations is not straightforward, however, as reliable values for the necessary parameters are hard to find, if not absent. Instead, it would be better to measure the free concentrations directly. We have for the first time, applied nd-SPME (negligible depletion-solid phase microextraction), an accepted method to measure free concentrations, to an *in vitro* assay. The free concentration of [^{3}H]estradiol was measured during an ER-CALUX assay, performed with different protein concentrations in the assay medium. As we expected, the dose-response curves shifted to the right with increasing protein concentration when using nominal concentrations. However, plotting the free concentration instead of the nominal concentration as dose parameter, resulted in one single dose-response curve. Calculated free concentrations, based on binding affinities and a multiple compartment equilibrium model, did not correspond well with measured free concentrations. These results not only confirm the importance of the free concentration in dose-response curves, but also show that directly measuring is better than calculating it. Nd-SPME proves to be applicable for measuring free concentrations in an in *vitro* assay.

747 OPTIMIZATION OF HUMAN LIVER AND KIDNEY SLICE INCUBATION IN DYNAMIC ORGAN CULTURE.

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Donated human liver and kidney, in the form of precision-cut tissue slices, are increasingly being used to predict drug metabolism and organ-specific toxicity of xenobiotics in man. In this study, an optimized procedure to incubate human liver and kidney slices has been developed. Slice viability was monitored by using various cellular viability parameters which included ATP content, K^+ retention, GSH content, morphology, and gene expression. This improved incubation procedure involved using organs which had been perfused with Viaspan cold preservation solution and selecting only those organs which were highly viable and fully functional. Immediately after the organs arrived at the laboratory, they were cut into 2 cm thick slabs, cored, and precision-cut into 200 μm thick slices using the Brendel/Vitron tissue slicer and V-7 cold preservation solution. Each slice was loaded onto a roller insert (Vitron, Inc.) which had a 13 mm Immobilon-NC HATF membrane disc (Millipore Corporation) placed on the medical-grade titanium screen. Each roller insert, containing the slice and membrane, was placed in a scintillation vial containing Waymouth's media with or without various supplements (ascorbate, sodium pyruvate, glucagon, insulin, epidermal growth factor, corticosterone, fetal calf serum, and heat-inactivated fetal calf serum. These vials were then placed in a 37°C Dynamic Organ Culture incubator with 95% O_2 and CO_2 flowing at 0.5 liters/min for 7 days. Each day an appropriate number of vials were taken out of incubation, the slices frozen in liquid nitrogen and stored in a -80°C freezer. To the remaining vials; their medium was changed every day with media prepared fresh that day and warmed to 37°C. This optimized incubation procedure allows one to incubate human liver and kidney slices for at least 7 days with only slight changes in cellular viability. The ability to incubate human tissue slices for longer periods of time may allow one to investigate correlations between viability, morphology, and gene expression and how they relate to the mechanism of toxicity.

748 A COMPARISON OF CYTOTOXICITY INDUCED BY SULFUR MUSTARD AND LEWISITE.

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Using a human leukocyte (HL) model, it was shown that both sulfur mustard (SM) and lewisite (L) (potent chemical warfare vesicating agents) caused a concentration and time dependent loss of cellular viability. Comparable levels of cytotoxicity were seen with both agents but at different concentrations and different post exposure times. HL from the same individual were exposed to either L or SM for 1, 2, 4, 8, 12, 16, and 20 hrs at 37oC, and then assayed by flow cytometry for their ability to exclude propidium iodide (PI). Cells that stained with PI were consider dead. Cytotoxicity was recorded after a 1-hr exposure to L. At concentrations of 3x 10-5 M and higher, L exposure reduced the number of viable cells to 20% of control level during the first 4 hrs post-exposure. During the next 16 hrs, there was no increase in cytotoxicity. When HL from the same individuals were exposed to SM at concentrations from 10-3 to 3x 10-6 M under the same conditions as the L exposure, there was no cytotoxicity detected during the first 4 hrs post exposure. However, after the 4-hr latent period, the number of PI positive cells increased in number and rate of appearance. At concentrations of 3x 10-4 M and higher, SM with time produced an almost linear increase in the number of PI positive cells that was independent of SM concentration, while at concentrations below 3x 10-4 M the cytotoxic responses were concentration-dependent.

749 OPTIMIZATION AND VALIDATION OF THE MCF-7 FOCUS ASSAY FOR ESTROGEN MODULATORS.

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It is now known that many commercial and natural substances in the environment have estrogenic or antiestrogenic activity. Inappropriate exposure to these compounds may result in health risks, both to humans and wildlife. It is also recognized by the pharmaceutical industry that potent estrogens and antiestrogens are useful for hormone replacement therapy and treatment of estrogen dependent cancer, respectively. The MCF-7 focus assay for estrogen modulators is based on an *in vitro* model of estrogen dependent breast cancer. Cultures of MCF-7 breast adenocarcinoma cells display pre-confluent cell proliferation in the presence or absence of 17β-estradiol (E2). Furthermore, in the presence of E2 (EC50 0.01nM), these cultures also exhibit focal accumulations of cells. These multicellular, multilayered nodules are discrete foci of estrogen dependent post-confluent cell proliferation analogous to tumor development in estrogen dependent breast cancer. This estrogen dependent foci formation provides a physiologically relevant endpoint for es-

trogen modulator activity. Using cloned MCF-7 cell lines, the MCF-7 focus assay conditions have been optimized for media, duration, and quantification. A validation series of 65 estrogens or antiestrogens, which include natural products, pesticides, herbicides, and other commercial products, has been tested in the MCF-7 focus assay. The results indicate that the MCF-7 focus assay can detect and differentiate between weak and strong estrogen modulators. These data indicate that the MCF-7 focus assay will be useful in bridging the gap between high throughput receptor-based screening assays and *in vivo* studies to address both pharmaceutical development as well as environmental concerns. Supported by NIH grant R42ES0946702

750 DIMETHYL SULFOXIDE AND ETHANOL, ORGANIC SOLVENTS MOST COMMONLY USED AS VEHICLES *IN VITRO*, AFFECT PRECISION-CUT LIVER SLICE VIABILITY.

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For *in vitro* studies, dimethyl sulfoxide (DMSO), a dipolar solvent, and ethanol, a polar low molecular weight alcohol, are the most commonly used vehicles for test agents that are not miscible with aqueous media. We have shown previously that DMSO is hepatoprotective *in vitro* and *in vivo*. DMSO has antioxidant properties among other effects, and ethanol can cause liver protein modification and impair protein synthesis and secretion. However, the relative effects of these vehicles in precision-cut liver slice assays have not been defined. Our goal was to investigate the effects of concentrations of DMSO and ethanol commonly used *in vitro* on the viability of precision-cut liver slices. A second objective was to determine whether either vehicle reacted with filters used to maintain the integrity of liver slices during incubation. Livers of Fischer F344 rats were cored (8mm) and precision-cut at 250μ. Some slices were placed on filters (10mm diameter, 0.45μ pores) prior to loading on titanium carriers. Incubation was in a Vitron rolling incubation system under 95% oxygen/5% carbon dioxide for 24, 48, or 72h in complete Waymouths medium. DMSO was added at 1 (0.1%), 3 (0.3%), 5 (0.5%), 10 (1%), or 20 μl/ml) (2%) and ethanol was added at 1, 5 or 10 μl/ml). At various time points, liver slices were harvested and weighed. As indices of slice viability, potassium was measured by flame photometry and lactate dehydrogenase (LDH) release was measured spectrophotometrically using a Sigma kit. At 48h incubation, DMSO in the medium resulted in dose-related significantly higher potassium levels and reduced release of lactate dehydrogenase (LDH) than in control slices with no additions. Ethanol in the medium caused reduced potassium, thus reduced slice viability. The presence of filters under the slices during incubation better retained the integrity of the slices but did not appear to alter the effects of the vehicles. This research demonstrates the necessity of studying the influence of vehicles that might alter or mask compound-specific effects.

751 DETOXIFICATION AND MUTAGENIC RESPONSE IN MEDAKA (ORYZIAS LATIPES) EXPOSED TO 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2-[5H]-FURANONE (MX).

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Ames assays have shown the drinking water disinfection by-product, MX, to be one of the most potent mutagens tested and accounting for about one-third of the mutagenicity of chlorinated drinking water. In this study, we investigated the effect of exposure to MX on cytochrome P4550E1 (CYP2E1)-like activity, total glutathione (GSH) levels, and mutation s in the liver of the medaka small fish model. The positive control chemical was methylazoxymethanol-acetate (MAMAc), an alkylating carcinogen in medaka. For mutation induction, we utilized the cII transgenic medaka strain that allow detection of *in vivo* mutations. The 96 hr LC50 of MX was 60 ppm in adult medaka. Both medaka liver microsome preparations and S-9 fractions catalyzed the hydroxylation of p-nitrophenol (PNP), suggesting CYP2E1-like activity. Male medaka exposed for 96 hr to the CYP2E1 inducers, ethanol and acetone under fasted conditions showed significant increases in PNP-hydroxylation activity. Furthermore, total reduced hepatic GSH was reduced in fish fasted for 96 hr, indicating that normal feeding is a factor in maintaining xenobiotic defenses. Exposure to MX and MAMAc induced significant increases in hepatic CYP2E1-like activity, but MX exposure did not alter hepatic GSH levels. Liver mutation induction was not observed in the cII transgenic medaka exposed to MX. However, a dose and time dependent increase was observed from the livers of fish exposed to 1 and10 ppm MAMAc. The lack of mutation induction following MX exposure correlates with mammalian studies which show MX to be effectively detoxified *in vivo*. These data strengthen the role of medaka as a suitable species for examining waterborne xenobiotic compounds. (This is an abstract of a proposed presentation and does not necessarily reflect the views of the EPA)

752 ACTIVITY OF BIOTRANSFORMATION ENZYMES IN EMBRYONIC TURKEY LIVER AND BIOACTIVATION OF 2-ACETYLAMINOFLUORENE.

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Avian embryos are potential alternative species for chemical carcinogenicity studies (Enzmann, Kaliner *et al.* (1992) Carcinogenesis 13(6) 943-946; Brunnemann, Enzmann, *et al.* (2002) Arch. Toxicol. http://link.springer.de/link/service/journals/00204/contents/02/00380/). In the present investigations, the capability of embryonic turkey liver to biotransform xenobiotics was investigated. Biochemical analyses of 21-day *in ovo* turkey liver post-mitochondrial fractions revealed average basal activity levels for biotransformation enzymes as follows: 7-ethoxycoumarin de-ethylase (ECOD), 3.7 nmol/g/min; 7-ethoxyresorufin de-ethylase (EROD), 0.20 nmol/g/min; aldrin epoxidase (ALD), 1.47 nmol/g/min; epoxide hydrolase (EH), 308 nmol/g/min; glutathione-S-transferase (GS-T), 25 umol/g/min; and UDP-glucuronyl transferase (GLU-T), 25 nmol/mg/min. Enzyme activities of ECOD, EROD, ALD, EH and GLU-T, but not of GS-T, were increased by two-fold or greater activity levels following the acute administration of phenobarbital (24 mg/egg) on day 20, 24 hours before harvesting. In contrast, acute administration of methylcholanthrene (5 mg/egg on day 20) only induced changes in ECOD and EROD activities. Bioactivation of the aromatic amine carcinogen 2-acetylaminofluorene (AAF) was also examined using ^{32}P-postlabeling for DNA adducts. Single doses of 1 or 5 mg AAF/egg administered on day 20 *in ovo* resulted in a dose-dependent formation of AAF adducts with chromatographic migration patterns similar to those of C8- and N^2-AAF adducts observed in rat liver. Moreover, high levels of two other AAF adducts with migration patterns similar to those of unidentified AAF adducts in rodents were also observed in embryonic turkey livers. We conclude that 20-day *in ovo* turkey liver is capable of biotransformation of chemical carcinogens. Thus, turkey embryos could be utilized for the identification of genotoxic carcinogens.

753 *IN VITRO* CYTOTOXICITY TESTING WITH CULTURED NORMAL AND IMMORTAL HUMAN MAMMARY CELLS.

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An *in vitro* study was conducted to determine the effects of trace metals on cultured human mammary cells. Confluent monolayers of human normal (MCF-12) and immortal (MDA-MB231) mammary epithelial cells were incubated in the absence or presence of increasing concentrations of trace metals for 24-hours, 4-days, and 7-days. Cell proliferation, MTT assay, and fluorescent-labeled probes, including calcein-AM and Sytox^R, were used to assess cytotoxicity. Inhibitory concentrations (IC50s) were extrapolated from concentration-effect curves after linear regression analysis. Arsenic, mercury, copper, nickel and cadmium were tested with each cell line. The data suggest that MDA-MB231 cells reveal some significant differences from normal mammary cells in their response to toxic insult. In addition, fluorescent probes are not as sensitive indicators of toxicity as the traditional MTT assay for cell viability. However, fluorescent indicators contribute to understanding the mechanisms of cytotoxicity. In combination with previously published reports, our study suggests that a basal cytotoxic phenomenon may only be used to compare either finite or continuous cell lines alone, but not to each other. In addition, the study suggests possible roles of metals in mammary cell carcinogenesis development.

754 SYNERGISTIC INTERACTION IN SIMULTANEOUS EXPOSURE TO *STREPTOMYCES CALIFORNICUS* AND STACHYBOTRYS CHARTARUM.

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Bioaerosols deriving from the microbial growth in moldy buildings have been associated with adverse health effects. However, the causal relationship between detected exposures and health end points are not known because the most important causative microbes among the mixed population of microbes and interactions between them have not been identified. In this study, the inflammatory responses and cytotoxicity induced by a low dose of *Streptomyces californicus* (10^5 spores/ml) alone and together with three different doses (10^4-10^6 spores/ml) of *Aspergillus versicolor, Penicillium spinulosum, Stachybotrys chartarum* and bacterial strains *Mycobacterium terrae, Bacillus cereus* and *Pseudomonas fluorescens* were studied in mouse RAW264.7 macrophages. Inflammatory mediators were measured from the cell culture medium; cytokines (IL-1β, TNF-α and IL-6) immunochemically and nitric oxide

(NO) by Griess-method. Cytotoxicity was determined by using the MTT-test. The low dose of spores of *S. californicus* alone induced only minor production of IL-6 (up to 11±5 pg/ml). Out of the studied microbes, *P. fluorescens* and *B. cereus* caused a dose-dependent production of IL-6 (up to 2160±30 and 72±25 pg/ml, respectively) but these responses were not significantly increased by co-exposure with *S. californicus*. Interestingly, no inflammatory responses were detected by the fungal species, but simultaneous exposure to the low dose of *S. californicus* and *S. chartarum* caused a synergistic increase (up to 560±270 pg/ml) in production of IL-6. The cytotoxicity of the *S. californicus* was also increased by simultaneous exposure to *S. chartarum*. Altogether this data indicate that *Stachybotrys chartarum* and *Streptomyces californicus* are able to induce synergistic inflammatory and cytotoxic effects in mouse macrophages, which may be one plausible explanation to the adverse health effects already at fairly low microbial concentrations in moldy buildings.

755 E-LLNA: AN ENHANCED, FLOW CYTOMETRY-BASED LOCAL LYMPH NODE ASSAY WITH IMMUNOPHENOTYPE ANALYSIS.

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The local lymph node assay (LLNA) is an alternative to the guinea pig sensitization test used to identify and characterize dermal sensitizers. Although the LLNA is a useful test, its disadvantages include: 1) the basic assay cannot readily differentiate some types of irritants and sensitizers, 2) it uses moderate amounts of radioactivity, generating hazardous waste, and 3) with idiosyncratic or highly irritating chemicals, increased numbers of mice are required to determine multiple endpoints (i.e., proliferation, immunophenotype) necessary to properly characterize the sensitization potential. We have applied flow cytometric techniques to the ICCVAM-validated LLNA protocol to enhance the basic test and to increase sensitivity/specificity. Proliferation of lymph node cells (LNC) is measured by BrdU incorporation, and immunophenotypic markers such as B220 (CD45R), CD69, CD3, CD4, CD8, I-A^K, CD80 and CD25, were evaluated for predictivity of sensitization potential. The most useful endpoints in the E-LLNA were the number of BrdU+ cells, B:T cell ratio, %B220+, %CD69+ and %I-A^K+ cells. Several known sensitizers including oxazolone, DNCB, hexylcinnamaldehyde, mercaptobenzothiazole and chlopromazine+UVA were correctly classified. SLS, which is a false positive in the standard radiometric LLNA, was correctly classified, failing to changes characteristic of true sensitizers in %B220+, %CD69+, %I-A^K+ and the B:T cell ratio. The E-LLNA yields stimulation indices (SI) comparable to those in the ICCVAM study for DNCB, HCA and several other chemicals. In the E-LLNA, the EC_3 (concentration which induces a SI > 3) for HCA ranges from 4.2% to 9.8%, and for DNCB it ranges from 0.01% to 0.05%, consistent with standard assay results using ^{3}H-thymidine. In summary, we have developed an enhanced, flow cytometry-based LLNA which 1) can sensitively and specifically identify and characterize dermal contact sensitizers; 2) is non-radiometric; 3) allows better discrimination of false-positive irritants from true sensitizers when compared to the standard LLNA.

757 PHOTO-LLNA: AN ALTERNATIVE PHOTOSENSITIZATION TEST USING FLOW CYTOMETRY AND IMMUNOPHENOTYPIC MARKERS TO IDENTIFY AND CHARACTERIZE PHOTOALLERGENS.

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Although the Local Lymph Node Assay protocol has been validated by ICCVAM for several years, factors have limited its widespread application and development. These include the propensity for irritants to cause false-positive results in the assay, and the need to use radioactive chemicals. We have developed an alternative photosensitization test which uses a non-radiometric method to assess proliferation of lymph node cells (LNC), using solar simulator irradiation to photo-activate test chemicals. The PHOTO-LLNA measures bromodeoxyuridine uptake into the DNA of proliferating cells using a BrdU-specific antibody and flow cytometry. Other immunophenotypic parameters including the B:T cell ratio, B220+, CD3+, CD4+, CD8+, CD69+, CD25+, I-A+ cells which permit the discrimination of false-positive irritants from true photosensitizers. The PHOTO-LLNA correctly classified several photosensitizers including chlorpromazine (EC_{3UV} = 0.095%), promethazine, bithionol, sodium omadine and tetrachlorosalicylanilide (TSCA), as well as the sensitizers DNCB and oxazalone, and the irritants benzalkonium chloride and SLS. In general, multi-component vehicles such as DMSO:Acetone:Ethanol (4:3:3) and Acetone:Olive Oil (4:1) were more useful than single-component vehicles. The immunophenotypic markers CD69, I-A^K, B220 (CD45R) and the B:T cell ratio were the most useful immunophenotypic endpoints for characterizing sensitizers and photosensitizers, and discriminating them from false positive irritants such as SLS. In summary, we have developed a modified non-radiometric PHOTO-LLNA test to identify and characterize photosensitizers, which is sensitive enough to characterize several weak photosensitizers. In addition, the enhanced PHOTO-LLNA can employ multiple immunophenotypic markers to discriminate between true photosensitizers and false-positive irritants which interfere with the interpretation of the results in standard sensitization tests.

758 EVALUATION OF MTT METABOLISM AS A MEANINGFUL INDICATOR OF VIABILITY IN HUMAN CORNEAL EPITHELIAL TISSUE MODELS.

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Cellular viability is routinely measured by the uptake and reduction of tetrazolium salt, MTT, to an insoluble formazan dye by cellular microsomal enzymes. With the emerging utilization of tissue equivalent models, the MTT bioassay has often been used for measuring cellular viability, however it has recently been reported that MTT only measures the viability in the basal layer of cells while cells in the suprabasal layers go undetected. To evaluate the ability of the MTT assay to measure the cell viability in tissue equivalent models, we examined the cellular viability of two commercially available human corneal models (MatTek EpiOcular OCL-200 and SkinEthic HCE) with multiple viability markers. First, we determined that the amount of formazan formed by healthy, non-treated tissue constructs increased linearly between 0.5 and 3 hr. of exposure to MTT. Histological analysis of cryopreserved tissue models incubated with MTT revealed that all nucleated cell layers in both tissue models reduced MTT demonstrating metabolically viable cells in the appropriate cell layers. An opposite staining pattern was visualized in tissue models treated with propidium iodide, a dye that penetrates nonviable cells, with only cells in the upper most (granular) layer retaining the dye. Further, rhodamine 123, which localizes to the mitochondria of viable cells, produced a similar distribution pattern to MTT in both tissue models. When the tissue constructs were treated with Triton X-100 to induce cellular damage, we measured expected decreases in MTT reduction after 10, 30 and 60 min exposures. Visual assessment of histological sections after Triton X-100 supports these quantified values with an approximate amount of cells retaining the formazan crystals. Our experiments demonstrate that the traditional MTT method exhibited conversion linearly with time and by multiples cell layers in the tissue constructs. In addition, results from the MTT method were comparable to other cytotoxicity dyes. Thus, we conclude that our data support the use of MTT as a cell viability marker as a stand-alone method.

759 APPLICATION OF AMPHITOX ASSAY TO DETERMINE THE TOXICITY OF DICHLOROACETIC AND TRICHLOROACETIC ACIDS.

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Epidemiological studies point out that chlorinated drinking-water could be related to cardiovascular disease, cancer, adverse pregnancy outcomes, etc. AMPHITOX, an *in vitro* toxicity test conducted with amphibian (Bufo arenarum) embryos may be valuable as an alternative method for screening agents for developmental effects. The acute (96 hr) and short-term chronic (7 days) toxicity of trichloroacetic acid (TCA) and dichloroacetic acid (DCA) was evaluated. Triplicates of 10 embryos, at complete operculum stage (S25) were maintained in AMPHITOX Solution containing TCA and DCA in concentrations ranging from 25 to 10, 000 mg/L. As DCA and TCA changed media pH markedly; normal embryo development was confirmed at this pH. TCA at 100mg/L reduced the pH to 4 and lethality was 50% at 24hr; DCA at 70mg/L also reduced the pH to 4 and lethality was 100%. Up to 8, 000 mg/L of TCA at pH 4.6 did not exert lethality in the amphibian embryos until 96 hr of exposure; DCA at 60mg/L resulted in 30% lethality; the toxicity of neither compound increased markedly when exposure period was extended to 7 days. The results indicate i) the toxicity of both DCA and TCA may related to the decreasing of pH in the maintaining media and ii) DCA is more toxic than TCA at equivalent pH. This may be related to the metabolic pathways of these haloacetic acids. The results are consistent with the developmental and reproductive potencies of these two compounds and demonstrate the validity of the AMPHITOX assay in the evaluation of the adverse effects of these disinfectant byproducts.

760 ESTABLISHMENT OF RAT LD50 REFERENCE VALUES FOR CHEMICALS TESTED IN A VALIDATION STUDY OF *IN VITRO* CYTOTOXICITY ASSAYS.

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The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM convened an international workshop in October 2000 that evaluated the validation status of *in vitro* methods for predicting

acute systemic toxicity (http://iccvam.niehs.nih.gov). Workshop participants recommended further validation of *in vitro* methods for predicting rodent and human acute toxicity. NICEATM and ECVAM subsequently designed a multi-laboratory validation study to evaluate two *in vitro* basal cytotoxicity tests using 72 coded chemicals representing the six globally harmonized acute oral toxicity hazard categories. In order to assess the predictive performance of the *in vitro* data, it was necessary to establish rodent LD50 reference values for each chemical. LD50 studies were located through literature searches and references from major toxicity databases such as RTECS. Studies were reviewed to identify the most appropriate LD50 reference value for each chemical. Criteria used to select reference LD50 values included: 1) consistency of age, gender, and species with that recommended in current acute oral toxicity testing guidelines, and 2) adequacy of the study based on documentation of experimental parameters such as method of administration, doses used, number of animals and deaths at each dose, and confidence interval for the LD50. Completeness of data for individual chemicals ranged from sparse summaries to detailed GLP compliant studies. Some LD50 values were unsupported by experimental details. The number of available rat oral LD50 values for each chemical ranged from 1 to 29, while oral LD50 values varied by as much as tenfold. Chemical-specific examples of the selection decisions for reference LD50 values will be provided. These reference data will be used to evaluate the extent that *in vitro* test methods can predict rodent LD50 values. Supported by NIEHS contract N01-ES 85424 and EPA IAG DW-75-93893601-0.

761 DESIGN OF A VALIDATION STUDY TO EVALUATE *IN VITRO* CYTOTOXICITY ASSAYS FOR PREDICTING RODENT AND HUMAN ACUTE SYSTEMIC TOXICITY.

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An international expert workshop convened by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM in October 2000 recommended that two *in vitro* basal cytotoxicity methods should be further evaluated to determine their usefulness for predicting rodent and human acute toxicity. NICEATM and ECVAM subsequently designed and initiated a multi-laboratory validation study to evaluate the relevance and reproducibility of two neutral red uptake assays using a mouse 3T3 fibroblast cell line and normal human epidermal keratinocytes. Seventy-two coded chemicals provided from a central repository and representing 12 chemicals from each of six hazard classification categories will be tested three times in each of three laboratories. The study is proceeding in three phases. Phase Ia established the historical databases for the positive control (sodium laurel sulfate) for each laboratory. The protocol will then be optimized to further minimize intra- and inter-laboratory variation after testing 3 chemicals in Phases Ib and 9 chemicals in Phase II. Sixty chemicals will then be tested in Phase III. The Registry of Cytotoxicity prediction model will be used to evaluate the prediction of rodent oral LD50 tests. Prediction of human toxicity will be evaluated using a prediction model based on human poisoning data. This study will characterize the usefulness of these cytotoxicity tests for predicting acute systemic toxicity and the extent that they may reduce or replace animal use. Supported by NIEHS contract N01-ES-85424 and EPA IAG DW-75-93893601-0.

762 CHARACTERIZATION OF DRUG METABOLIZING ENZYMES IN LLC-PK1 CELLS.

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The biochemical mechanism leading to para-aminophenol (PAP) toxicity in LLC-PK_1 cells, a porcine-derived proximal tubule cell line, is unknown. While investigating the mechanism of para-aminophenol bioactivation, the drug metabolizing capabilities of this cell line were determined. Four enzyme systems were chosen based on their wide substrate specificity and their tissue distribution. These included cytochrome P450, flavin monooxygenase (FMO), lipoxygenase (LO), and prostaglandin H synthase (PHS). Activities of each enzyme were determined in LLC-PK_1 S9 and compared to activities in rat liver and kidney S9. The expression of each enzyme was also determined by Western blotting. CYP450 1A1/1A2 activity and expression was greatest in hepatic S9, markedly lower in renal S9 and virtually undetectable in LLC-PK_1 S9. Similar results were obtained for CYP 2E1. FMO and PHS activities and expression were greatest in hepatic S9, reduced in renal S9 and barely detectable in LLC-PK_1 S9. LO activity and expression was highest in liver S9, very low in renal S9 and intermediate in LLC-PK_1 S9. These data suggest that the drug metabolizing capability of LLC-PK_1 cells is very low. For toxicants that require metabolic bioactivation, LLC-PK_1 cells may not represent a suitable model. Further, this lack of drug metabolizing ability needs to be considered when using LLC-PK_1 cells.

763 ICCVAM/NICEATM EXPERT PANEL RECOMMENDATIONS FOR THE STANDARDIZATION AND VALIDATION OF *IN VITRO* ESTROGEN RECEPTOR (ER) AND ANDROGEN RECEPTOR (AR) BINDING ASSAYS.

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A number of published studies indicate that a variety of natural and anthropogenic substances can interact with the endocrine system. As a result, legislation was enacted requiring the USEPA to develop a screening and testing program to identify substances with endocrine disrupting activity. Within the Tier 1 battery of screening test methods, *in vitro* ligand binding assays are proposed to identify substances that might interact with the ER or AR. The *in vitro* results would be considered with data from other Tier 1 assays in a weight-of-evidence evaluation of the need for testing in the more definitive Tier 2 *in vivo* assays. A comprehensive literature review indicated no adequately validated *in vitro* ER or AR binding assays. After considering the available data, an ICCVAM/NICEATM-sponsored Expert Panel developed recommendations for future standardization and validation efforts. For both types of binding assays, the Panel recommended recombinant human receptors and high-throughput procedures for validation; however, patent issues with the human AR may make it necessary to use a recombinant receptor derived from a non-human primate. For ER binding assays, the Panel recommended the use of intact human ERa or b proteins or the equivalent proteins from the rat. When screening for ecological effects, recombinant receptors from wildlife should be evaluated. Recommendations were also provided for minimum procedural standards and substances for validation studies. These recommendations should facilitate standardization and validation of protocols for ER and AR binding assays. Supported by NIEHS Contract N01-ES-85424.

764 ASSESSMENT OF PROTOCOL VARIABLES IN CYTOTOXICITY ASSAYS UTILIZING BALB/C 3T3 CELLS AND NORMAL HUMAN KERATINOCYTES.

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The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the European Center for the Evaluation of Alternative Methods (ECVAM) are currently sponsoring a study to determine the usefulness of two *in vitro* basal cytotoxicity methods [employing BALB/c 3T3 (3T3) cells and normal human keratinocytes (NHK)] for predicting rodent and human acute toxicity. Although cytotoxicity protocols for these two cell types were available (and were used to qualify these cell types with the Registry of Cytotoxicity prediction model), it was thought prudent to reevaluate certain variables before starting a formal study. We examined acceptable solvent concentrations, the effect of exposure time on correct toxicity predictions, and the appropriate seeding density to match the desired exposure time. For solvent concentration, we concluded, based on cytotoxicity at higher doses, that 0.5% should be the highest concentration for both ethanol and DMSO for both cell types. To assess exposure time, we tested 6 chemicals whose cytotoxicity had been reported to increase significantly at exposures greater than 24 h [Riddell, *et al.* (1986) ATLA 14:86-92]. After testing the chemicals using 24, 48 and 72 h exposures, we concluded that a 48 h exposure was optimal for either 3T3 or NHK cells. Finally, we found that the appropriate cell seeding densities that permitted just subconfluent growth in the solvent-alone control wells after a 48 h exposure period were $2.5X10^3$ cells/well (96-well plates; 24 h prior to treatment) for 3T3 cells and $2.5X10^3$ cells/well (96-well plates; 48-72 h prior to treatment) for NHK cells. These new parameters have been incorporated into the protocols that are now being used in the ICCVAM/ECVAM sponsored multi-laboratory study to evaluate *in vitro* cytotoxicity assays.

765 HUMAN MICROVASCULAR ENDOTHELIAL CELLS AND COCAETHYLENE-INDUCED VASCULAR TOXICITY.

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Cocaethylene (CE) is a toxic compound formed in the human liver after co-exposure to cocaine and ethanol. Such exposure occurs in over 80% of cocaine abusers. CE is more potent, lethal, and persistent relative to either cocaine or ethanol. Case

and clinical studies have revealed an association between peak CE serum levels, vascular pathology, and ischemic organ damage and failure. To further investigate the pathogenic mechanisms uderlying these findings, we evaluated the direct effects of CE on immortalized human microvascular endothelial cells (HMEC-1). When HMEC-1 were exposed to physiologically relevant doses of CE (0, 0.1, 0.5, and 1mM) during log-phase growth, a dose-dependent decrease in average adherent cell number (n=8, p<0.05) was observed in treated versus non-treated HMEC-1 (controls). Trypan blue exclusion testing showed decreased average viability in 1mM-treated cells (83%) versus controls (94%, n=8, p<0.01) during log-phase growth. However, significant changes in cell number and viability were not observed when exposure occurred in confluent cultures of HMEC-1 versus controls. Analysis of media from 1mM-treated post-confluent cultures resulted in decreased pH (n=9, p<0.05) and changes in gas consumption as reflected by decreased media bicarbonate levels (n=9, p<0.05) versus controls. Cation analysis in media samples from 1mM CE-treated HMEC-1 showed significant decreases in Ca(2+) and Na(+), and increased K(+), versus controls (n=9, p<0.05 for all). Glucose levels in media from 1mM-dosed HMEC-1 were significantly higher (n=9, p<0.01) than controls. Such changes suggest a loss of cellular regulation of cations and mitochondrial metabolism after exposure to CE. MTT assays showed significantly increased metabolic activity in CE-treated cells (n=18, p<0.05) versus controls. All significant changes were time-dependent and observed over 72 hours after dosing. These results showed that CE is capable of direct, toxic interaction in HMEC-1.

766 PROGRESS REPORT ON THE DSSTOX DATABASE NETWORK: NEWLY LAUNCHED WEBSITE, APPLICATIONS, FUTURE PLANS.

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Progress will be reported on development of the Distributed Structure-Searchable Toxicity (DSSTox) Database Network and the newly launched public website that coordinates and serves as the central hub for this effort. DSSTox is an EPA-sponsored, community-wide project whose goals are to: 1) create and promote the use of standard format, chemical structure-inclusive data files for storing public chemical toxicity data; 2) involve the international user community in the effort to migrate more public toxicity data into the standard format for sharing; and 3) facilitate structure-searchability across toxicity databases and more complete access to data for use in toxicity prediction model development. DSSTox toxicity data files for a variety of public toxicity databases have been created for carcinogenicity, mutagenicity, aquatic toxicity, pesticide toxicity, and estrogen-receptor binding; a variety of other database files are in development. Importing of these files into commercial chemical relational database applications and other public web-based applications provide sophisticated structure-searching capability for exploring the structural basis for toxicity across multiple types of biological endpoints. Essential to the ultimate success of this effort will be community involvement in propagating the proposed data standards and hosting DSSTox standard format data files. Hence, one of the most important roles of the central DSSTox website is that of enlisting and coordinating the efforts of the larger toxicology and modeling communities in contributing to this public database project. This abstract does not reflect EPA policy.

767 ASSESSMENT OF COLONY NUMBER AND MORPHOLOGY HIGHLIGHTS TOXICITY FOR HEMATOPOIETIC AND MESENCHYMAL PROGENITORS.

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Standardized and robust clonogenic assays for myeloid, erythroid and megakaryocytic progenitors can be used to assess the toxicity of lead compounds on the various precursors of mature blood cells. In addition, assays for mesencymal progenitors can be used to assess potential toxicity of compounds being developed for use in tissue engineering. Inhibitory activity can be determined in media formulations containing optimal cytokine combinations. This allows the identification of either gross effects i.e. reduction of colony numbers or subtle effects where colony number is consistent but the morphology of the colony is compromised. This morphological discrimination may be important to identify compounds that may have escaped toxic surveillance using other assays, and which may be used over an extended time period clinically. Unlike other surrogate assays that rapidly assess antigen expression or cell death, these clonogenic assays require culturing bone marrow, mobilized peripheral blood or cord blood cells for 14 days. This promotes the proliferation and differentiation of primitive cells into lineage specific colonies. In the semi-solid methycellulose based assay, the simultaneous proliferation of erythroid and myeloid colonies allows the identification of compounds that have lineage specific effects. Studies performed have shown that when cells are cultured in the presence of a toxic compound, colony number or morphology of one lineage type can be unaffected but there can be complete ablation of the other progenitors. The flexibility of these assays also facilitates the assessment of potential stimulatory molecules like chemokines or cytokines using sub-optimal cytokine concentrations. Using specific cytokine combinations, direct or synergistic effects of compounds on hematopoieitic progenitors can be elucidated. Although not useful for high throughput preliminary screening, these assays have proved important in pre-clinical studies.

768 EPIDERM™ FULL THICKNESS (EPIDERM-FT), A DERMAL-EPIDERMAL SKIN MODEL WITH A FULLY DEVELOPED BASEMENT MEMBRANE.

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Paracrine signaling between dermal fibroblasts (FB) and epidermal keratinocytes (KC) is believed to modulate skin responses during contact irritant or allergic reactions. Dermal FB also play an important role in photo-aging and photo-damage, wound healing and cancer progression. To enable *in vitro* study of these and other dermal phenomena in which FB-KC interactions are important, a full thickness skin model composed of a FB-containing dermis/KC-containing epidermis was developed. Normal human epidermal KC and dermal FB were cultured to produce highly differentiated full-thickness tissues extending wall-to-wall in cell culture inserts. Histologic examination of the tissue shows a collagen dermis populated by numerous viable FB and an epidermis consisting of stratified KC including basal, spinous, granular and stratum corneum components. The ultrastructure of the dermal/epidermal junction was examined by transmission electron microscopy. A well-developed basement membrane was evident. Hemidesmosomes were observed at the basal membranes of KC, with associated tonofilaments extending into the cytoplasm. Well-defined, continuous lamina lucida and lamina densa and fine anchoring filaments were present beneath the basal KC. Anchoring fibrils with characteristic striated structure connected the lamina densa to the underlying collagen matrix. Tissue responses to ultraviolet irradiation (UVR) were also evaluated. Twenty-four hours after irradiation with 40 J/cm2 of UVR, tissues were examined histologically, and culture media was assayed for pro-MMP-1 secretion by ELISA. Irradiation produced numerous sunburn cells and disruption of basal KC organization compared to control tissues. Also, pro-MMP-1 secretion was significantly increased compared to controls. EpiDerm-FT overcomes shortcomings of previous models in terms of providing a wall to wall tissue as well as appropriate *in vivo*-like basement membrane development. These attributes will enable more realistic *in vitro* toxicological studies of dermal/epidermal phenomena.

769 VALIDATION AND AUTOMATION OF AN *IN VITRO* TOXICITY SCREEN USING ACTIVTOX C3A HEPATOCYTE CELL LINE ON A FAMILY OF NSAIDS.

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Rational: Approximately half of all drug candidates fail due to poor toxicological profiles that are not identified prior to the commitment of lead compounds to costly trials. Despite advances in the development of high throughput technologies that benefit early phases of discovery, there is a lag in selection of early lead compounds that will prove non-toxic in pre-clinical and clinical development. By creating a way to conduct high throughput screening technologies with a cell line that accurately and reproducibly detects toxicity, a novel method for drug development could be established. Structure-activity relationships (SAR) can be derived allowing undesirable properties of molecules to be designed out early in the development, without necessarily affecting the potency of the compound. **Experimental Procedure:** All assays were performed using Amphioxus Cell Technologies' ACTIVTox human hepatocyte cell line. All liquid handling, fluorescence detection and incubations were fully automated on a high throughput system (HTS). Toxicity was evaluated using proliferation inhibition and membrane integrity in two different fluorescent based assays. **Results:** The nonsteroidal anti-inflammatory drugs (NSAIDS) present a structurally related group of compounds where there is an extensive scientific literature that can be used for standardization and comparison. . Upon ranking of the NSAIDS according to their toxicity, a clear structural progression was observed. As part of testing the reliability and quality of these high throughput toxicity screens, Z-factor analysis was performed in order to demonstrate the ease and reliability of these assays when adapted to a fully automated system. **Conclusions:** Our results demonstrate that ACTIVTox provides a set of high throughput toxicity assays that can be used to sort and rank compounds according to structure. Additionally, Z-factor values of 0.8 demonstrate it's use as a HTS early toxicity discovery tool.

769a EVALUATION OF THE METABOLISM OF TOXIN T514 (PEROXISOMICINE) IN HEPATOCYTES AND LIVER MICROSOMES.

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Toxin T-514 (Peroxisomicine) is a dimeric hydroxyantrhacenone with selective citotoxic effect for malignant cells.In this study we have evaluated the metabolism of this compound in two *in vitro* systems (liver microsomes from rat, mouse, dog and monkey, and rat and human hepatocytes).The microsomes (12.5, 125 and 250 micrograms/mL) and hepatocytes (1x106 cel/mL) were incubated with the toxin (25 micromolar) for 0.5, 1, 3, 6, 9, 12 and 24 hours, and the samples were examined by chromatographic analysis and UV-Vis spectra. Two chromatographic peaks were detected besides the toxin peak in the rat microsomes samples and one in the monkey microsomes.The Tr and UV-Vis spectra of the peaks were very similar to those of the toxin suggesting that they would corresponde to semiquinone metabolites.The cytotoxicity of the two metabolites was evaluated in Chang liver cells and Hep G2 cells and they did not show the selective cytotoxic effect on tumoral cells as the original compound.

770 LOW-LEVEL CYCLO-SARIN (GF) VAPOR EXPOSURE IN RATS: EFFECT OF EXPOSURE CONCENTRATION AND DURATION ON PUPIL SIZE.

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Objective of the study was two-fold: a) to determine the EC50 for GF vapor-induced miosis and associated probit slope in the rat and b) to model the relationship between GF vapor concentration (C), exposure duration (t) and the probability of miosis. The probability of GF vapor-induced miosis (defined as a post-exposure pupil diameter 50% or less of pre-exposure pupil diameter) was estimated in rats exposed to various combinations of exposure concentration and duration. Groups of male and female Sprague-Dawley rats were exposed to one of a series of GF vapor concentrations for a single duration (10, 60 or 240 min) in a whole-body dynamic chamber. Miosis was measured using an infrared camera technique. For each exposure time, separate effective concentrations for miosis in 50% of the exposed population (EC50) and the corresponding dose-response slopes were determined by the Bliss probit method. Contrary to Haber's rule, ECt50 values increased with exposure duration (i.e., the Ct for 50% of the exposed population to show miosis was not constant over time). Female rats were more sensitive to GF vapor toxicity than male rats. Miosis was the only clinical sign noted following GF vapor exposure. Possible depression of blood esterase (acetylcholinesterase, butyrylcholinesterase and carboxylesterase) activities due to low-level range of GF vapor concentrations was also investigated. GF was regenerated from blood samples of vapor-exposed rats by the addition of fluoride ion at pH 4 and the samples were analyzed by GC-FPD and GC-MS. Levels of regenerated GF in the red blood cell (RBC) fraction of the samples were four to 20 times lower than in plasma. All controls were negative for regenerated GF. The primary conclusions were: a) the probability of GF vapor-induced miosis was not described by a toxic load model and b) no correlation was found between miosis and circulating esterase activity.

771 INHALATION TOXICITY OF SARIN (GB) VAPOR IN THE GOTTINGEN MINIPIG: LOW-LEVEL THRESHOLD EFFECTS.

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In order to estimate the potential impact of a chemical warfare agent vapor contamination on battlefield operations, it is necessary to fill critical gaps in data involving acute low-level exposures. While the initial signs of such an exposure may not be immediately life threatening, it is possible that they may become a physiological dysfunction, affecting performance of some military tasks. The ability to estimate threshold conditions of exposure (concentration and duration) that are likely to result in physiologically significant effects is an essential prerequisite for predicting the potential impact on task performance or military operations. The goal of these experiments was to determine the lowest sarin (GB) vapor concentration of physiological significance. During a whole-body exposure to GB vapor, the first noticeable effect, at such concentrations, is constriction of the pupil (miosis). The minipig was chosen as a model for studying the effects of GB vapor on the pupil because of anatomical and physiological similarities to humans. The minipigs were secured in a sling that allowed continuous monitoring of one eye. The pigs were individually exposed to fixed concentrations of GB vapor ranging from 0.03-2.0 mg/m^3 for 60 minutes. Pupil constriction was assessed, under dim-light conditions, using an infrared light sensitive video camera. Pupil size was continuously monitored by capturing high-resolution images of the eye before, during and after exposure to GB vapor. Pupil area was then quantified, off-line, using a custom-designed software package. Pupil area was graphed as a function of time. The relationship between the dose-response curve (percent pupil constriction as a function of time) and vapor concentration was investigated. The calculated EC_{50} (miosis) was 0.04267 mg/m^3 with 95% confidence intervals of 0.041 to 0.047 mg/m^3.

772 SUBCLINICAL DOSES OF THE NERVE GAS SARIN IMPAIR T CELL RESPONSES THROUGH THE AUTONOMIC NERVOUS SYSTEM.

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The nerve gas agent sarin is a lethal tool in the hands of terrorists, and exposure to it may lead to neurotoxicity and death. Even low doses of sarin may cause subtle electrical changes in the brain. Because the brain communicates with and regulates the immune system, we examined the effects of inhalation of subclinical doses of sarin on the immune system. Spleen cells from F344 rats, inhaling low levels (0.2-0.4 mg/m3) of sarin for 1 h/day for 5 or 10 days, but not for 1 day, exhibited significant reductions in the primary antibody-forming cell response and T cell proliferation in response to concanavalin A and the anti-T cell receptor mAb. Moreover, sarin suppressed the increase of the intracellular calcium concentration in response to anti-T cell receptor mAb, indicating that sarin might affect the antigen-mediated signaling in T cells. The immunological effects of sarin were not associated with increased serum corticosterone levels or changes in the brain cholinesterase activity. However, pretreatment of animals with the ganglionic blocker chlorisondamine abrogated the effects of sarin on T cell mitogenesis. These results suggest that subclinical doses of sarin may suppress the immune system *via* the autonomic nervous system.

773 FLUORIDE ION REGENERATION OF GB FROM MINIPIG TISSUE AND FLUIDS AFTER GB INHALATION EXPOSURE.

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Fluoride ion regenerated sarin (R-GB) was found in minipig blood and tissue after whole-body sarin vapor exposure at miosis and lethal levels. R-GB in these samples was analyzed by GC-FPD or GC-MS after a C18 solid-phase extraction (SPE) sample preparation that included fluoride ion addition and pH adjustment. The highest levels detected after a lethal level exposure were seen in the lungs (22.8 ng/g) and red blood cells (18.8 ng/g) as expected after an inhalation exposure. Concentrations of R-GB in red blood cells were approximately 100 times greater than in the plasma. Outside of the lungs and blood, sections of the brain had significant levels of R-GB such as the frontal lobes (2.28 ng/g), the temporal lobes (1.68 ng/g) and the caudate nucleus (5.46 ng/g), which by itself was comparable to liver levels (5.84 ng/g). Other significant depots of regenerated GB included saliva (137 ng/g), nasal discharge (72 ng/g), and lachrymal discharge (186 ng/g). Limited amounts of free GB were also isolated from saliva (6.91 ng/g) and nasal discharge (0.65 ng/g). Serial blood samples taken before and during miosis-level GB vapor exposures resulted in red blood cell R-GB levels that steadily rose during the exposure. This clearly demonstrated the ability of the R-GB assay to monitor systemic dosage. The slopes of the lines created by plotting R-GB versus time closely correlated to the experimental exposure level. Therefore, the rates of GB absorption in these animals were proportional to the GB exposure concentration. Acetylcholinesterase activity, which is typically used to verify and monitor exposure to nerve agents, was an ineffective indicator of exposure at miosis levels in these experiments.

774 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR HYDROGEN CYANIDE.

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AEGL values represent one-time exposures for emergency situations. AEGL values were developed for hydrogen cyanide (HCN), a colorless, rapidly-acting, highly poisonous gas having an odor of bitter almonds. HCN is a systemic poison that inhibits cytochrome oxidase, thus preventing cellular utilization of oxygen. Data on

human exposures were limited to monitoring studies. Animal studies with several species addressed lethal as well as sublethal effects (incapacitation and changes in respiratory and cardiac parameters). Time scaling was based on C^n x t = k. Regression analysis of the exposure duration-concentration relationships for incapacitation and lethality for the monkey determined that n = 2 and that for the relationship for lethality in the rat, n = 2.6. The AEGL-1 values for 10 and 30 min and 1, 4, and 8 hr of 2.5, 2.5, 2.0, 1.3, and 1.0 ppm, respectively, were based on human monitoring studies in which the preponderance of data indicated that an 8-hour exposure to 1 ppm would be without adverse health effects for the general population. Time scaling utilized the default value of n = 3. The AEGL-2 values (17, 10, 7.1, 3.5, and 2.5 ppm for 10 and 30 min and 1, 4, and 8 hr, respectively) were based on exposure of cynomolgus monkeys to a concentration of 60 ppm for 30 minutes. This exposure was the threshold for a physiological response of increased respiration and central nervous system depression. The 60 ppm value was adjusted by a total uncertainty factor (UF) of 6. For time-scaling, n = 2 was used. AEGL-3 values (10 and 30 min and 1, 4, and 8-hr values of 27, 21, 15, 8.6, and 6.6 ppm, respectively) were based on the threshold for lethality in the rat following various exposures (15- and 30-min and 1-hour LC_{01} values of 138, 127, and 88 ppm, respectively). Values were adjusted by a total UF of 6; time scaling to the 4- and 8-hr exposure durations was based on the empirical data for lethality in the rat in which n = 2.6.

775 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR NERVE AGENTS.

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Nerve agents are potent anticholinesterase compounds. For AEGL development, human and laboratory animal exposure data were analyzed. In comparison to agent GB, the data sets characterizing toxicity of agents GA, GD, GF and VX are sparse. AEGL estimates for these latter agents were developed by a comparative method of relative potency from the more complete data set for agent GB. The critical effect for agent GB AEGL-1 values (0.0069, 0.0040, 0.0028, 0.0014, and 0.0010 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) is EC50 for miosis in female SD rats, with supportive studies for other species. Because the miotogenic response of mammal eyes to GB vapor exposure is similar across multiple species, the interspecies uncertainty factor (UF) for AEGL-1 is 1; the intraspecies UF of 10 is for protection of potentially susceptible individuals. AEGL-2 values for agent GB (0.087, 0.050, 0.035, 0.017, and 0.013 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) were developed from human experimental data for miosis plus dyspnea, RBC-ChE inhibition, and single fibre electromyographic changes. The interspecies UF is 1, and the intraspecies UF is 10. AEGL-3 values for agent GB vapor exposure (0.38, 0.19, 0.13, 0.070, and 0.051 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) are based upon LC01 values for female SD rats exposed to GB vapor for durations of 3 to 360 min. The interspecies UF is 3, and the intraspecies UF is 10. Scaling of exposures to AEGL-specific durations was linear (C^n x t = k, where n=2). Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the US Department of Energy under contract DEAC0500OR22725

776 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR SULFUR MUSTARD (AGENT HD).

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Sulfur mustard (Agent HD) is an alkylating chemical vesicant that affects any epithelial surface with which it comes in contact; it has been historically developed and used as a warfare agent. For AEGL estimation, both human and animal exposure data were analyzed. AEGL-1 values (0.40 mg/m3, 0.13 mg/m3, 0.067 mg/m3, 0.017 mg/m3, and 0.008 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) were based upon data from informed human volunteers. An exposure concentration-time product (Ct) of 12 mg-min/m3 represented a threshold for conjunctival injection and minor discomfort but no functional decrement. An intraspecies uncertainty factor (UF) of 3, applied to protect potentially sensitive individuals, was considered appropriate. AEGL-2 values (0.60 mg/m3, 0.20 mg/m3, 0.10 mg/m3, 0.025 mg/m3, and 0.013 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) were based upon human exposure data showing a Ct of 60 mg-min/m3 as a lower margin of the concentration-effect zone to the eye that resulted in ineffective military performance. Variability in the ocular responses was minimal among the volunteers, thereby justifying an intraspecies adjustment of only 3. A 3-fold reduction accommodated potential onset of long-term ocular or respiratory effects. AEGL-3 values (3.9 mg/m3, 2.7 mg/m3, 2.1 mg/m3, 0.53 mg/m3, and 0.27 mg/m3 for 10 min, 30 min, 1 hr, 4 hrs, and 8 hrs, respectively) were based upon an estimated lethality threshold of 21.2 mg/m3 in mice exposed for 1 hr. A 3-fold downward adjustment accounted for intraspecies variability and a UF of 3 was applied to account for interspecies variability in the lethal response to sulfur mustard. Scaling of exposures to AEGL-specific durations utilized Cn x t = k, where n=1 or 3. Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

777 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR ARSINE.

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AEGL values represent one-time exposures for increasingly severe effects with AEGL-1 being least and AEGL-3 being most severe. AEGLs were developed for arsine, a colorless gas and potent hemolytic agent, using exposure-response data from animal studies. Values for AEGL-specific exposure durations of 0.5 hr, 1 hr, 4 hr, and 8 hr were scaled from the experimental exposure duration using exponential scaling (Cn x t = k, where n= 3 when extrapolating to shorter durations and n = 1 when extrapolating to longer durations). Numeric values for AEGL-1 were not recommended due to the lack of data and the inability to define an exposure that would be consistent with AEGL-1 effects. AEGL-2 values (0.21 ppm, 0.17 ppm, 0.04 ppm, and 0.02 ppm for 0.5 hr, 1 hr, 4 hr, and 8 hr, respectively) were based on experimental exposures that did not result in significant alterations of hematologic parameters in mice exposed to arsine for 1 hr. Uncertainty factor application included a factor of 10 to account for possible interspecies variability and a factor of 3 was applied for individual variability. The AEGL-3 values (0.63 ppm, 0.50 ppm, 0.13 ppm, and 0.06 ppm for 0.5 hr, 1 hr, 4 hr, and 8 hr, respectively) were based upon an exposure producing hemolysis and lethality in mice exposed to arsine for 1 hr. Total uncertainty factor adjustment was 30; 10 for interspecies variability and 3 for intraspecies variability. The AEGLs reflect the narrow margin between exposures resulting in minor effects and those producing lethality. The approach used to develop the AEGLs for arsine was justified by the known steep exposure-response relationship, the induction of hemolysis by arsine at extremely low concentrations, and the potential of arsine-induced hemolysis to irreversibly progress to life-threatening renal failure. All of the AEGL values are near or below the odor threshold for arsine. Oak Ridge National Laboratory is managed by UTBattelle, LLC for the US Department of Energy under contract DEAC0500OR22725.

778 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR PHOSGENE.

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AEGL values represent one-time exposures for increasingly severe effects with AEGL-1 being least and AEGL-3 being most severe. Appropriate data were not available for deriving AEGL-1 values for phosgene. Odor cannot be used as a warning for potential exposure. The odor threshold is reported to be between 0.5 to 1.5 ppm, a value above or approaching AEGL-2 and AEGL-3 values, and tolerance to the pleasant odor of phosgene occurs rapidly. Furthermore, following odor detection and minor irritation, serious effects may occur after a clinical latency period of 24 hours. AEGL-2 values (0.6 ppm, 0.6 ppm, 0.3 ppm, 0.08, and 0.04 ppm for 10-min, 30-min, 1-hr, 4-hr, and 8-hr, respectively) were based on chemical pneumonia in rats exposed to 2 ppm for 90 min. The 90 minute value was then scaled to the 30-minute, 1-, 4-, and 8-hour AEGL exposure periods, using cn x t = k, where n=1. The 30-minute value was also adopted as the 10-minute value because extrapolation would yield a 10-minute AEGL-2 value approaching concentrations producing alveolar edema in rats. The 30-min, 1-, 4-, and 8-hr AEGL-3 values (1.5 ppm, 0.75 ppm, 0.20 ppm, and 0.09 ppm, respectively) were based on the highest concentration causing no mortality in rats after a 30-minute exposure (15 ppm). The value was scaled to the 1-, 4-, and 8-hour AEGL periods, using cn x t = k, where n=1. The 10-min AEGL-3 value (3.6 ppm) was based on the highest concentration causing no mortality in the rat or mouse (36 ppm) after a 10-minute exposure. For all AEGL-2 and -3 values, an uncertainty factor (UF) of 3 was applied for interspecies extrapolation because little species variability is observed for both lethal and non lethal effects after exposure to phosgene. A UF of 3 was applied to account for sensitive individuals due to the steep concentration-response curve

which implied limited variability. Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

779 EXPOSURE OF HUMAN EPIDERMAL KERATINOCYTES TO SULFUR MUSTARD INDUCES THE FORMATION OF HIGH MOLECULAR WEIGHT PROTEIN AGGREGATES CONTAINING KERATIN 5 AND KERATIN 14.

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The chemical warfare agent sulfur mustard (SM) is a vesicant and alkylating agent that has the capacity to crosslink biological molecules. We are interested in identifying specific proteins that are altered upon SM exposure. Keratins are particularly important for the structural integrity of skin, and several genetically inherited blistering diseases have been linked to mutations in keratin 5 (K5) and keratin 14 (K14). We examined whether SM exposure alters keratin biochemistry. Cultured human epidermal keratinocytes (HEK) were exposed to SM. Western blotting with specific monoclonal antibodies revealed the formation of stable high molecular weight aggregates containing K14 and K5. These aggregates begin to form within 15 minutes after SM exposure, form only at SM concentrations that are considered vesicating-equivalent doses, and persist until cell death. These aggregates display a complex gel electrophoresis pattern between ~100 kD and ~200 kD. Biochemical purification of these aggregates allowed analysis of the aggregate components by mass spectrometry. These approaches confirmed the presence of K14 and K5 in the aggregates and suggest that at least some of the aggregates are composed of K14-K14, K14-K5, or K5-K5 dimers. These studies demonstrate that SM is capable of inducing keratin aggregation in HEK and support further study into the role of keratin aggregation in SM-induced vesication.

780 ACUTE ORAL TOXICITY OF NITROSO DEGRADATION PRODUCTS OF HEXAHYDRO-1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX).

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Anaerobic degradation of the munition hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) in the environment and during bioremediation results in nitroreductase-catalyzed formation of hexahydro-1-nitroso-3, 5-dinitro-1, 3, 5-triazine (MNX), hexahydro-1, 3-dinitroso-5-nitro-1, 3, 5-triazine (DNX), and hexahydro-1, 3, 5-trinitroso-1, 3, 5-triazine (TNX). Although parent RDX has been identified as a central nervous system toxicant in both humans and rats upon acute oral exposure, toxicity of the degradation products is unknown. The objective of this study was to determine the most potent RDX N-nitroso product in acutely exposed rats, then estimate its oral LD50 and identify critical adverse effects. An abbreviated up-and-down procedure (UDP) with dose progression of 2.13 (1/3 log unit) and 400 mg/kg starting dose, chosen to exceed previous estimates of RDX LD50 of ~100 mg/kg, were used for comparative studies. Compounds emulsified in corn oil plus 4% DMSO were administered by gavage to female Sprague-Dawley rats (175 - 200 gm) and clinical observations made over 14 days. Lethality at 400 mg/kg of MNX, DNX and TNX was 2/2, 2/3 and 2/3 and at 187 mg/kg was 2/3, 0/3 and 0/3, respectively. CNS toxicity, as indicated by onset of forearm clonic seizures as early as 10 min, occurred with a prevalence of 2/2, 3/3 and 2/3 at 400 mg/kg and 2/3, 0/3 and 1/3 with 187 mg/kg MNX, DNX and TNX, respectively. A complete UDP test with starting dose of 87 mg/kg MNX and analyzed with the AOT425StatPgm yielded a maximum likelihood LD50 estimate of 187 mg/kg and 95% confidence interval of 118 to 491 mg/kg. Seizures were again observed, while histopathological splenic congestion and siderosis were noted for 14-day survivors dosed with 187 mg/kg MNX. These results indicate that MNX, with an LD50 comparable to parent RDX, is the most potent N-nitroso degradation product and shares CNS toxicity as a critical toxic effect. Support: US Army Corps of Engineers

781 VARIATION OF SERUM BUTYRYLCHOLINESTERASE IN A MILITARY POPULATION.

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Butyrylcholinesterase (BChE) is often used in the occupational monitoring of individuals exposed to carbamate or organophosphorus insecticides. BChE is used as a surrogate marker for acetylcholinesterase inhibition. One drawback of BChE is the large variation it exhibits. This variation makes this enzyme impractical for spot analysis. In practice, baseline values must be obtained for later comparison to individual values. To date, the majority of variation has not been explained. A military population may be more homogeneous in unknown variables which may reduce the variation. Elucidation of the baseline variability in this population may aid in the development of spot testing procedures among groups of military members without the use of individual baseline results. This study involved analysis of previously collected samples for BChE activity. The samples were taken from an active duty male population with an age distribution of 18 to 57 years. After removal of unique identifiers, samples were frozen at minus 20C until analyzed. A modified version of the Ellman assay, using butyrylthiocholine as the substrate, was used to measure BChE. A total of 445 unique samples were analyzed over a twelve-month period. The mean activity and range were 9.1 University/mL and 0.1 to 23.0 University/mL respectively. The overall variation (37% coefficient of variation) of these samples indicates that the variability is not lower than civilian counterparts. Samples from individuals under age 26 had lower ($p = 0.008$) variation as compared to those over age 26. There was also a statistically significant difference ($p < 0.001$) between the total BChE activity measured among the months of the year. These findings suggest that the amount of variation in a military population does not warrant spot individual testing of BChE activity.

782 RELATIONSHIP BETWEEN THE DOSE-RESPONSE CURVES FOR LETHALITY AND SEVERE EFFECTS FOR CHEMICAL WARFARE NERVE AGENTS.

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In recent years, the US Environmental Protection Agency has developed a categorical (or ordinal) logistic regression approach for regressing ordered categories of toxic responses to a toxicant exposure on one or more factors. This approach permits the simultaneous fitting of two types of dose-response curves (severity of effect and percent of individuals versus dose). It is also possible to statistically demonstrate and quantify the steepness of both types of dose-response curves for acute exposures to organophosphate-type chemical warfare agents. Data from three previous acute inhalation studies were separately reanalyzed using a probit link-function: monkeys exposed separately to GA (tabun), GB (sarin) or GF (cyclosarin) (from a 1957 study); rats exposed to GB; and rats exposed separately to GB or GF. For the three studies, vapor concentration and exposure time were varied; and in the recent rat studies, identifying differences between genders was an objective. Clinical signs and mortality were recorded in all three studies, from which three categorical responses were defined: death, severe effects and less than severe effects. An animal was categorized as having severe effects if it exhibited at least one of the following signs (yet did not die): convulsions, gasping, collapse or prostration. The regression analysis found that for all three studies slightly more than one standard deviation (1.0 to 1.4) separated an effective concentration (ECXX) for severe effects from a lethal concentration (LCXX) for XX% effected. The immediate benefit from this finding is the establishment of a method for estimating threshold lethality for nerve agents. Threshold lethality (approximately a LC01 or LC05) is roughly equivalent to an EC16 (severe) for the data analyzed. The 16% effect level can be estimated with greater confidence from experimental quantal data *via* probit analysis than the 1% effect level. Thus, questionable extrapolation of the dose-mortality curve down to the 1% level can be avoided by using the dose-severe effect curve in its place.

783 INHALATION MEDIAN LETHAL DOSES OF *BACILLUS ANTHRACIS*, AMES, AND VOLLUM STRAINS IN THE RHESUS MONKEY.

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Bacillus anthracis, the etiologic agent of anthrax in both humans and animals, is a large, Gram-positive rod in its vegetative form. More importantly, *B. anthracis* is capable of forming spores that are highly resistant to environmental deterioration. Aerosolized spores can cause inhalational anthrax, which is greater than 50% fatal in humans, despite appropriate treatment. Thus, weaponized *B. anthracis* is considered a serious threat. This study was to determine the median lethal dose (LD_{50}) of *B. anthracis* Ames and Vollum strains in a rhesus macaque model of inhalational anthrax. Rhesus macaques were exposed to Ames or Vollum strains of *Bacillus anthracis* by the pulmonary route. The cumulative average mass median aerodynamic diameter of the generated particles for all exposures was 1.31 mm, with a geometric standard deviation of 1.8 as determined by an API Aerosizer. For each strain, probit dose-response models were fitted to dose-lethality data using the method of maximum likelihood. Estimated parameters of probit dose-response models were used to compute LD_{50} values for each strain. Fieller's method was used to compute a 95% confidence interval for the inhaled LD_{50}. In rhesus macaques, the inhaled LD_{50}s and 95% confidence intervals were 10, 900 (1, 320 to 241, 000) and 6, 750

(21 to 116, 000) CFUs for the Ames and Vollum strains, respectively. There was no statistical significant difference in the survival time for either strain with an average day to death of 6.8 days for Ames strain, and 4.7 days for Vollum strain. These results demonstrate that both strains are lethal by the inhalation route. The inhaled LD_{50}s of the Ames and Vollum strains of *B. anthracis* were not significantly different from each other and are within the expected experimental difference of previously reported values in the rhesus monkey. The results of these data are important for the estimation of the human inhalation *B. anthracis* dosage.

784 EFFECTS OF SULFUR MUSTARD ON SKIN TOXICITY IN COX-1- AND COX-2-DEFICIENT MICE.

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Sulfur mustard (SM) is a potent vesicant employed as a chemical-warfare agent. Exposure results in erythema, edema, and blisters. The evolution of tissue damage is associated with an inflammatory response including vasodilatation, polymorphnuclear infiltration, and production of inflammatory mediators and cyclooxygenase (COX) activity. In order to evaluate the role of COX-1 and COX-2 in SM-induced skin toxicity, we tested the effect of SM on the ear of COX-1- and COX-2-deficient mice. In the latter, the degree of ear swelling 24 and 48 hr- after SM exposure was significantly reduced by 54% and 33%, respectively, compared to the wild-type mice. Histopathological analysis 48 hr after exposure revealed a statistically significant reduction of 58% and 64% in epidermal necrosis and acute inflammation, respectively. A similar trend was observed in subepidermal microblister formation, hemorrhage, and dermal necrosis. Although not statistically significant, COX-1-deficient mice showed a higher degree of subepidermal microblister formation, epidermal ulceration, and epidermal necrosis in comparison to the wild type. These findings indicate that COX-2 plays a central role in SM-induced skin toxicity and that COX-1 might exert a protective function against this chemical insult. (This study was supported by US Army Medical Research and Material Command under Cooperative Agreement DAMD17-98-2-8009.)

785 PROTECTIVE EFFECTS OF TOPICAL IODINE CONTAINING ANTI-INFLAMMATORY DRUGS AGAINST SULFUR MUSTARD-INDUCED SKIN LESIONS.

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Previous studies with sulfur mustard (SM), a potent vesicatory chemical-warfare agent, showed the antidotal efficacy of topical iodine at short intervals of 15 and 30 min between exposure and treatment. We demonstrate efficacy at longer intervals (20-, 30-, 45-, and 60-min) in mouse and guinea pig using an improved topical povidone-iodine preparation termed N66 containing steroidal and non-steroidal anti-inflammatory agents. In the mouse, ear edema was reduced by 43%, 47%, 44%, and 36%, ear epidermal ulceration by 74%, 58%, 45%, and 58%, and epidermal necrosis by 54%, 34%, 26%, and 31% at the respective intervals; a similar effect was seen with encrustation. The grade of acanthotic area, a healing marker, showed dramatic increases of 40-, 25-, 21-, and 22-fold, respectively. Two dermal parameters showed marked improvement; acute inflammation was reduced by 63%, 34%, 34%, and 38% and dermal necrosis by 80%, 54%, 54%, and 59%, respectively. In the guinea pig, topical treatment 45 min after exposure reduced the SM-induced ulceration area by 75%. Histologically, subepidermal microblister formation, epidermal ulceration, epidermal necrosis, and encrustation were reduced by 63%, 61%, 41%, and 41%. The grade of acanthotic area was elevated by 73% compared to control. N66 also caused a statistically significant reduction in acute inflammation (33%) and dermal necrosis (48%). Reduced damage was also observed in skin areas adjacent the treated sites. These findings suggest that the povidone-iodine preparation combined with anti-inflammatory agents functions as an antidote against skin lesions induced by SM (supported by USAMRMC DAMD17-98-2-8009).

786 SOY PHYTOESTROGENS PROTECT AGAINST GAMMA IRRADIATION.

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Chemical compounds that provide protection from ionizing radiation induced injury are known as radioprotectors. They have applications in clinical oncology, space travel, radiation site cleanup, radiological terrorism, and military scenarios. Soy phytoestrogens, such as the isoflavones genistein and daidzein, have beneficial health effects. We previously reported that both oral and subcutaneously administered genistein are radioprotective in mice. In this study, we further characterized the radioprotective effects of genistein and also evaluated daidzein for protective efficacy. Pharmacologic doses of the isoflavones were administered to CD2F1 male mice. The traditional 30-day survival endpoint for the evaluation of radioprotectors was used. In the first experiment, mice received a single subcutaneous dose of 3 to 400 mg/kg of genistein either 1 hr or 24 hr before cobalt-60 gamma irradiation (9.5 Gy at 0.6 Gy/min). When administered 24 hr before irradiation, doses of 25 to 400 mg/kg of genistein, with the optimal dose being 200 mg/kg, provided significant ($p < 0.01$) radioprotection compared with that provided by vehicle controls. In contrast, the 30-day survival rates of mice treated with genistein 1 hr before irradiation were not significantly different from those of the vehicle control group. In a preliminary experiment, mice were injected subcutaneously with 200-mg/kg daidzein 24 hr before 9.5 Gy irradiation. Significantly more daidzein-treated mice survived than did control mice ($p < 0.01$). The level of protection was similar to that observed with 200 mg/kg of genistein. The effect of administration of daidzein at 1 hr before irradiation is currently under investigation. These results demonstrate that both genistein and daidzein provide protection against ionizing-induced radiation injury.

787 A 7-DAY MOUSE MODEL TO ASSESS PROTECTION FROM SULFUR MUSTARD (SM) SKIN INJURY.

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The mouse ear vesicant model (MEVM) has been successfully used as a screening tool to identify protective compounds against sulfur mustard [bis(2-chloroethyl)sulfide]-induced skin injury. The MEVM provides an edema response and histopathological endpoints as measurements of inflammation and tissue damage 24 h following a topical SM (0.16 mg) exposure. Compound effectiveness is defined as a statistically significant ($p<0.05$) reduction in these endpoints. To further evaluate compounds, the MEVM was modified for use as a 7-day model. Dose response studies were conducted with SM (0.01 mg to 0.16 mg) to determine the optimal dose for producing widespread necrosis at day 7. A modified Draize scoring system of 0-4 (no damage to extensive necrosis) was incorporated as an endpoint to evaluate the extent of ear tissue damage out to day 7. Draize results revealed an optimum SM dose of 0.08 mg. Edema response and the existing histopathological scoring system were not feasible endpoints in the 7-day model. Octyl homovanillamide (OHV), a protective compound in the MEVM, and 2 pharmacologically inactive analogs were tested as topical pretreatments 15 min prior to a 0.08 mg SM challenge. OHV significantly reduced Draize scores whereas the inactive analogs had no effect. OHV was also evaluated as a pretreatment 15 min before exposure to 0.06, 0.08, and 0.16 mg SM. OHV protection at day 7 was similar for the 0.06 and 0.08 mg doses but not effective against the severely necrotizing 0.16 mg SM dose. OHV also significantly reduced SM injury when a single topical 2 µmol dose was administered 10 min after SM challenge. These data support the use of the Mouse Ear Vesicant Treatment Model (MEVTM) for evaluating candidate antivesicant compounds. This work was supported by the US Army Medical Research and Materiel Command under Contract DAMD17-99-D-0010, Task Order 0002.

788 ANTI-INFLAMMATORY EFFECT OF PARENTERALLY INJECTED H2A HISTONE FRAGMENT AGAINST SULFUR MUSTARD-INDUCED SKIN LESIONS.

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Skin exposure to sulfur mustard (SM), a potent chemical-warfare agent, results in erythema, edema, and blisters. Previous studies demonstrated the protective effect of topical iodine treatment against SM-induced skin damage. Iodine exerts its beneficial activity by induction of endogenous protective peptide, identified as 9 amino acids fragment of histone H2A (termed IIIM1). The anti-inflammatory/anti-vesicating activity of the peptide was evaluated by the mouse ear edema model. Intravenous administration of 1 and 10mg/kg IIIM1 5 min prior to SM exposure caused statistically significant reduction of 33% and 28%, respectively, in mouse ear swelling as compared to control animals injected with saline. Injection of 10mg/kg IIIM1 2 hrs prior to SM exposure significantly reduced mouse ear swelling by 23%. Post-exposure administration of metabolically stable N-methylated analog of IIIM1 reduced ear swelling by 19%. These findings indicate that parenteral administration of H2A fragment and its analogs decreases SM-induced skin toxicity. It is suggested that the antidotal effect of IIIM1 and its analogs might be of therapeutic value in protecting other sensitive tissues such as lung and bone marrow and in treatment of inflammation-associated disorders. (This study was supported by US Army Medical Research and Material Command under Cooperative Agreement DAMD17-98-2-8009.)

789 MODULATION OF CYTOKINE GENE EXPRESSION BY ANTI-INFLAMMATORY AGENTS FOLLOWING *IN VIVO* SULFUR MUSTARD INJURY.

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Sulfur mustard [bis(2-chloroethyl)sulfide, SM] is a chemical warfare agent that penetrates the skin rapidly and causes extensive blistering after a latent period. We have used the mouse ear vesicant model for cutaneous SM injury quantitation to evaluate pharmacological agents in a number of drug classes, including anti-inflammatory drugs. Topically applied anti-inflammatory agents, including indomethacin and vanilloids, have been shown to reduce SM-induced skin inflammation and tissue damage. We previously identified an early increase in the *in vivo* expression of the inflammatory cytokines GM-CSF, IL-1β, and IL-6 following murine cutaneous exposure to SM. The goal of this study was to determine the effect of topically administered anti-inflammatory agents in reversing inflammatory mediator gene expression following SM-induced injury. Alterations in mouse (n=6) cutaneous GM-CSF, IL-1β, and IL-6 gene expression from ears with and without pre-treatment with indomethacin, heptylisovanillamide, and octyl homovanillamide were examined using real time quantitative RT-PCR. Indomethacin pretreatment produced a significant reduction in the SM-mediated increase of IL-1β and IL-6 mRNA levels. Heptylisovanillamide pretreatment produced significant reductions of GM-CSF, IL-1β, and IL-6 mRNA levels. Octyl homovanillamide produced an apparent reduction of cytokine mRNA levels; however, the reduction did not reach statistical significance at the present animals numbers. SM-induced inflammation was significantly modulated by all three anti-inflammatory agents as determined by reduction in tissue edema (n=10). The alteration in cytokine gene expression suggests that these drugs play a role in modulating SM-induced injury by reducing inflammatory pathways. Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0002.

790 TEMPORAL AND DOSE ANALYSIS OF MURINE GENE EXPRESSION BY MICROARRAY DURING CUTANEOUS SULFUR MUSTARD INJURY.

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The chemical warfare agent sulfur mustard (SM) produces skin blister formation with a severe inflammatory reaction in exposed individuals. The development of efficacious countermeasures against SM vesication requires an understanding of the cellular and molecular mechanism of SM-induced tissue injury. Our previous studies identified alterations in gene expression by cDNA array for 6 SM doses at a single 24h timepoint using an *in vivo* mouse skin model. This study examined SM-induced alterations in gene expression in mouse skin using microarrays (5002 genes) to identify transcriptional events associated with SM toxicity. Using 3 mice/experiment, paired exposed/unexposed (right/left) mouse ear transcript comparisons were made using either 40, 80, and 160μg exposure SM ear skin doses, and tissue harvested at 1.5, 3, 6 and 12h post-exposure. The 40μg dose was selected as representative of a low level exposure that would not produce sufficient damage to increase ear inflammation at 24h. The 80μg and 160μg doses are used for screening candidate treatments in the mouse ear vesicant model. Using a gene transcript alteration criteria of >2-fold increase for all 3 mice in the same dose-time group, 498 transcripts were altered with exposure to SM, 135 transcripts at 40μg, 247 transcripts at 80μg and 126 transcripts at 160μg SM, respectively, for the currently analyzed 1.5, 3, and 12 h time points. Although a few of these genes have previously been reported as differentially regulated by SM exposure, many new target genes were identified. Interestingly, only 3 transcripts were detected as increased at all doses and 32 transcripts for any 2 doses without regard to time; and only 3 transcripts were found at the same dose but at a different time. These data will be further mined for temporal cell pathway changes, which can be used as biomarkers in drug treatment studies.

791 GENE EXPRESSION CHANGES FOLLOWING LOW-LEVEL EXPOSURE TO SARIN (GB) VAPOR.

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The acute effects of chemical weapons have been widely studied since World War I and are well understood. Since the description of Gulf War and Balkans Syndromes, however, there has been increased concern for exposure of personnel to sub-acute and low-level doses of chemical warfare (CW) agents that may occur as a result of decontamination procedures or due to use of such agents by an adversary. There is a considerable body of literature suggesting that exposures to organophosphorus (OP) compounds used as pesticides result in significant cognitive and neurological decrements. However, there is a paucity of literature regarding the molecular effects of low-level exposures to such agents. Although low-level exposures may not cause obvious pathology at the time of exposure, they may cause genetic alterations or changes in gene expression that may predispose personnel to injury or disease later in life. In this study, male and female Sprague-Dawley rats were exposed to low-level doses (0.010-0.033 mg/m3) of the aerosolized nerve agent Sarin (GB) *via* whole body inhalation for 4 hours. Control animals were exposed to air under the same conditions for the same time period. Alteration of gene expression levels in the liver and brain of the exposed animals was assessed using DNA microarray analysis. To date, our results indicate that low-level inhalation exposure to Sarin results in the alteration of expression of a number of important genes. Many of the altered genes participate in cellular processes critical to detoxification pathways and neurological homeostasis. Furthermore, our analyses reveal several differently altered genes between the female and male animals. Additional studies with Sarin and other OP nerve agents such as GF will reveal whether these differences translate into true sex-linked susceptibility to increased OP toxicity, and whether a genetic fingerprint can be defined for a class or a particular OP agent.

792 TEMPORAL TRANSCRIPTIONAL CHANGES IN RAT LUNG TISSUE FOLLOWING AN INTRAVENOUS EXPOSURE TO SULFUR MUSTARD (SM).

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Sulfur mustard [bis (2-chloroethyl) sulfide; SM] is a well-known chemical warfare agent and a suspected industrial carcinogen. SM is known for its vesicating properties following dermal exposure. However, SM can pose a particularly insidious threat to the respiratory tract, with exposure resulting in acute lung edema characteristic of an unregulated inflammatory response. Owing to the physico-chemical nature of SM, aerosol inhalation models are particularly difficult because of the uncertainty of delivered dose. This study was designed to deliver SM intravenously in a dose-response manner to investigate possible molecular markers of lung injury. Male Sprague-Dawley rats (240-270 g) were anesthetized i.p. with 50 mg/kg sodium pentobarbital. Following anesthesia, rats were placed in a supine position, and a small surgical incision was made in the femoral region of the left leg. The femoral vein was isolated, and vehicle (isopropanol) or vehicle plus SM in a concentration of 1, 3, or 6 mg/kg was slowly injected over 3-4 min using a sterile 1-mL syringe. The injection volume was 600-650 μ L/kg body weight. Rats were allowed to recover and had full access to food and water. At 0.5, 1, 3, 6, or 24 hr after exposure, rats were euthanatized with 100% CO_2, and the left lung was dissected, perfused in situ with RNA-Later, stored at 4° C overnight, and frozen at -80° C. Frozen lungs were ground in liquid nitrogen with a mortar and powder homogenized in buffered 4M guanidium isothionate with a Polytron. Total RNA was purified and 33P-labeled cDNA prepared for probing Clontech rat arrays containing 1, 176 cDNAs. Multiple transcripts were induced over dose and time. Preliminary results showed induction of specific pro-inflammatory and stress genes including the predominant expressed genes cytochrome oxidase I, IkB, cysteine-rich protein 2, and glutathione S-transferase P, subunit 7 over dose and time. Numerous gene transcripts were expressed at lower levels following SM exposure.

793 FURTHER EVIDENCE THAT VANILLOIDS MODULATE CUTANEOUS SULFUR MUSTARD INJURY *IN VIVO.*

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Presently, no pre- or post-treatment pharmacological medical countermeasure is established to protect against sulfur mustard (SM)-induced skin injury. In previous studies using the mouse ear vesicant model, we found that three analogs (retro olvanil [ROLV], olvanil [OLV], and retro synthetic capsaicin [RSCAP]) of the vanilloid, capsaicin, effectively modulated cutaneous SM injury. The present study was undertaken to exploit our premise that a structure-activity relationship is involved in the effectiveness of vanilloids against SM injury. We first demonstrated that both natural capsaicin (NCAP) and synthetic capsaicin (SCAP) significantly modulated cutaneous SM injury as effectively as ROLV, OLV, and RSCAP. Secondly, pharmacologically inactive structural analogs of the active RSCAP and SCAP did not modulate SM injury. Finally, a structure activity relationship study was conducted, yielding 17 new synthetic vanilloid candidates based on the structure of RSCAP. Several of these new analogs modulated SM injury as effectively as

the previously evaluated vanilloids. A pharmacologic role for vanilloids is the blockage of cutaneous vascular leakage (anti-edema) following exposure to certain irritants. Since vascular leakage occurs early after SM exposure, it is reasonable to suggest that the anti-edema action of the vanilloids observed throughout these studies is consistent with their known pharmacology. Furthermore, we conclude that the data demonstrate a strong case for a structure-activity relationship for vanilloids involved in protecting against SM injury, warranting specific investigation into the mechanism of action of vanilloids against SM. This work was supported by the US Army Medical Research and Materiel Command under Contract DAMD17-99-D-0010, Task Order 0002.

794 ANALYSIS OF 2-IMINOTHIAZOLIDINE-4 CARBOXYLIC ACID (ITCA) AS A METHOD FOR CYANIDE MEASUREMENT.

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Methods of directly evaluating cyanide levels in tissue are limited by the volatility of cyanide and by the difficulty of establishing steady state cyanide levels with time. To circumvent the challenge of measuring cyanide in tissue, we are interested to see whether measurement of a stabile, toxic metabolite, ITCA, could replace measurement of cyanide as the method of detecting and monitoring cyanide toxicity. This study focused on a spectrophotomeric ITCA determination in the presence of CN, SCN, cysteine, rhodanese, thiosulfate and other sulfane sulfur donors, e.g., organic thiosulfonates. Specific detection of ITCA involves a thiazolidine ring opening in the presence of p-hydroxy-mercury benzoate, followed by the reaction of diphenylthiocarbazone. The absorbance was measured at 625 nm in organic solvent carbon tetrachloride. The method was validated; the standard curve was linear with a regression line of $Y = 2.687X - 0.0168$ ($r^2 = 0.987$). The validity of this method in the presence of the cyanide antidotes is discussed in comparison with other analytical methods. CN, thiocyanate and cysteine significantly shifted the standard curve for analysis. The method of determining presence of ITCA should serve as a substitute for the measurement of the less stable cyanide and allow for a more reliable assay.

795 IDENTIFICATION OF BIOTOXIN VARIANTS AND VIRAL SIGNATURES USING THE HAND PORTABLE μCHEMLAB™/CB DETECTION SYSTEM.

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μChemLab™ /CB is a hand portable rapid detection system that can be used to identify chemical, biotoxin and viral agents in the liquid phase. The technical approach makes use of parallel electrophoretic analyses combined with a highly sensitive laser-induced fluorescence detector that are integrated at the microchip scale. Using capillary zone electrophoresis and capillary gel electrophoresis as independent separation techniques, the goals of these studies was to determine whether current μChemLab methods are sufficient for: 1) detecting all toxin isoforms of the protein biotoxin ricin; 2) discriminate between close but functionally distinct toxin isoforms of six other biotoxins and; 3) identify specific protein signatures for viral agents. Analysis of ricin isoforms revealed the separation profiles between ricin species was similar. For other biotoxins, such as S. enterotoxins, we were able to clearly distinguish between closely related specie isoforms (S.enterotoxin A & B) using two-channel analysis. In a separate set of experiments we performed analysis of the Tphage virus proteome. The analysis was rapid, complete in 10 minutes, and indicated good correlation with slab gel electrophoretic analysis of viral stocks. From these studies we conclude that: 1) ricin isoforms can be readily identified by μChemLab, 2) variant isoforms known to have different functional qualities could be discriminated by two-channel analysis and 3) viral protein signatures observed by dual channel analysis may be useful for phenotypic discrimination of such organisms.

796 SYSTEM OF DECONTAMINATION OF RADIOACTIVE EFFLUENTS.

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An on-site radioactive element decontamination facility, or preferably, a mobile radioanalysis laboratory which can be deployed in the field to detect these contaminating radioelements that can pollute the environment. Before being released into the environment, it is necessary to assure that there are negligible amounts of radioelements. The purpose of this study is to establish the binding to ion exchange resin, a technique of treatment of these contaminating radioelements for the environment. We have considered two cases. -The radioelements in free form in solution -The radioelements which are found in a complex form such as bond to Diphoterine. The binding of the principal toxic radio elements Ce137, Sl90, U235, Pu239, as well as tritium to ion exchange resins such as Amberlite (cationic or anionic) has been studied by passing solution containing these radioelements into columns containing these resins. The decontamination factor varies according to the radioelement and according to the resins between 1/1000 and 500/100. In the case of complexed radioelements, the factor of decontamination varies between 1/1000 and 10/100 except in the case of tritium that is 9/200 and 50/100. The different decontamination factor will be discussed. From these results, a system of treatment of these contaminating radioelements is proposed which comprise the two types of resins and one system of measurement of the radioactivity by release of the contaminating radioelements.

797 ASSESSMENT OF RSDL AS A DECONTAMINANT AGAINST SULFUR MUSTARD AND VX.

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Reactive Skin Decontamination Lotion (RSDL), developed by the Canadian Defense Research Establishment Suffield, has been proposed for use by US troops as a substitute for the M291 Skin Decontamination Kit (SDK) against exposure to chemical agent. Approval of RSDL as a medical device by the US Food and Drug Administration requires safety testing and demonstration that RSDL efficacy is "substantially equivalent to" that of the existing predicate device. Rabbit models previously used to establish the efficacy of M291 SDK were used to evaluate and compare RSDL with M291 SDK against topical challenges of sulfur mustard (SM). In SM studies, a grid with eight test sites was drawn on each rabbit's back, and a 1-μL dose of SM applied at each test site. At 2 min after SM application, each test site was treated with either the M291 SDK or RSDL decontamination applicator, or a gauze applicator wetted with distilled water. Lesion areas were estimated at 24 h after dosing and normalized by dividing by the lesion area at the no-treatment site on each rabbit to determine lesion area ratios (LARs). Mean LARs were 0.13 for RSDL, 0.25 for M291 SDK, and 3.16 for water-treated sites relative to the no-treatment control sites. RSDL was significantly more effective than M291 SDK against SM. In studies with VX, 24 h median lethal doses (LD_{50}s) were determined from separate dose-response curves for rabbits either left untreated, or treated with either M291 SDK or RSDL. The LD_{50}s (and protective ratios) were 0.33 mg/kg (9.6) for M291 SDK, and 2.3 mg/kg (66) for RSDL. Untreated rabbits had an LD_{50} of 0.035 mg/kg. RSDL was significantly more effective than M291 SDK against VX. The results indicate that significant protection is afforded by RSDL against SM and VX and establish its equivalency with M291 SDK. This work was conducted under the USAMRMC Contracts DAMD17-99-D-0010, Task Order 0008, with support from the US Marine Corps Foreign Comparative Testing Skin Decontamination Project.

798 INVESTIGATING THE *AEDES AEGYPTI* MOSQUITO LARVAE AS AN *IN VIVO* BIOASSAY FOR CHEMICAL AND BIOLOGICAL WEAPONS AGENTS (CBWA).

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CBWA are an emerging threat and at present there are no reliable detection/identification systems. Thus, there is a need to develop novel assays that can identify the presence of CBWA in environmental samples. A system that can measure CBWA-specific markers of toxicity in a living organism represents a viable solution. We investigated a novel *in vivo* detection system that monitors the toxicity of CBWA in Aedes aegypti mosquito larvae. Larvae were incubated in water that contained a test agent. Tests included chemical (aflatoxin: AF), protein (ricin: RC and botulinum toxin: BT) and bacterial (B. globigii: BG, a simulant for anthrax) agents. The endpoints were LD50 (2-hour exposure), lactate dehydrogenase (LDH) activity in the exposure media, and CBWA-induced alterations in proteins that are known to play key roles in cell signaling, apoptosis, and metabolism. Protein activity levels were monitored using 2 separate ELISA systems (FITC- and peroxidase-conjugated antibody). Based on LD50, larvae were susceptible to the acute toxic effects of several CBWA (RC>AF>>BT>>BG). When exposed to RC and AF, a significant increase in LDH activity was detected in the exposure media (2-hour exposure), suggesting a direct toxic effect on larvae gut epithelial cells. We also detected changes in protein activities at the LD50 dose (1-hour exposure) that were CBWA-specific. In an attempt to increase the number of proteins examined per experiment, we investi-

gated an antibody microarray using the toxic industrial chemical trichloroethylene (TCE). Of >250 proteins examined, we detected TCE-induced alteration in 16 proteins, suggesting this application may be useful in further developing this detection system. Collectively, these observations suggest that larvae are sensitive to the acute toxic effects of certain CBWA and from this we have been able to generate CBWA-specific toxicity biosignatures. This information may be useful in identifying the presence of CBWA in an unknown sample. (Supported by DARPA)

799 EVALUATING THE THREAT OF CHEMICAL AND BIOLOGICAL WARFARE *VIA* MAIL OR DRINKING WATER DELIVERY SYSTEMS.

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A literature survey of known potential, biological, chemical, explosive and radiological threats that could be delivered through the public mail delivery system (e.g., US Postal Service, UPS, etc.) was conducted. In addition, a survey of known potential biological, chemical and radiological threats to the public drinking water system was undertaken. For mail threats, it was found that biological agents posed a moderate to high risk due to the ease of production and ease of mailing. Infectious biological agents (e.g., smallpox, plague, etc.) may be of particular concern because they pose epidemic potential. In an aerosolized form, they are easily spread person-to-person, and they require more than the standard infection control precautions. Chemical agents were found to pose low to moderate risk as a mail threat. They are relatively easy to obtain or synthesize, but are not well suited to delivery by mail since they are typically in the form of liquids or gases and would likely be shipped in appropriate containers which have a reasonable chance of being detected. The risk from explosive agents was found to be relatively small for letter-sized pieces of mail with the possible exception of plastic explosives. Radiological agents were found to pose low to moderate risks. The greatest threat for radiological sources comes from the accidental ingestion or inhalation of these substances. For water delivery systems, the magnitude of the threat is dependent on the solubility of the agent in water. Biological agents are soluble and generally have a low LD50 but are unstable in the presence of chlorine. Chemical toxins are likely a viable threat *via* water delivery systems although overall stability remains a question. Finally, radiological threats also seem to be a low to moderate threat based on their solubility, low hydrolysis potential and stability under chlorinated conditions.

800 *IN VITRO* STUDIES OF DIPHOTERINE FOR DECONTAMINATION OF ORGANOPHOSPHORUS COMPOINDS, INCLUDING DFP AND THE NERVE AGENTS SOMAN AND VX.

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Diphoterine is a proposed organophosphate (OPs) decontaminant. To evidence the potent OP-scavenging property of Diphoterine, measurements of decreases in OP concentration were done indirectly measured using OP-interacting enzymes (human butyrylcholinesterase). BuChE activity was measured by the Elman's method. Human paraoxonase (PON1) and Pseudomonas diminuta phosphotriesterase (PTE), and the G117H mutant of BuChE were used. OPs were paraoxon, DFP), echothiophate, racemic soman and VX). The final concentrations of Diphoterine® in buffers ranged from 0 to 0.6 M. Diphoterine did not promote hydrolysis of OPs when incubatated with paraoxon. Diphoterine did not inhibit or activate wild-type BuChE and its G117H mutant, even with 24h preincubation at 20° C. Diphoterine in the medium slowed down the progressive inhibition of BuChE caused by OPs. The effect was greater with increasing the Diphoterine concentration. Increasing incubation time for OPs and Diphoterine® had no effect. This can be explained by depletion of OP in the medium by rapid formation of an inactive complex. The apparent estimated dissociation constant of OP-Diphoterine complex about 10-20 mM. The affinity of Diphoterine for OPs ranked as follows: Echothiophate>VX>Soman>DFP>paraoxon. Diphoterine alone did not inhibit PON1 up to 0.1 M. PTE was not inactivated in the presence of higher Diphoterine concentrations. With increasing concentrations of Diphoterine, the OP-hydrolase activity of PON1, PTE and G117H mutant of BuChE towards different concentrations of paraoxon as a substrate progressively decreased. This indicates that Diphoterine caused a reduction in substrate concentration, corroborating inhibition data. Inhibition of BuChE by OPs in the presence of Diphoterine as well as results about activity of OP hydrolases under similar conditions support the conclusion that Diphoterine acts by scavenging OPs.

801 ADVANCES IN TOXICOGENOMICS: NIEHS NATIONAL CENTER FOR TOXICOGENOMICS.

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Toxicogenomics represents the interface between toxicology and gene expression technology; it combines studies of genetics, genomic-scale mRNA expression, cell and tissue-wide protein expression, metabolite profiling and bioinformatics in order to understand the role of gene-environment interactions in disease. The NIEHS National Center for Toxicogenomics was established to facilitate research to improve our understanding of mechanisms underlying toxic responses of environmental agents and to develop an extensive knowledge-base of Chemical Effects in Biological Systems (CEBS). In order to catalyze these efforts the NCT has established a Toxicogenomics Research Consortium, comprised of six institutions including Duke University, MIT, Oregon Health Sciences Center, University of North Carolina, The Fred Hutchinson Cancer Center and the NIEHS Microarray Center. Studies conducted by this consortium will facilitate the development of the field of toxicogenomics.

802 INTEGRATION OF GENOMIC AND PROTEOMIC APPROACHES TO STUDY TOXICOLOGICAL PHENOTYPES.

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To define the molecular state of a given phenotype resulting from toxicant exposure a comprehensive measure of genes and their products expressed in a given organ or cell system will greatly facilitate the identification of potential pathways and their interactions. To develop these methods we utilized the genotoxin methylazoxymethanol (MAM), a developmental neurotoxin implicated in western Pacific Amyotrophic Lateral Schlerosis (ALS), Parkinson's disease, and an Alzheimer-like dementia (ALS/PDC). The mechanism of MAM induced acute or chronic brain injury is poorly understood. To characterize the gene and protein expression changes in MAM induced toxicity and the developing nervous system, C57BL6 (postnatal mice, 3-days old), were administered saline or a sub-lethal dose of MAM (43 mg/kg, s.c.), and 1, 8, 15, and 22 days later RNA and protein were isolated from various brain regions (cortex, hippocampus, striatum, thalamus, midbrain, cerebellum). Using high-density cDNA arrays we examined expression levels of ~26, 000 mouse sequence verified clones. Using 2D-electrophoresis and MALDI-TOF methods we examined the protein expression changes. "In silico" we combined the gene and protein expression patterns to generate clusters that are unique to this toxicant. We used the combined approaches to identify pathways regulated by MAM in the developing CNS.

803 COMPLEX RESPONSES TO ALKYLATING AGENTS.

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Using Affymetrix oligonucleotide GeneChip analysis, we previously found that, upon exposure to the simple alkylating agent methylmethane sulfonate (MMS), the transcript levels for about one third of the Saccharomyces cerevisiae genome (~2, 000 transcripts) are induced or repressed during the first hour or two after exposure. This increased the number of S. cerevisiae genes previously known to be responsive to a DNA damaging agent by about 100 fold. In order to determine whether the responsiveness of these genes has any relevance to the protection of cells against alkylating agents we have undertaken several follow-up studies. First, we explored the specificity of this global transcriptional response to MMS by measuring the global response of S. cerevisiae to a broad range of agents that are known to induce DNA damage. We found that each agent produced a very different mRNA transcript profile, even though the exposure doses produced similar levels of toxicity. We also found that the selection of genes that respond to MMS is highly dependent upon what cell cycle phase the cells are in at the time of exposure. Computational clustering analysis of the dataset derived from a large number of exposures identified several promoter motifs that are likely to control some of the regulons that comprise this large set of genes that are responsive to DNA damaging agents. However, it should be noted that these agents damage cellular components other than DNA, and that the responsiveness of each gene need not be in response to DNA damage per se. We have also begun to study the response of other organisms to alkylating agents, and these include E. coli, cultures of mouse and human cells, and mice. Finally, we have developed a high throughput phenotypic screening method to interrogate the role of all non-essential S. cerevisiae genes (about 4, 800) in protecting S. cerevisiae against the deleterious effects of alkylating agents; we

have termed this analysis 'genomic phenotyping'. This study has uncovered a plethora of new pathways that play a role in the recovery of eukaryotic cells after exposure to toxic/mutagenic agents.

804 AN APPROACH TO THE CLASSIFICATION OF TOXICOLOGICAL EFFECTS USING MICROARRAY AND PROTEOMIC TECHNOLOGIES.

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Data from microarray analysis of tissues of rats exposed to selected chemicals/drugs have shown that specific patterns can be recognized that are representative of chemical class (Hamadeh et al., Toxicology. Sciences. 67:219). It has also been possible to demonstrate that even with a relatively small learning set, compound classification is possible (Hamadeh et al., Toxicology. Sciences. 67:232, 2002). Such "signature patterns" of gene expression changes were derived from tissues within 24 hours to two weeks of exposure to nontoxic doses of agent and thus are likely to be representative of the pharmacological properties of the agents. It is therefore important to determine if similar signature patterns can be identified representing toxic effects. Agents selected for the induction of prototype toxicities (e.g., hepatic portal necrosis) are being analyzed in experiments in which target and non-target tissues are examined. Microarray and proteomic methodologies are applied to identify candidate biomarkers of toxicity.

805 USING TOXICOGENOMIC ANALYSIS TO ASSESS THE PROTECTIVE EFFECTS OF ENHANCED GLUTATHIONE SYNTHESIS IN GLUTAMATE-CYSTEINE LIGASE TRANSGENIC MICE.

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Glutathione (GSH) is a major free radical scavenger and is important in detoxification of reactive oxygen species and xenobiotics. The rate-limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GCL) which is composed of catalytic (GCLC) and modifying (GCLM) subunits. GCL expression has been shown to be variable in a number of disease states, including cancer, fibrotic lung diseases and cardiovascular disease. We have created transgenic mice that conditionally overexpress GCLC and/or GLCM in the liver. In order to test the hypothesis that enhanced GSH synthesis can ameliorate toxicant-induced oxidative stress, we are examining the influence of GCLC and GCLM overexpression on CCl4 or acetaminophen induced liver injury, as assessed by histopathology, lipid peroxidation and global gene expression changes using cDNA microarrays. Transgenic mice overexpressing GCL show diminished histopathological changes in the liver, less lipid peroxidation and lower serum ALT activity after CCl4 treatment. Relatively few changes in gene expression were noted when mice were treated with the inducing agent alone (mifepristone) to up-regulate GCL expression. Experiments are currently underway to examine the effects of GCL overexpression on CCl4 and APAP -induced changes in global gene expression. Combining toxicogenomic analyses with transgenic mouse models can provide additional insights into the mechanisms of toxicant induced injury. Supported by NIH Grants 1P42ES04696, 1P30ES07033 and 1U19ES11378.

806 DISSECTING THE ROLE OF PLEIOTROPIC TOXICANTS AND CELL TYPE SPECIFIC RESPONSES USING DNA MICROARRAYS.

C. M. Perou. *Lineberger Comprehensive Cancer Ceneter, University of North Carolina, Chapel Hill, NC.* Sponsor: R. Tennant.

Many environmental toxicants have pleiotropic effects, and it is often uncertain which of the mechanisms of action are responsible for observed toxic endpoints. The toxicant doxorubicin (DOX) represents an excellent model compound for dissecting the pleiotropic effects of a toxicant that induces oxidative stress. DOX is a commonly used chemotherapeutic that has been found to inhibit topoisomerase II (TOP2) and cause oxidative damage. Using RNA from treated cell lines, we performed DNA microarrays to obtain detailed information on networks of DOX-responsive genes. We treated a panel of breast epithelial cell lines with DOX using two doses: the first dose corresponds to the IC50 for each cell line and the second was a lower dose, common to all cell lines. Gene expression profiles associated with topo-II inhibition and oxidative stress were identified in these cell lines by comparison with a panel of toxicants thought to only inhibit TOP2 activity or only to induce oxidative stress. Sets of genes were identified that were repressed in all treatments and are largely involved in proliferation and cell cycle progression including v-myb2, TOP2 and cyclin A2. Sets of genes that were potentially p53-dependent, notably p21, were induced following DOX and etoposide treatment. The potency of a toxicant is also determined by the genetic background of the treated cells. Recent studies have identified breast tumors with characteristics of basal epithelium that show a poor prognosis and potentially, a different response to therapy than tumors derived from luminal cells. Thus, we compared the expression profiles of to DOX treated cell lines derived from basal and luminal epithelium and found unique sets of genes that were specifically induced in either the basal or luminal lines, indicating that the cell type of origin impacts upon the response to therapeutics. Notably, RERG was induced in basal epithelial lines and not in luminal lines in response to DOX, with the induction of this gene being previously shown by our lab to correlate with decreased cell proliferation rates.

807 MOLECULAR MECHANISMS OF CARDIOVASCULAR TOXICITY OF METALS AND METALLOIDS.

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Cardiovascular toxicity of metals and metalloids has long been recognized. However, molecular mechanisms leading to cardiovascular injury by these agents are not understood. A major reason for the lack of this knowledge is that toxicologists have not investigated cardiovascular toxicology in general to the same extent as the toxicology of the liver, kidneys, lungs, or brain. As a result, cardiovascular toxicology is extremely under-represented in the discipline of toxicology. Advances in molecular biology of cardiovascular system have provided tremendous opportunities to explore cardiovascular toxicology at cellular and molecular levels. Improved understanding of signaling pathways and molecular mechanisms leading to cardiovascular diseases such as acute myocardial infarction, heart failure and hypertension requires expanding our knowledge to the interaction between the myocardium and environmental agents including metals and metalloids. There is no doubt that environmental and medical exposure to metals and metalloids play a significant role in cardiovascular diseases. Interactions between toxic metals such as cadmium with essential minerals such as calcium and zinc can directly interfere with signal transduction and gene expression. Crucial molecules such as vital protein thiols in cardiovascular system are very sensitive to toxic metalloid arsenic. The role of toxic metals and metalloids in activation of transcription factors and post-translation modifications is also of great concerns in cardiovascular medicine. A comprehensive discussion of up-to-date understanding of these molecular and cellular events involved in the cardiovascular toxicity of metals and metalloids will thus provide novel insights into the role of metals and metalloids in the development of cardiovascular diseases.

808 NOVEL INSIGHTS INTO THE CARDIOVASCULAR TOXICITIES OF METALS AND METALLOIDS.

M. P. Waalkes. *NIEHS, Research Triangle Park, NC.*

Metallic compounds, including heavy metals and metalloids, cause a wide variety of toxic manifestations in humans. Although understudied, the cardiovascular system is often a critical target of inorganic compounds, such as cadmium, nickel, lead and arsenic. Through as yet poorly defined etiologies, exposure to metals and metalloids can induce dramatic cardiovascular effects, ranging from hypertension to peripheral vascular disease. Metals can also have effects on myocardial and/or vascular function by attacking endothelial or myocardial cells and causing dysfunction. Indirect attack, as through disruption of mediators of vascular tone, can also play a role. Toxic metallic agents have many intricate effects within cells, including aberrant gene expression, disruption of signal transduction, perturbation of membrane transport, and mimicry of essential elements, that all likely contribute to cardiovascular toxicity. Our understanding of the mechanisms of metallic cardiotoxicity is, at present, incomplete. The advent of powerful molecular techniques, such as toxicogenomics and proteomics, as well as the use of transgenic animals should allow for significant advances in our understanding of the mechanistic basis of metal-induced cardiovascular toxicities. The knowledge of the impact of metal exposure on gene and protein expression profiles in cardiovascular target tissue should provide important candidate genes or pathways for more detailed mechanistic study. Targeted disruption of genes involved in metal metabolism or tolerance should allow for the definition of the role of such genes in cardiovascular toxicity. As with all areas of toxicology, the recent advances in the methods of molecular toxicology will likely create many new and exciting research avenues in defining the mechanisms of the cardiovascular toxicity of metals.

809 INTERACTIONS OF NICKELS AND CADMIUM WITH CARDIAC SR CALCIUM RELEASE CHANNELS.

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The heavy metals Cd^{2+} and Ni^{2+} are known to block cardiac contraction. These divalent cations are known to inhibit the L-type Ca^{2+} channel but it is not known if they also interfere with the Ca^{2+} release process itself. The purpose of this study was to investigate the direct effects of these metals on cardiac sarcoplasmic reticulum (SR) Ca^{2+} release channels (ryanodine receptors, RyRs). Purified RyRs was isolated from dog ventricular heavy SR and single channel activity was recorded in artificial planar lipid bilayers. Cd^{2+} (10-30mM) caused a time-dependent reduction in single channel activity that eventually resulted in complete channel block. Unitary current amplitude was decreased immediately before block occurred, probably as the result of a rapid flicker-type interaction with the channel. Block occurred when Cd^{2+} was applied to either side of the bilayer. Reducing [Cd^{2+}] by successive dilutions did not permit recovery of channel activity but temporary recovery from block did occur in the presence of the methane thiosulfonate derivative MTSES (1mM) shortly before permanent block occurred. Ni^{2+} also blocked single channel activity but this block could not be relieved, even temporarily, by MTSES. These results indicate that Cd^{2+} probably interacts with free sulfhydrl groups in the pore of the channel protein. However, this interaction can be broken by the preferential binding of the MTSES with the sulfhydryl groups, displacing the Cd^{2+} and allowing the temporary recovery of channel activity. The slower interaction between MTSES and the sulfhydryl groups then is responsible for the eventual and permanent block of the channel. The interaction between Ni^{2+} and the channel protein is fundamentally different but also leads to block of channel activity. The effects of these heavy metals to inhibit the activity of the SR Ca^{2+} release channel might contribute to their negative inotropic effects in the heart

810 MYOCARDIAL APOPTOSIS AND CARDIOMYOPATHY INDUCED BY ARSENIC.

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Arsenic is an environmental toxic agent, however, it has been used in the form of arsenic trioxide in the treatment of acute promyelocytic leukemia (APL) due to its irreplacible efficacy. Recent clinical trials in the United States have shown a serious ventricular tachycardia at the therapeutic doses of arsenic trioxide in APL patients. Using a mouse model, we have observed that treatment with arsenic trioxide at 5 mg/kg/day for 30 days produced plasma concentrations of arsenic within the range of those present in arsenic-treated APL patients and caused cardiac toxic manifestations similar to humans. The cardiac toxic effects monitored by electrocardiogram include prolonged QT intervals and prominent University waves, a torsade de pointes form of ventricular tachycardia. An in situ left ventricle performance analysis revealed that arsenic caused a siginificant decrease in the maximum rate of rise in intraventricular pressure during ventricular contraction and significant increases in the end diastolic pressure and ventricular minimum diastolic pressure. Arsenic-induced cardiomyopathy was also observed by histopathological and ultrastructural examination. Myocardial apoptosis along with increased voulme of the remaining cardiomyocytes were predominant cellular events. Caspase-3 activation and mitochondrial cytochrome c release were accompanied with apoptosis. Interestingly, elevation of myocardial metallothionein concentrations significantly prevented all of these toxic manifestations along with suppression of arsenic-induced decrease in the ratio of reduced glutathione to glutathione disulfide. The inhibition of thiol-containing enzymes such as oxidoreductases by arsenic was also prevented by metallothionein. These results thus demonstrate that arsenic-induced cardiotoxicity is associated with apoptosis mediated by mitochondrial cytochrome c release. The interaction of arsenic with essential thiols is likely involved in the mechanism of arsenic cardiotoxicity.

811 SIGNALING PATHWAYS INVOLVED IN ARSENIC-INDUCED VASCULAR DISEASE.

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Chronic, low level exposure to arsenite increases the incidence of proliferative cardiovascular diseases, such as arteriosclerosis and ischemic heart disease. Arsenic-associated changes in blood vessels may also contribute to the vascular components of diabetes and tumor growth. Angiogenesis is fundamental to these pathological changes and may underlie effects of arsenic on vascular remodeling. In support of this hypothesis, nanomolar to low micromolar levels of arsenite increased blood vessel density *in vivo* in both chicken and mouse models. However, higher levels of arsenite inhibited blood vessel growth. Investigation of the potential angiogenic factors induced by arsenite revealed increased expression of both vascular endothelial cell growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1). Mechanistic studies indicated that separate signaling pathways mediated induction of these two angiogenic factors. These pathways were associated with increased signaling through a Src family tyrosine kinase, protein kinase C, and stabilization of hypoxia inducible factor-1alpha. Induction of VEGF in vascular smooth muscle cells was directly correlated to increasing doses of arsenite, whereas PAI-1 induction was stimulated at low doses and inhibited at higher levels. This may indicate that signaling mechanisms for expression of PAI-1 may be better predictors of the pro-angiogenic potential of arsenite. Supported by SBRP grant ES07373.

812 NOVEL INSIGHTS INTO THE TOXICOLOGY OF LUNG OXIDATIVE STRESS.

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Oxidative stress is derived from an overproduction of oxidants such as reactive oxygen and nitrogen species relative to the detoxification of the oxidants. The increased production of such oxidants can lead on one hand to fairly nonspecific damage to lipids, proteins, and DNA as well as to rather specific changes related to receptor binding and signal transduction. Oxidative stress is present in all lung disease states and has been associated with toxicity induced by radiation and most, if not all, lung toxicants. Lung oxidative stress can be derived from both endogenous sources (*e.g.*, induction of inflammation and disruption of normal O_2 metabolism) and exogenous sources (*e.g.*, exposure to gaseous and particulate air pollutants). The possibility exists that extrapulmonary organ systems can be affected by lung oxidative stress as increased production of oxidant species in the lung can lead to transport of the oxidant species or reaction products outside the lung with possible biological effects. The focus of this session will be to present recent advances in the measurement of oxidative stress primarily in the lung as well as new insights related to the pathobiology of oxidant formation within this organ. Findings that will be presented will range from the detection of endogenously and exogenously derived oxidants in pulmonary and extrapulmonary tissue, to involvement of oxidants in lung responses and clinical disease. The role of oxidant-induced activation of important lipid signaling pathways related to acute lung injury and strategies to attenuate the injury will be described. Validation studies of air pollutant biomarkers of exposure and effects using ozone and other lung toxicants will be shown. Relevant to the great interest in particulate matter (PM) toxicology, the mechanisms by which lung cells transport and metabolize transition metals on PM will be discussed, as will early markers of oxidant production with PM using cobalt as a model PM. [This abstract does not necessarily reflect EPA policy.]

813 ROLE OF OXIDATIVELY MODIFIED PHOSPHOLIPIDS IN ACUTE LUNG INJURY.

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The Platelet-Activating Factor (PAF) signaling system mediates cellular activation, and its dysregulation contributes to pathologic inflammation and thrombosis in syndromes that include shock, sepsis and acute lung injury (ALI). The PAF receptor, a member of the seven membrane spanning G protein-linked family, is constitutively expressed on human platelets and myeloid leukocytes, which are key effector cells in thrombotic and inflammatory disorders. The PAF receptor recognizes not only PAF (1-0-alkyl-2-acetyl-sn-glycero- 3-phosphorylcholine), a phospholipid that is enzymatically synthesized by endothelial cells and several other cell types in response to inflammatory agonists and oxidants, but also a group of structurally-related ligands termed PAF-like lipids or oxidatively-modified phospholipids (OX-PL) that are generated by oxidant attack on membrane phospholipids rather than by regulated enzymatic synthesis. This provides a mechanism by which ligands that activate the PAF signaling system are generated in uncontrolled fashion. A variety of observations indicate that this mechanism operates in experimental ALI and in acute respiratory distress syndrome (ARDS), the most severe form of human ALI. The signals triggered by PAF and oxidatively-modified PAF-like lipids are terminated by their hydrolysis by a group of intra - and extracellular enzymes termed PAF acetylhydrolases (PAF AH); the extracellular, or plasma, form of PAF AH is particularly important because it limits the half-life of PAF to minutes in human blood. Plasma PAF AH is inactivated by oxidants *in vitro* and *in vivo*, establishing a mechanism for pathologic imbalance between generation of ligands for the PAF receptor and their degradation. Plasma PAF AH has been cloned and characterized at

the molecular level, and a recombinant form is under study as a novel anti-inflammatory agent. Preliminary evidence indicates that it terminates signals in human ALI.

814 USE OF OXYGEN-18 ISOTOPE LABELING FOR MEASUREMENT OF OXIDATIVE STRESS.

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Oxygen-18 (18-O) labeling provides a sensitive means for quantifying oxygen binding that occurs during *in vivo* oxidations. Oxidants (ozone, nitrogen oxides, hydrogen peroxide, etc.) are synthesized using 18-O, then cells or tissues are exposed to the labeled oxidants, after which the tissues can be assayed for excess 18-O to indicate the extent of oxidation that occurred in them. Use of this stable isotope allows samples to be stored indefinitely. In addition to exposure to labeled oxidants, animals can be exposed to 18-O_2 gas at the same time they are exposed to free radical generating toxicants (metal, CCl_4, etc.) to yield an estimate of "secondary oxidations." Separation of tissue fractions (protein, lipid, DNA, etc.) provides individual assessment of oxidation in these pools, yielding valuable information about mode of action of the oxidants studied. Measurement of the elimination of 18-O in urine appears to provide a quantitative assessment of tissue repair following oxidative injury. Since 18-O is not radioactive this isotope is easily used in human studies. We have used 18-O isotopically labeled pollutants to compare human and animal ozone dose to target sites in the nose and lung. Interactions between ozone and inhaled diesel particles have also been elucidated using 18-O. Comparisons between 18-O measurements and more traditional measures of oxidative stress indicate that the former method is often more sensitive and less subject to interferences and artifacts. However, further work is needed to distinguish between pathological and "normal" oxidations. In summary, use of oxygen-18 provides a unique research tool to study oxidative processes occurring *in vivo*.

815 OXIDATIVE DAMAGE INDUCED BY CCL4 AND OZONE: VALIDATION OF BIOMARKERS.

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Oxidation products of lipids, proteins and DNA in the blood, plasma and urine of rats were measured as part of an international multilaboratory validation study searching for non-invasive biomarkers of oxidative stress. Acute CCl_4 poisoning and the ozone exposure were used as two different rodent models of oxidative stress. The time (2, 7, and 16h) and dose-dependent effects of CCl_4 (120 and 1200 mg/kg ip) or ozone (1 and 5 ppm) on concentrations of lipid hydroperoxides, thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), isoprostanes, protein carbonyls, methionine sulfoxidation, tyrosine products, 8-hydroxy-2-deoxyguanosine (8-OHdG), and leucocyte DNA-MDA adducts and DNA-strand breaks were investigated to determine whether the oxidative effects of CCl_4 or ozone would result in increased generation of these oxidation products. Plasma concentrations of MDA and isoprostanes (measured only by GC/MS) and urinary concentrations of isoprostanes (measured with an immunoassay) were increased in CCl_4 treated rats. All other products were not changed by CCl_4 and ozone, or showed only high-dose and/or single time point effects. Interestingly, in the ozone exposed rats no changes in the plasma isoprostanes were found. Measures of oxidation products of proteins and DNA were not reliable biomarkers for free radical damage induced by CCl_4 and ozone.

816 MODULATION OF TRANSITION METAL-INDUCED INJURY BY METAL TRANSPORT PROTEINS.

A. Ghio. *NHEERL\HSD\Clinical Research Branch, USEPA, Research Triangle Park, NC.* Sponsor: M. Madden.

The capacity of divalent metal transporter 1 (DMT1) to transport iron and its ubiquitous expression make it a likely candidate for transferrin-independent uptake of iron in peripheral tissues. Airway epithelial cells increase both mRNA and expression of that isoform of DMT1 without an iron response element (-IRE) following exposure to iron. We tested the hypothesis that -IRE DMT1 participates in the detoxification of metals in the lung. Exposure for 24 hours of human bronchial epithelial cells to oil fly ash particles that were high in iron and other transition metals content unexpectedly resulted in a decrement in mRNA by RT-PCR for the -IRE isoform of DMT1 and the protein by Western blot analysis. Similar decreases in mRNA and protein expression were demonstrated following exposure of bronchial epithelial cells to vanadyl sulfate, a metal in high concentration in this specific oil fly ash. Pre-exposures of respiratory epithelium to iron and vanadium were associated with an increased and a decreased uptake of metal respectively. Pre-exposure of an animal model to iron was associated with both an increased expression of DMT1 and diminished injury after particle exposure. In contrast, vanadium pre-exposure decreased DMT1 expression and increased injury after the same particle. Exposure of Belgrade rats (an animal model deficient in functional DMT1) to the particle resulted in a greater injury relative to control animals. We conclude that levels of -IRE DMT1 expression are associated with both metal transport in the lower respiratory tract and injury following exposure to a particle. [This abstract may not reflect EPA policy.]

817 ACTIVATION OF THE HEXOSE MONOPHOSPHATE SHUNT: AN EARLY MARKER OF OXIDATIVE STRESS CAUSED BY COBALT PARTICLES.

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Airborne cobalt-containing particles can lead to the development of interstitial lung disease and lung cancer in workers. In cell free experiments, it has been shown that Co ions (Co^{2+}) are able to react with H_2O_2 to produce reactive oxygen. Moreover, freshly suspended hard metal (cobalt tungsten carbide, CoWC = 6% Co + 94% tungsten carbide) particles generate activated oxygen species in water as a result of the WC-catalyzed reduction of oxygen and, consequently, the oxidation of the metal cobalt (mCo) at the surface of the carbide. Changes in the activity of the hexose monophosphate shunt (HMS) or pentose phosphate pathway of glucose oxidation, as well as increased thiol oxidation have been shown in hamster lung exposed in vivo to $CoCl_2$ and in hamster lung slices exposed *in vitro*. Recently we have shown that mCo particles freshly immersed in medium lead to an early stimulation of the HMS activity in primary rat alveolar type II pneumocytes (AT-II) in a dose dependent way. The combination of mCo and WC, as present in hard metal, also increased the HMS activity in rat AT-II. However, this increase did not differ from that caused by an equivalent amount of pure mCo. We were not able to show a simple relationship between the stimulation of the HMS activity and the subsequent cell damage as measured using the microtetrazolium (MTT) cytotoxicity assay. The HMS activity was not stimulated by other particles (such as pure WC, positively or negatively charged latex beads or PVC particles), by "aged" mCo particles, or by $CoCl_2$. The peroxides H_2O_2 and t-BOOH stimulated the HMS activity in a comparable way to mCo, and catalase abolished the mCo-induced stimulation of the activity, suggesting that the observed stimulation of the HMS may be due to the production of peroxide. Consequently, the early observed HMS stimulation is possibly a sign of toxicity. Although the exact mechanism of toxicity still needs to be elucidated fully, in particular with regard to type of Co (e.g. metalic, ionic) and the role of other substances (e.g. WC), the evidence exists for an oxidant-mediated toxicity of Co upon inhalation.

818 INTRODUCTION: CHALLENGES OF THE DEVELOPMENTAL NEUROTOXICITY STUDY.

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The developmental neurotoxicity (DNT) study is a key study for identification of potential damage to the nervous system of the developing organism. In this study, exposure to the test substance occurs during fetal and postnatal development. Neurobehavioral and neuropathological assessments are performed on treated individuals in early life, and as adults following a period without exposure. These evaluations include functional observational battery, motor activity, auditory startle, and cognitive (learning and memory) testing, and histopathology of nervous system tissues, including morphometry of several areas of the brain. This Workshop will summarize the evolution of the DNT study as a regulatory guideline and the use of the rat model as a predictive species for nervous system effects in the young human. Some of the challenges of conducting a study with its inherent complexity in terms of the number of animals evaluated, the number and types of neurological tests, and the large amounts of data to process and interpret, will also be included. Finally, the use of the DNT study for hazard identification and risk assessment of new chemicals will be discussed in light of the current regulatory framework. Ample time will be allotted for a full discussion of the technical and regulatory challenges involved in conducting and interpreting the DNT.

819 OVERVIEW OF THE DEVELOPMENTAL NEUROTOXICITY (DNT) STUDY.

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Behavioral impairments in humans following prenatal and early postnatal exposures to several drugs, physical, and chemical environmental agents have been recognized for over a half century and have raised concerns that such effects might re-

sult from exposure to a wider array of agents. Due to the concerns of regulatory agencies that recognized the need to standardize testing for such effects, reliable and routine testing procedures were developed. The Collaborative Behavioral Teratology Study (CBTS), a six-laboratory comparison study funded by FDA and NIOSH, showed that behavioral tests conducted in a standardized manner could provide reliable and reproducible findings. The DNT testing battery developed in the late 1980s by EPA built on earlier studies and was evaluated for its ability to detect agents known to cause DNT in humans. The final EPA DNT protocol published in 1991 was designed to include apical tests of a number of structural and functional aspects of nervous system development. These include measures of growth and development, motor activity, auditory startle habituation, learning and memory, and neuropathology. The challenge of DNT testing is to ensure that exposure is adequate during the major periods of nervous system development, that the major domains of neurobehavioral function are evaluated, that the appropriate times for evaluation are included, and that appropriate signals will result from the testing battery to indicate when there is a need to conduct further testing of potentially more subtle effects.

820 COMPARATIVE SCHEDULES OF DEVELOPMENT IN RATS AND HUMANS: IMPLICATIONS FOR DEVELOPMENTAL NEUROTOXICITY TESTING.

J. Buelke-Sam. *Toxicology Services, Greenfield, IN.* Sponsor: M. Weiner.

The USEPA developmental neurotoxicity study (DNT) guideline provides a relatively fixed design approach in rats for hazard identification following maternal exposure during gestation and a portion of lactation. The CNS is not a unitary organ; component morphology and function develop on separate schedules in both rats and humans. In spite of a multitude of subsystem schedules, the postnatal day 10 (PND10) rat brain has been considered at a similar developmental stage to that of a term infant, and by PND21 at a similar stage to that of a 2-year-old. Maternal exposure begins on GD6 and ends on PND10 (or PND21) in the current EPA guideline. Continuing maternal treatment throughout lactation continues some level of offspring exposure through a portion of additional CNS development, eg, blood brain barrier maturation and myelination processes. Other organ systems that have implications for developmental exposure in a DNT study may develop on similar or rather different schedules. For example, nephrogenesis is complete at ca PND7 in rat and ca 35 weeks gestation in the human, with renal glomerular filtration rates reaching adult rates by ca PND21-28 in rats and ca 2 years of age in humans. Hepatic conjugation patterns in the rat do not reach mature levels until a few weeks after puberty. Behavioral assessment and neuropathology evaluations are conducted during the preweaning period through ca PND60, a range of development from infancy through mid-adolescence in the rat. Many of these test procedures have been used effectively to characterize CNS damage in rats following developmental exposure to known human developmental neurotoxicants, e.g., methylmercury, lead, PCBs, alcohol, and several anticonvulsants. At present, the DNT study approach is expected to function as other current toxicological screening tools: high dose effects observed in rats should be assumed to be predictive of an increased risk to the human fetus, infant and/or toddler up to 2 years of age following maternal or direct infant exposure to lower doses.

821 EXPERIENCE CONDUCTING THE DEVELOPMENTAL NEUROTOXICITY STUDY.

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The developmental neurotoxicity study (OPPTS 870.6300) is a very large and technically demanding study, with behavioral and morphometric assessments that are unfamiliar to many industry toxicologists. Approximately 1000 animals are included in the study design, representing 20-25 litters at each of four dose levels, with the treatment administered *via* the dam (e.g., by gavage or *via* the diet) from gestation day 6 through postnatal day 10. Subsets of the offspring are assessed using detailed observations or tests of motor activity, auditory startle habituation and cognitive function at specific days of age, with neural tissues collected at specific ages for microscopic examination and morphometry. Challenges associated with this study type include managing the logistics, maintaining attention to details that may affect the behaviors, the selection and validation of test devices and procedures, development and validation of analysis software, and data interpretation. This paper examines these and other practical issues (e.g., the selection of doses and means of administration), along with some study results, and considers how certain changes in experimental design that were recently instituted by the EPA (e.g., extending the dose interval) affect the logistics and introduce confounding factors that compromise interpretation. Discussion will include consideration of the interpretation and value of findings at maternally-toxic doses and the interpretation of findings during the period of exposure, prior to weaning.

822 APPLICATION OF THE DNT STUDY RESULTS TO RISK ASSESSMENT UNDER FQPA.

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The EPA Office of Pesticide Programs (OPP) requires the DNT study for chemicals that have neurotoxic potential. A weight-of-evdence assessment is conducted, and information on structure activity relationships, mode of action, and toxicological response (e.g., neurobehavioral or neuropathological, alterations in development of the nervous system, or endocrine perturbations) are used to determine the need for DNT testing. OPP may request a more specific, tailored study to assess special sensitivity to the young. Such studies are often adapted from the DNT protocol and evaluate specific endpoints not included in the DNT. Studies requested to date include cholinesterase activity, glutamine synthetase activity, and thyroid hormones. These data are requested if a known effect is seen in adult animals and may impact offspring. DNT data studies are useful in evaluating potential risk to the fetus, and to infants and children. DNT results are used most frequently for acute and short-term risk assessments. In a few cases the results were used for intermediate-term and chronic endpoints. Treatment-related effects observed in DNT testing are used to assess the level of concern, and residual uncertainties (i.e., those not addressed by the traditional uncertainty factors). The presence of residual uncertainties may justify the retention of a special FQPA safety factor. Evaluation of DNT studies submitted to OPP has provided a basis for determining the need for (and magnitude of) uncertainty factors. These factors may be applied to the risk assessment to account for the absence of the guideline DNT study, the absence of other data that are expected to more fully characterize the potential for developmental neurotoxicity, or the identification of treatment-related effects or offspring susceptibility in DNT testing.

823 MODE OF ACTION IN ASSESSING HUMAN RELEVANCE OF ANIMAL TUMORS: IMPROVING THE FRAMEWORK FOR ANALYSIS.

P. A. Fenner-Crisp and S. M. Cohen. *Pathology/Microbiology, University of Nebraska Medical Center, Omaha, NE.*

Developing an understanding of the mode(s) of action (MOA) by which chemicals induce tumors in animals has become a key element in determining whether or not the tumors observed are relevant/applicable for use in human cancer risk assessment, and, if so, what might be the characteristics of the human dose response. This workshop, the product of an ILSI Risk Science Institute administered project funded by USEPA and Health Canada, includes 1) Discussion of proposed enhancements to the IPCS MOA Framework and EPA's MOA Framework in its 1999 draft cancer guidelines to strengthen the building of the bridge from "I think I understand the animal MOA(s)" to "I am able to articulate the importance of this animal tumor and its MOA(s) for the human, " 2) Implications of the Human Relevance Framework for cancer risk assessment, using several well-examined tumor types as examples; 3) An update on the understanding of the MOA(s) by which PPARα agonists induce liver and other tumors in rodents; and 4) Application of the enhanced Framework to PPARαagonist-induced tumors as an example of a newly-developed consensus on MOA.

824 UPDATING CANCER ASSESSMENT PRINCIPLES: AN EPA PERSPECTIVE.

V. L. Dellarco. *US Environmental Protection Agency, Washington, DC.* Sponsor: P. Fenner-Crisp.

In 1996, University.S.EPA proposed revisions to its 1986 Guidelines for Carcinogen Risk Assessment. Several areas were given particular attention. They included the development of a new set of hazard descriptors; an expanded explication of the nature and use of default assumptions; guidance on the use of the margin of exposure analysis; the consideration of the relevancy of the assessment for children, and an analytical framework for identification and characterization of the mode(s) of action for tumors observed in animal bioassays to determine the applicability and relevance of the tumors for human cancer assessment. Beginning in 1996, the Agency began testing the usefulness of the proposed revised guidelines. At the same time, it was attempting to develop a consensus on the relevance and use of tumors produced by peroxisome-proliferating chemicals. The International Programme on Chemical Safety also was developing a framework for use worldwide. Application of these frameworks soon revealed that, while they were adequate in providing guidance on determining whether or not the animal mode of action was adequately understood, they were not sufficiently robust to guide the analysis for establishing the applicability/relevance of the tumor MOA(s) for use in human cancer risk assessment. The Agency asked the ILSI Risk Science Institute to convene an expert

panel to define an expanded scientific process for utilizing a chemical's mode of action in animals to evaluate the human relevance and applicability of this information, with special attention to be given to the peroxisome proliferators. The subsequent presentations will describe the efforts of the panel with regard to the development of an expanded Framework and an update on the state-of-the-science of our understanding of the mode(s) of action of rodent tumors induced by certain peroxisome proliferators followed by application of the Framework to reach conclusions about the applicability of these tumors for human cancer assessment. (The opinions expressed are those of the author and should not be construed as Agency policy).

825 DEVELOPING THE HUMAN RELEVANCE FRAMEWORK.

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To evaluate the human relevance of a particular mode of action for a tumor type observed in an animal bioassay and its applicability in cancer risk assessment, a process involving three questions was developed: 1) Is the animal mode of action known? 2) Are the key events qualitatively applicable to humans? 3) Are the key events quantitatively applicable to humans? To conduct this exercise, the frameworks developed by the IPCS and EPA were used as the initial basis for evaluation. It was soon noted that these frameworks provided adequate guidance for answering the first question, but relatively little for answering the other two questions. It became necessary to modify the existing frameworks to incorporate guidance for reaching conclusions about the applicability of the tumor type and its MOA to human cancer assessment. As part of this process, the specific key events in the animal mode of action were identified, and the concordance between these key events in the animal model and in humans were explicitly evaluated. Human data could be chemical-specific or related to what is known about mode of action based on information with other chemicals or the biology of the process. If there was concordance qualitatively for each of the key events, they were then evaluated on a quantitative basis, taking into account toxicokinetic and toxicodynamic parameters, physiologic processes, and relative sensitivities in the animal and human. Case studies involving seven chemicals with known information regarding mode of action in animals were used in an iterative process to develop an approach for incorporating mode of action into the risk assessment process. Case studies were acrylonitrile, d-limonene, atrazine, melamine, chloroform, phenobarbital, ethylene oxide and DEHP.

826 THE MODE(S) OF ACTION FOR PPARα AGONIST-INDUCED RODENT TUMORS.

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Peroxisome proliferators are rodent nongenotoxic carcinogens. The mode of action for liver tumors is *via* activation of the peroxisome proliferator activated receptor α (PPARα) which regulates gene transcription leading to perturbations in cell proliferation, apoptosis and peroxisome proliferation (PP). Suppression of apoptosis coupled with cell proliferation allows DNA damaged cells to persist and proliferate giving rise to pre-neoplastic foci and ultimately to tumors *via* further clonal expansion. PPARα ligands also may cause oxidative stress, inhibit GJIC and stimulate non-parenchymal hepatic Kupffer cells; these effects could contribute to the MOA *via* several routes such as induction of cell proliferation or oxidative DNA damage. Several PPARα ligands also have been shown to induce Leydig cell tumors (LCTs) and/or pancreatic acinar cell tumors (PACTs) in rats. PPARα agonists do not induce peroxisomes in either tissue, and hence, may induce these extrahepatic tumors *via* different mechanisms. LCTs may be secondary to the changes in liver function associated with exposure to PPARα agonists since these substances induce liver aromatase and may thus alter levels of circulating hormones. Alternatively, LCTs could arise from direct inhibition of testosterone biosynthesis within the testis. For PACTs, data suggest that PPARα ligands decrease bile acid flow, a phenomenon associated with choleostasis and tumorigenesis in PACs. For both LCTs and PACTs, activation of PPARα and subsequent changes in target gene expression may play a role, but this remains to be determined. Based on an evaluation of the published literature, we propose a number of key steps in PPARα-mediated rodent liver, Leydig cell or pancreatic acinar cell tumorigenesis. We propose for each key step an evaluation of causality (role of the event in the tumors), strength (strength of the evidence for or against) and specificity (a specific response to PPARα ligands or ubiquitous?). This reductive approach to evaluating rodent MOA(s) facilitates the evaluation of the relevance of each MOA to humans and provides guidance on the required data elements.

827 APPLICATION OF THE FRAMEWORK TO THE PPARα AGONIST CASE EXAMPLE.

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An update and summarization of our understanding of the mode(s) of action by which PPARα agonists (peroxisome proliferators) induce liver, Leydig cell and pancreatic acinar cell tumors in rodents has recently been conducted. This information is necessary for a scientifically based assessment of the carcinogenic potential of this group of agents in humans. The determination of the rodent tumor mode of action for PPARα agonists is not sufficient by itself for reaching a conclusion about whether or not these modes of action are applicable for use in human cancer risk assessment. Two additional questions must be addressed: 1) Are the key events of the mode of action qualitatively applicable to humans? and 2) Are the key events quantitatively applicable to humans? This presentation will describe the application of the proposed Human Relevance Framework to the experimentally supported mode(s) of action for selected PPARα agonists that have been shown to induce hepatocyte peroxisome proliferation as well as hepatic, Leydig and/or pancreatic acinar cell tumors in rodents following long-term administration. A comparative analysis will show whether or not the data for each of four case study chemicals (clofibrate, DEHP, oxadiazon, PFOA) are sufficient to support the characterization of the MOA(s) for each tumor type as being that/those hypothesized for PPARα agonists. For those substances that satisfy the criterion of sufficiency, additional analysis will be presented to demonstrate how the conclusions were reached with regard to the qualitative and quantitative applicability of these data to the human and the relevance of this information for human cancer risk assessment.

828 EVIDENCE FOR EGFR PATHWAY MEDIATION OF CLEFT PALATE INDUCTION BY TCDD.

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is teratogenic in C57BL/6J mice, producing cleft palate (CP) after exposure *in utero*. Epidermal growth factor receptor (EGFR), EGF and TGF-alpha (TGF) expression in the palate is disrupted by TCDD affecting proliferation and differentiation. The key role of EGF in mediating the teratogenicity was confirmed using EGF knockout (KO) mice. The EGF KO embryos were less responsive for the induction of cleft palate than the wild type mice (WT=C57BL/6J x 129). However, exposure of TGF KO mice (having C57BL/6J background) produced incidences of CP that were similar to that observed in C57BL/6J wild type mice. In this study, the pivotal role of EGF in the response to TCDD was confirmed using palatal organ culture. Embryonic mouse palates from C57BL/6J, TGF KO, WT and EGF KO mice were placed in organ culture on gestation day (GD) 12 and cultured with or without TCDD ($1x10^{-8}$ M) in presence or absence of growth factor supplements (2 ng/ml EGF or TGF). After 5 days in culture, the effects of the treatments on palatal fusion were evaluated. The palates from all of the genotypes fused in the serum-free, defined control medium. Similar to the *in vivo* response, EGF KO palates exposed to TCDD fused and the rate of fusion did not differ from the control cultures. EGF KO palates exposed to TCDD in presence of EGF (2ng/ml) failed to fuse ($p<0.05$ vs control medium or TCDD without EGF). The responses of TGF KO palates differed as only TGF supplementation significantly reduced fusion rates in the presence of TCDD. Our lab and others have reported a requirement for serum for many cell types to respond to TCDD in culture. The present study demonstrates that addition of EGF or TGF alone can be sufficient to mediate responses to TCDD. The palatal culture model also confirms that providing EGF to the palates of EGF KO mice restores the responsiveness to TCDD. These studies support the hypothesis that the mechanism for induction of CP by TCDD is mediated *via* the EGFR pathway. Disclaimer: This abstract does not necessarily reflect EPA policy.

829 DIVERSE NON-SPECIFIC MATERNAL IMMUNE ACTIVATION PROCEDURES REDUCE SEVERITY OF DIABETIC EMBRYOPATHY IN MICE.

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Diabetic embryopathy is a complication occurring in pregnant women with type-1 or insulin-dependent diabetes mellitus. Several biochemical pathways have been proposed for its pathogenesis, including myo-inositol and prostaglandin deficiencies and free radical toxicity. In the present study, we hypothesized that the local immune response in the uterus may be disrupted by diabetes, which may play a role in producing teratogenic results. Maternal immune stimulation prevents or reduces

diabetes-induced dysmorphogenesis, possibly through normalizing of the uterine cytokine production and secretory profile. Streptozocin (STZ)-induced diabetic mice were used to produce malformed fetuses. Tree techniques of immune stimulation, complete Freunds adjuvant (CFA), granulocyte-macrophage colony stimulating factor (GM-CSF), and interferon-g (IFNg) were used to stimulate immune cells of pregnant ICR mice. Approximately 50% of live fetuses from diabetic mice were malformed with neural tube defects. Immune stimulated diabetic mice produced significantly lower numbers of malformed fetuses: CFA 19.6%; GM-CSF 22.1%, IFNg 15.0%. These results suggest that diverse procedures to activate nonspecific maternal immune cells cause, for unknown reasons, approximately equal suppression of the dysmorphogenic effects of diabetes mellitus.

830 PERINATAL EXPOSURE TO THE PESTICIDE HEPTACHLOR PRODUCES ALTERATIONS IN IMMUNE FUNCTION PARAMETERS IN SPRAGUE-DAWLEY RATS.

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Pesticides, and other environmental contaminants, recently have been implicated as potential causes of recent increases in childhood asthma and leukemia. The objective of this study was to determine the effect that the pesticide heptachlor (H) has on the developing immune system of Sprague-Dawley rats. Dams were dosed from gestational day 6 (GD6) through post-natal day 21 (PND21), at which time the pups were weaned and dosed directly with H until PND42. The doses of H were 0 (control), 0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg/day. Rats were tested for the delayed type hypersensitivity (DTH) response to bovine serum albumin (BSA), the phagocytic ability of peritoneal macrophages, and the IgM and IgG antibody responses to sheep red blood cells (SRBC). H did not affect the DTH response in eight week-old male rats, while the females at eight weeks showed a dose-related increase in the DTH responses from control to 3.0 mg H/kg/day. The phagocytic ability of peritoneal macrophages from 12 week-old rats was suppressed at the lowest dose of H, with higher doses not significantly different from controls. Primary (IgM) antibody responses were significantly suppressed in a dose-dependent manner in eight week old rats treated with H (i.e., a mean $\log_2$ titer of 9.0 in controls versus 7.5 in 3.0 mg H/kg/day dose group). Rats re-immunized with SRBC and evaluated for IgG titers revealed a dose-dependent effect, with control rats at a mean $\log_2$ titer of 11.3 and the high dose group at a mean log2 titer of 8.1. These results support and extend our earlier work with H, indicating suppression of the primary and secondary response to SRBC following perinatal exposure to H is consistently observed. (This abstract does not reflect EPA policy. Supported in part by the American Chemistry Council under CRADA 0215-02.)

831 MATERNAL IMMUNE STIMULATION REDUCES BOTH PLACENTAL MORPHOLOGIC DAMAGE AND DOWN-REGULATED PLACENTAL GROWTH-FACTOR AND CELL CYCLE GENE EXPRESSION CAUSED BY URETHANE: ARE THESE EVENTS RELATED TO REDUCED TERATOGENESIS?

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Activation of the maternal immune system in mice decreased cleft palate caused by the chemical teratogen, urethane. Direct and indirect mechanisms for this phenomenon have been suggested, including maternal macrophages that cross the placenta to find and eliminate pre-teratogenic cells, or maternal immune proteins (cytokines) that cross placenta to alleviate or partially alleviate toxicant-mediated effects in the developing fetus. A third mechanism to explain improved fetal developmental outcome in teratogen-challenged pregnant mice might involve beneficial effects of immune stimulation on the placenta. In the present experiments, urethane treatment altered placental morphology and impaired placental function, the latter indicated by down-regulated activity of cell cycle genes and of genes encoding cytokines and growth factors. Maternal immune stimulation with either Freund's Complete Adjuvant (FCA) or interferon-gamma (IFNg) reduced morphologic damage to the placenta caused by urethane, and normalized expression of several genes that were down-regulated by urethane. Urethane treatment also shifted placental cytokine gene expression toward a T cell helper 1 (Th1) profile, while immunostimulation tended to restore a Th2 profile that may be more beneficial to pregnancy and fetal development. These data suggest that the beneficial effects of maternal immune stimulation on fetal development in teratogen-exposed mice may, in part, result from improved placental structure and function.

832 FUMONISIN-INDUCED NEURAL TUBE DEFECTS: DISRUPTION OF SPHINGOLIPIDS AND FOLATE TRANSPORT.

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Fumonisin B1 (FB1), a mycotoxin produced by F. verticillioides, is a contaminant of corn involved in the etiology of various diseases in different species. FB1 disrupts sphingolipid biosynthesis by inhibiting the enzyme ceramide synthase, and has recently been implicated as a potential teratogen. Using a murine model, the current study investigates maternal FB1 exposure, sphingolipid alterations, and subsequent disruption of folate transport systems in the induction of neural tube defects (NTDs). FB1 exposure during early gestation caused an increase in apoptosis in the dorsal neuroepithelium and subsequent NTDs (exencephaly) in inbred LMBc mice. The timing of maternal FB1 exposure was critical, and the number of affected pups/litter increased in a dose-dependent manner. The ability of FB1 to cross the placenta was demonstrated by injecting pregnant dams with 14C-labeled FB1 and measuring radioactive uptake in embryos and maternal tissues. In addition, sphinganine levels were shown by HPLC analysis to be significantly elevated in both embryos and placentas following FB1 exposure. Maternal folinic acid supplementation by daily oral gavage reduced the incidence of FB1-induced NTDs by approximately 50%. However, co-administration of ganglioside GM1 during FB1 exposure was able to almost completely rescue the phenotype (95%). The impact of FB1 on folate transport was investigated *in vivo* by measuring 3H-folate uptake in control and FB1 embryos. 3H-folate uptake was significantly inhibited by FB1 exposure, but partially reversed by GM1 co-administration. Immunohistochemical staining for both the folate receptor and GM1 was decreased following FB1 exposure. Since GM1 is co-localized with the GPI-anchored folate receptor in lipid rafts, it is proposed that GM1 is an important component of the membrane microdomain necessary for proper folate receptor function, and adequate uptake of folate during development to protect against NTDs.

833 MICROARRAY ANALYSIS OF STRAIN-SPECIFIC RESPONSES TO ETHANOL.

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The striking parallelism possible with cDNA microarray analysis allows functional relationships in the genomic response to ethanol to be uncovered. Ethanol is a human teratogen that can act early in development to cause Fetal Alcohol Syndrome (FAS). In addition to craniofacial abnormalities and neurological deficiencies, FAS-like symptoms can include effects to the developing eye (microphthalmia and optic nerve hypoplasia). Whereas CD-1 mice are relatively less sensitive to the teratogenic effects of ethanol, C57BL/6 mice are susceptible. Strain differences imply a genetic basis to ethanol-related birth defects; therefore, the availability of a mouse model that exhibits differential susceptibility to acute ethanol exposure makes possible studies to elucidate the mechanism(s) related to these outcomes that are currently undefined. Pregnant mice were dosed with 2.9g/kg ethanol i.p. on gestation day 8. Embryonic headfold collected 3h post-exposure provided a source of RNA for microarray analysis. After checking QA/QC standards, the RNA from treated (ethanol) and control (vehicle) samples was labeled for competitive hybridization to MPS621 microchips followed by tyramide signal amplification and two-color fluorescent labeling (PerkinElmer Life Sciences). Data analysis used GeneSpring v 4.2 (Silicon Genetics). Genes were filtered for a 1.5-fold change and a consistent response between replicate arrays. Hierarchical clustering of the 383 genes (17% of those arrayed) meeting these criteria revealed distinct differences between the strains. Genes (240) related to transcription, stress response, and mitochondrial processes were found to change in C57BL/6 embryos whereas only 10% of these genes changed in CD-1 embryos, about half of them coding ribosomal proteins. Genes (121) related to chromosomal structure (HMG Y/1), protein synthesis, and cytoskeletal structure were found to change in CD-1 embryos. Organizing responsive genes into larger clusters should provide clues to mechanisms of differential susceptibility. (Supported by NIH grants AA13205 and ES07282)

834 THE PRESENCE OF XENOBIOTIC TRANSPORTERS IN RAT PLACENTA.

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Understanding the role of various transporters in placental handling of xenobiotics across the maternal-fetal interface is essential to evaluate the pharmacological and toxicological potential of therapeutic agents, drugs of abuse and other xenobiotics

to which the mother is exposed during pregnancy. Therefore, the purpose of this study was to assess mRNA levels of various transporters in placenta and to compare these to levels in maternal liver and kidney, the predominant organs of excretion, in an attempt to determine which transporters are most likely to have a role in xenobiotic transfer within the placenta. During late stage pregnancy, relative mRNA levels of three transporters of the multidrug resistance family (Mdr), six members of the multidrug resistance-associated protein family (Mrp), eight members of the organic anion transporting polypeptide family (Oatp), three members of the organic anion transporter family (Oat), five members of the organic cation transporter family (Oct), two bile acid transporters (Na+/taurocholate cotransporting polypeptide [Ntcp] and bile salt excretory protein [Bsep]), as well as four metal (ZnT1, DMT1, Menkes and Wilsons), a prostaglandin, two peptide, two sterolin, and four nucleoside transporters were assessed in placenta with respect to corresponding maternal liver and kidney mRNA levels. Of the 40 genes evaluated, the mRNA of 16 genes (Mdr1a and 1b, Mrp1 and 5, Oct3 and N1, Oatp3 and Oatp12, four metal, the prostaglandin, one sterolin [Abcg8], and two nucleoside transporters [ENT1 and ENT2]) was present in placenta at levels similar to or higher than those observed in maternal liver and kidney. The abundance of these transcripts in placenta suggests a role for these transporters in placental transport of xenobiotics. These data may further aid in the prediction of maternally-mediated developmental toxicities resulting from xenobiotic exposures. (Supported by NIH grants ES-03192, ES-09716, and ES-09649 and Training Grant ES-07079)

835 AN EMBRYO-FETAL DEVELOPMENT STUDY IN CYNOMOLGUS MONKEYS WITH RITUXIMAB, AN ANTI-CD20 ANTIBODY.

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Rituximab (Rituxan, Genentech, SSF, IDEC, San Diego) is approved by the USFDA for the treatment of CD20+, B-cell non-Hodgkin's lymphoma (NHL). This chimeric anti-CD20 antibody is being evaluated in non-oncologic indications, including immune thrombocytopenia purpura (ITP) and rheumatoid arthritis (RA). Although rituximab has been used to treat over 200, 000 oncology patients, it was not previously investigated for effects on reproduction. A study to investigate rituximab's effect on embryo-fetal development was undertaken. Forty-eight cynomolgus monkeys were divided into 4 groups (12/group). Rituximab was administered (IV) 1X/week during organogenesis (gestation days, GDs, 20-50). In order to provide consistent exposure during this period, loading doses were administered on GDs 20, 21 and 22. Rituximab was administered at doses of 0, 20, 50 and 100 mg/kg (loading doses were 0, 15, 37.5 and 75, respectively). Animals were monitored for clinical signs, pregnancy by ultrasound, body weight, food consumption, hematology, serum biochemistry, anti-drug antibody, and PK. Upon cesarean section at GD 100, fetal examinations included an external exam (viability, sex, body weight and placental weight), a visceral exam (gross observations of organs and tissues, with subsequent immunohistochemistry of spleen and lymph nodes), and a skeletal exam. There were 5 spontaneous abortions during the study; however, this is within the historical control data (10%) and does not appear to be treatment related. There were no effects of rituximab on any of the parameters measured, other than the expected pharmacologic effect of B-cell depletion. In addition, there were no unusual findings in the fetal examinations. Therefore, rituximab administration appears to have no adverse effect on embryo-fetal development in cynomolgus monkeys.

836 AMBIENT OXYGEN REGULATES INTRACELLULAR REDOX POTENTIAL AND TRANSCRIPTION FACTOR ACTIVITY IN CULTURE PRIMARY HUMAN MYOBLASTS.

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Muscle satellite cells are mononucleate stem cells that generate muscle or fat in an O2-dependent manner: High O2 (20%) in culture favors adipogenesis and low O2 (6%) promotes myogenesis. The free radical trap PBN reduces adipocyte differentiation by 50% in satellite cells cultured in 20% O2, suggesting that ROS produced as a function of environmental O2 concentrations play a pivotal role in the regulation of specific pathways in cultured mesenchymal-derived stem cells. Human skeletal myoblasts (hSKM) were loaded with a ROS indicator, DCF, and cultured in either 6% or 20% O2 for 24 hr. DCF fluorescence was significantly reduced in 6% O2, 40% less than at 20% O2. Intracellular redox potentials were calculated from GSH and GSSG concentrations, (determined by HPLC) following culture at either 6% or 20% O2 for 7 days. At 20% O2, hSKM GSH concentrations were 27% lower and GSSG concentrations were 6-fold higher, yielding a significant redox difference of +30 mV in 20% vs. 6% O2. Nuclear GSH was 78% lower in 20% O2 cultures as compared to 6% O2, suggesting that the nuclear environment is more oxidative than the cytosol. It is believed that +/-15 mV shift can modulate redox-sensitive transcription factors. Electromobility shift assays showed enhanced activity in the pro-adipogenic transcription factors C/EBP, CREB and RXR at 20% O2. These findings support environment-induced ROS increases and intracellular redox potential as key factors in regulatory pathways of both myogenic and adipogenic differentiation.

837 EXPRESSION OF MHC CASS II PROTEINS ON SPLENOCYTES AS A BIOMARKER FOR MODELING AND PREDICTING THE EFFECTS OF CHEMICAL STRESSORS.

S. B. Pruett, R. Fan and Q. Zheng. *Cell. Biol. & Anatomy, LSU Health Sciences. Center, Shreveport, LA.*

Previous studies have shown that the area under the corticosterone concentration vs. time curve (AUC) can be used as a quantitative measure of a chemical-induced stress response, allowing prediction of the effects of propanil on selected immunological parameters. The present study was designed to extend these findings using two additional chemical stressors (atrazine and ethanol) and to determine if a single immunological end point might serve as a predictive biomarker for chemical stressors. Ethanol (4, 5, 6, or 7 g/kg by gavage) and atrazine (100, 200, or 300 mg/kg i.p. in corn oil) increased serum corticosterone levels in female B6C3F1 mice 1-8 hr after dosing. Values for the corticosterone AUC were dose-responsive, and these values were related in a linear manner to changes in several immunological parameters including: splenic NK cell activity, IgG1 and IgG2a responses to keyhole limpet hemocyanin (KLH), spleen and thymus cellularity, B and T cell subpopulations in the spleen, CD4 and CD8-definsed subpopulations in the thymus, and expression of MHC class II proteins on splenocytes. Comparison of these results with previous results using exogenous corticosterone and restraint stress indicate that most of the linear models were more similar to those derived from restrained mice. As expected, there were a few immunological parameters (e.g., NK cell activity in ethanol-treated mice) that were affected more than predicted by stress models, suggesting chemical-specific immunotoxicity independent of the stress response. Finally, using the restraint models for each of these parameters and the chemical dosage at which 50% inhibition of MHC class II expression occurred, it was possible to accurately predict the quantitative change in almost all the other immunological parameters. This and other considerations suggest that MHC class II expression on splenocytes may be a useful biomarker and for chemical-induced stress and predictor or other immunological effects of this stress. This work was supported by a grant from NIEHS (ES09158).

838 MODELING AND PREDICTING IMMUNOSUPPRESSION BY CHEMICAL STRESSORS IN MICE USING BLOOD PARAMETERS.

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Previous studies have shown that the area under the corticosterone concentration vs. time curve (AUC) can be used to model and predict the effects of restraint stress and chemical stressors on a variety of immunological parameters in the mouse spleen and thymus. However, if this approach is to be used to complete a risk assessment parallelogram, similar data are needed with blood as the source of immune system cells, because this is the only tissue routinely available from human subjects. Therefore, studies were conducted using treatments for which the corticosterone AUC values are already known: Exogenous corticosterone, restraint, propanil, atrazine, and ethanol. The following immunological parameters were measured using peripheral blood from mice treated with a series of dosages of each of these agents: flow cytometry was used to quantify MHC II, B220, CD4, and CD8 cells, and leukocyte counts and differential counts were done. Cytokine (IL-2, IL-4, and IFN-g) production by isolated peripheral blood mononuclear cells was also measured. Spleen cell number and NK cell activity were evaluated (using a standard 4 hr 51Cr-relase assay with YAC-1 target cells) to confirm similarity to previous studies. Immune parameter data from mouse blood indicate that MHC II expression has consistent quantitative relationships to corticosterone AUC values, similar to those in the spleen. However other the immune parameters tended to have greater variability in the blood than observed in the spleen. The pattern observed in the spleen in which the chemical stressors generally produced very similar effects as noted for restraint stress (at the same corticosterone AUC values) was not observed using blood. Nevertheless, MHC class II expression seems to provide a consistent indication of stress exposure, regardless of the source of cells. The completed analysis of these two corners of a parallelogram has brought this predictive model one step closer to completion. This work was supported by a grant from NIEHS (R01 ES09158).

839 ETHANOL SUPPRESSES SIGNALING THROUGH TOLL-LIKE RECEPTOR 3 (TLR 3).

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Acute and chronic exposure to ethanol (EtOH) can be immunosuppressive, and cytokine responses to LPS, which acts through TLR 4, are suppressed by EtOH. However, it is not clear if suppression of signaling through other TLRs occurs or what components of the signaling pathways are affected. The purpose of the present study was to investigate these questions. Mice were treated with polyinosinic-polycytidylic acid (poly I:C), which induces cytokine production by acting on TLR 3, primarily on dendritic cells and macrophages. Some groups of mice were also treated by EtOH (6 g/kg) using our binge drinking model. The results indicate that EtOH profoundly suppresses poly I:C-induced cytokine production and accumulation of neutrophils in the peritoneal cavity (the site of poly I:C injection). This was associated with decreased activation of NF-kappa B in peritoneal macrophages (a late event in TLR signaling) and with decreased degradation of IRAK-1 in these same cells (an early event in TLR signaling). The results strongly suggest that EtOH acts to inhibit signaling through TLR 3 by acting at an early step in the signaling pathway. Further studies are needed to determine if decreased activation of NF-kappa B constitutes an additional effect of EtOH or is simply a consequence of inhibition of early steps in the TLR signaling pathway. To determine if IL-10 induced by poly I:C plus EtOH contributes to suppression of signaling, spleen cells from IL-10 knockout mice were stimulated *in vitro* with poly I:C or poly I:C plus EtOH (0.5% w/v). Similar experiments using normal mice indicated that EtOH significantly suppresess poly I:C-induced IL-12 production. In contrast, EtOH significantly enhanced IL-12 when splenocytes from IL-10 knockout mice were used. This suggests a possible role for IL-10 in suppression of poly I:C-induced signaling that leads to IL-12 production. This work was supported by a grant from NIAAA (R01 AA09505).

840 ETHANOL SUPPRESSES CYTOKINE PRODUCTION INDUCED BY LIGANDS FOR TOLL-LIKE RECEPTOR (TLR) 9 AND TLR 6.

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Oligodeoxynucleotides containing unmethylated CpG dinucleotides stimulate cells of the innate immune system, in part by inducing the production of several cytokines. Yeast polysaccharides such as zymosan also induce cytokine production, and both of these agents act through TLRs. Considering the prevalence of binge drinking and alcoholism, it seems appropriate to test the effects of ethanol (EtOH) on CpG DNA- and zymosan-induced cytokine production. The effect of acute EtOH exposure on the induction of IL-12 by CpG DNA was evaluated by ELISA. B6C3F1 mice were treated intraperitoneally with CpG DNA (40 μg/mouse) and given EtOH (6 g/kg by gavage in a 32% solution in water) at the same time. CpG DNA significantly increased IL-12 cytokine production *in vivo* compared to untreated mice, and EtOH significantly suppressed this increase (measured in the serum 2 hr after dosing). We also examined CpG DNA at lower dosages of 20 μg/mouse and 10 μg/mouse *in vivo* and observed dose-dependent production of IL-12, with near peak levels at 20 μg/ml. This indicates 20 μg/ml would be an appropriate dosage for future studies. Zymosan (5 mg/mouse, i.p.) induced the production of IL-12 (measured in serum 3 hr after dosing). Administration of EtOH (6 g/kg) at the same time as zymosan significantly decreased IL-12 production and significantly increased the production of the immunosuppressive cytokine IL-10. Previous studies by this and other laboratories indicate that EtOH suppresses cytokine production stimulated through TLR 3 and 4. The ligands examined here signal through TLR 9 (CpG DNA) and TLR 2/6 (zymosan). This strongly suggests that EtOH is a general inhibitor of TLRs. If confirmed in additional studies, this finding would have important implications as a mechanism for the well-characterized immunosuppressive effects of EtOH. This work was supported by a grant from NIAAA (R01 AA09505).

841 SUPPRESSION OF INFLAMMATORY CYTOKINE PRODUCTION BY CIGARETTE SMOKE IS MEDIATED BY ACROLEIN.

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While cigarette smoking is known to inhibit T and B lymphocyte response in the lungs, little is known about the nature of the immunosuppressive compounds in cigarette smoke. We have found that pretreatment of human peripheral blood mononuclear cells with cigarette smoke extracts derived from a single high-tar (unfiltered *Camel*) or low-tar (*Carlton*) cigarette suppressed anti-CD3/PMA-stimulated IL-1β, IL 2, IFN-γ, and TNF-α production by >90%. The inhibitory effect of the cigarette smoke extract did not require direct contact with the cells. Rather, the effect carried over in the vapor phase to untreated cells in neighboring wells. Cells closest to the extracts exhibited >95% suppression of cytokine production, while cells further away on the plate exhibited progressively less inhibition. Using gas chromatography/mass spectrometry, we identified several aldehydes (acetaldehyde, acrolein, and propionaldehyde) and other volatile compounds (toluene, xylene, styrene, and limonene) in the vapor phase of the cigarette smoke extracts. Among these compounds, only the thiol-reactive acrolein exhibited significant inhibition of cytokine production. The effect of acrolein could be mimicked by another α, β-unsaturated aldehyde, crotonaldehyde, but not by the saturated acetaldehyde, butyraldehyde or propionaldehyde. Cigarette smoke extracts and acrolein blocked induction NF-κB and AP-1 DNA binding activity without affecting expression of p65, p50, Fos and Jun proteins. Finally, acrolein directly inhibited the binding of preformed NF-κ and AP-1 to their respective promoters, suggesting that it alkylates critical thiols in their DNA binding domains. All of these effects could be completely prevented by treating the cells or the extracts with the thiol protectant, N-acetylcysteine.

842 PARTICLE SIZE AND COMBINATION WITH ANTIGEN ARE IMPORTANT IN MACROPHAGE ACTIVATION BY PARTICULATE MATTER.

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Various epidemiological and animal studies have shown a correlation between exposure to airborne particulate matter (APM) and airway hypersensitivity. Recently, we have shown that intranasal exposure of mice to carbon black (CB) as a model substance for APM enhanced the immune response to a co-exposed antigen (ovalbumin or OVA). Subsequent studies by others and us have indicated that alveolar macrophages might be important in these particle-induced adjuvant effects. In addition it became evident that certain particle characteristics, in particular charge and size, are crucial for the effect. Therefore, we have tested the effects of different model particles (CB, titanium dioxide (TiO2), polystyrene particles (PSP) of different sizes, and silica oxide (SiO2) on two different murine macrophage cell lines, the RAW 267.4 (monocyte/macrophage) and MH-S (alveolar macrophage). Effects of particles alone were compared with those of the antigen OVA or a combination of both. All particles were phagocytized, and most particles induced pro-inflammatory cytokines (TNF-a and IL-1b), and NO by the macrophages. SiO2 induced very high levels of TNF-a but was also the most toxic of all particles used. CB and TiO2 (1-200ug/ml) induced TNF-a release only at the highest concentrations. Using PSP of different size made it possible to determine the importance of particle size and surface area for the effects on macrophages. Whereas no difference in phagocytosis was observed, there is a correlation between total surface area and TNF-a release. Although exposure to OVA induced the release of both TNF-a and high levels of NO, the combined exposure to CB particles and antigen was even more effective. Results indicate that particulate matter, OVA and the combination of both have a stimulatory effect on macrophages. This effect depends on certain particle characteristics. Together, these data support the idea that macrophages may provide adjuvant-help to antigen-specific immune responses in the airways.

843 NEUROTOXICITY AND IMMUNOTOXICITY ASSESSMENT IN CBA/J MICE WITH CHRONIC *TOXOPLASMA GONDII* INFECTION AND MULTIPLE LOW DOSE EXPOSURES TO METHYLMERCURY.

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The present study was conducted to determine the effect of multiple low doses of methylmercury (MeHg) on the course of a chronic Toxoplasma gondii infection. Four groups of six-week-old, female CBA/J mice were either fed 25 T. gondii tissue cysts of the ME-49 strain or vehicle control. Six weeks later, half of each group were orally gavaged with 8-mg/kg body weight doses of MeHg on days 0, 2, 4, 7, 10, 13. Mice were sacrificed on day 17 or 18 post MeHg exposure. Flow cytometric analysis of lymphocyte subpopulations in the thymus demonstrated a significant increase in the percentage of CD4-8+ T-cells in mice exposed to MeHg with a concurrent T. gondii infection. Groups of mice exposed to MeHg showed a decrease in total thymic cellularity and cellularity of all T-cell subpopulations when compared to control mice, but viability of these cells was unaffected. Splenic cell viability was decreased in mice exposed to MeHg, but alterations in T-cell subpopulations were not noted. These data indicate that multiple low doses of MeHg may not exacerbate

chronic toxoplasmosis but MeHg-induced effects on the immune system were evident. (The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US Environmental Protection Agency.)

844 THE ROLE OF METABOLISM IN BENZO(*A*)PYRENE-INDUCED IMMUNOSUPPRESSION IN A FISH MODEL.

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Benzo(a)pyrene (BaP) is a ubiquitous aquatic contaminant and mammalian immunosuppressant. Using Japanese medaka (*Oryzias latipes*) as the animal model, studies in this laboratory demonstrated that *in vivo* exposure to BaP (2 - 200 μ g/g BW) suppresses both innate and humoral-mediated immunity. Further studies examining the molecular mechanism(s) by which the observed effects on immune function may have occurred demonstrated that BaP activates biotransformation pathways both in the intact animal and within immune cells. Amelioration of BaP metabolism either by blocking CYP1A protein induction with the aryl hydrocarbon receptor (AhR) agonist alpha-napthaflavone or inhibiting CYP1A enzyme activity with ellipticine, resulted in the diminution of BaP-induced suppression of antibody production. In addition, IP injection of fish with the structurally-related weak AhR agonist, benzo(e)pyrene had no effect on immune function (compared to vehicle-treated control). To determine what role, if any, BaP metabolites may have played in the observed immunosuppression, non-adherent and adherent immune cells were treated *in vitro* with either BaP, benzo(a)pyrene-7, 8-dihydrodiol (BD), or benzo(a)pyrene-7, 8-dihydrodiol-9, 10-epoxide (BPDE). Although co-incubation of immune cells with CYP1A inhibitors alleviated the immunotoxicity of BaP and BD, BPDE-induced immunosuppression was unaffected. This suggests that BPDE represents the ultimate immunotoxicant in fish as appears to be true for mammals. Results of this study not only demonstrate that BaP-induced immunosuppression in fish proceeds *via* the AhR, but that CYP1A-mediated metabolism of BaP into its toxic metabolites is also necessary. These investigations have also revealed a plausible non-mammalian alternate species for assessing the immunotoxicity of PAHs - critical in light of intense social and political pressure for developing alternative animal models for scientific studies. US Army DAMD 60-1-8109/17-99-9011.

845 ALLERGEN-INDUCED GENE CHANGES IN MURINE LYMPH NODE CELLS.

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Prolonged (13 day) topical exposure of BALB/c strain mice to the contact sensitizer 2, 4-dinitochlorobenzene (DNCB), or to the respiratory allergen trimellitic anhydride (TMA) results in the induction of preferential T helper (Th-) 1 and Th2 patterns of cytokine expression, respectively. In order to characterize gene expression changes occurring in the draining lymph node during the early stages of exposure we have employed a transcript profiling (microarray) approach. Groups of female BALB/c strain mice were exposed topically on the dorsum of both ears to 10% TMA or 1% DNCB, concentrations of these chemicals that are of equivalent immunogenicity with respect to lymphocyte activation. Seventy-two hours following initiation of exposure the draining lymph nodes were excised and mRNA isolated using oligo dT beads. Radiolabelled cDNA probes, generated by reverse transcription of sample mRNA in the presence of 33P ATP, were hybridized to nylon microarray filters comprising 588 known murine genes representing cellular mechanisms such as stress response, transcription factors and cell-cell communication including cytokines and associated receptors (Clontech). Differential gene changes were assessed by phosphorimager and ArrayVision software (Molecular Dynamics). Data were normalized for inter-membrane variation and stringent controls for background and duplicate spot variation were applied to exclude false positives. Changes of 1.5 fold or greater were considered significant. In two independent experiments the majority of genes on the array remained unchanged by exposure to chemical allergen. However, 13 genes were consistently up-regulated following exposure to TMA compared with DNCB and a further 7 genes were more highly expressed in DNCB-treated samples. Of the latter group, increased expression of interferon γ receptor (IFNγR) is consistent with the development of a Th1 profile and may play a role in polarization of the immune response.

846 EXAMINATION OF GENE EXPRESSION CHANGES IN PERIPHERAL BLOOD-DERIVED DENDRITIC CELLS FOLLOWING EXPOSURE TO A CONTACT ALLERGEN.

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An important step in the induction of allergic contact allergy is the activation and subsequent migration of Langerhans cells, the antigen presenting dendritic cells (DC) of the skin. Therefore, a possible approach for the development on an *in vitro* test for skin sensitization is to examine at the cellular and molecular levels the effects of exposure to contact allergens on DC. DC were generated from the adherent cell fraction of peripheral blood mononuclear cells by culture in the presence of granulocyte/macrophage-colony stimulating factor and interleukin-4 for 7 days. The resulting immature DC were treated with either 1 mM or 5 mM dinitrobenzene sulfonic acid (DNBS), the water soluble analog of the strong contact allergen dinitrochlorobenzene, for 24 hours. Total RNA was obtained and changes in gene expression were analyzed using Affymetrix U95Av2 Genechips®. Pairs plots indicated good correlation between the replicates for the treated and untreated groups. In the principal component (PC) analysis, a treatment related effect was clearly observed for the first PC (the component representing the highest source of variance). Comparison of mean signal values from the replicate cultures revealed 185 genes that were significantly different ($p < 0.001$) between DC treated with 1 mM DNBS versus control DC and 1340 significant gene changes between 5mM DNBS treated DC and untreated DC. It is our aim to utilize data achieved through this approach to develop endpoint measures that can be used as the basis of an *in vitro* approach for the identification of skin sensitizing chemicals.

847 DIVERGENT CYTOKINE RESPONSES ELICITED IN MICE BY PEANUT LECTIN AND PURIFIED PROTEIN DERIVATIVE (PPD).

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The majority of food allergies are caused by a limited range of produce, including peanuts. Allergic sensitization is associated usually with high titer IgE antibody, consistent with the preferential induction of a T helper (Th) 2 cell population. Using C57BL/6 strain mice, a strain which displays a Th1 type phenotype in response to PPD (a bacterial protein extract that is associated in humans with a selective type 1 response), we have compared cytokine expression profiles induced following exposure to PPD with those provoked by peanut lectin, a minor allergenic constituent of peanuts. Groups (n=15) of mice were immunized with peanut lectin or PPD by intradermal injection on the dorsum of both ears on days 0 and 7. On day 14, the auricular lymph nodes draining the site of exposure were excised and a single cell suspension prepared for culture in the presence of peanut lectin, PPD, the T cell mitogen concanavalin A (con A) or medium alone. Following 24, 72 and 120 hours in culture, supernatants were harvested and total RNA isolated from the cultured cells. Cytokine gene expression was assessed by ribonuclease protection assay and protein secretion by enzyme-linked immunosorbent assay. Restimulation of peanut-primed cells *in vitro* with peanut lectin, but not with con A, PPD or medium alone, induced the selective expression of the Th2 cytokines interleukin (IL) -4, IL-5 and IL-13, with relatively low levels of interferon (IFN)-γ. In contrast, cells from mice both primed *in vivo* and restimulated *in vitro* with PPD exhibited high level expression of IFN-γ and IL-10. These patterns of mRNA expression were reflected by similar profiles of cytokine protein secretion. These data demonstrate the selective induction of a Th2 response following exposure to an allergenic protein.

848 BONE MARROW-DERIVED DENDRITIC CELLS UTILIZE DIFFERENT INTERNALIZATION PATHWAYS FOR UPTAKE OF THE NATIVE AND RECOMBINANT FORMS OF LACTOFERRIN.

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Lactoferrin (LF, 79.5kDa) is a member of the transferrin family of iron-binding proteins. The native (n) and recombinant (r) forms of human LF, available from breast milk and produced in *Aspergillus*, respectively, exhibit identical amino acid sequences and 3-D X-ray crystallographic structures. However, nLF and rLF differ with respect to their glycosylation patterns and their ability to provoke differential IgG and IgE antibody responses following systemic exposure to BALB/c strain mice. To investigate further the mechanistic basis for the differences observed in immune responses induced by nLF and rLF, we have examined the internalization pathways utilized by bone marrow-derived dendritic cells (BM-DC) for the uptake of LF. BM-DC, isolated from female BALB/c strain mice and harvested on day 8 of culture, were pulsed for 30 minutes at either 37°C or 4°C with various concentrations of nLF or rLF (0.1 to 1000μg/ml). LF uptake was visualized by flow cytometry using an anti-human LF antibody, which recognized both LF species equally when tested by Enzyme Linked Immunosorbent Assay. At all doses of LF examined, BM-DC exposed to rLF expressed 2-5 fold higher levels of internalized protein than did cells treated with nLF. Following pre-treatment of BM-DC with mannose, an inhibitor of the macrophage mannose receptor and other C-type lectins with mannose specificity, the active uptake of rLF at 37°C was inhibited by at least 80%. In contrast, uptake of nLF was compromised only partially (approximately

45%) by competition with mannose. These data suggest that whereas rLF is internalized almost entirely through a mannose-dependent pathway, uptake of nLF occurs *via* both mannose-dependent and independent routes; an observation that may ultimately impact upon the quality of immune response induced.

849 ANALYSIS OF DRAINING LYMPH NODE DENDRITIC CELL ACCUMULATION PROVOKED IN MICE BY CHEMICAL CONTACT AND RESPIRATORY ALLERGENS.

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It has been reported previously that preferential activation of T helper (Th) 1 and Th2 cells is observed following repeated topical exposure of mice to chemical contact and respiratory allergens, and that only those chemicals that provoke respiratory allergy in humans induce IgE antibody responses. Temporal differences in the kinetics of Langerhans cell (LC) migration stimulated by these classes of chemical allergens have been reported recently. To explore further the dendritic cell (DC) response to such chemicals, we have performed kinetic and phenotypic analyses of DC accumulating in the draining lymph node (DLN) of BALB/c strain mice in response to the contact allergen 2, 4-dinitrochlorobenzene (DNCB) or the respiratory allergen trimellitic anhydride (TMA), at doses shown previously to be of equivalent immunogenicity. Excision of DLN 24 hours following exposure revealed increases in DC accumulation, enumerated by flow cytometry on the basis CD11c/Ia expression, that were significantly greater for DNCB ($p<0.05$, n=3) than for TMA; an observation that is in agreement with the delayed kinetics of LC migration observed for TMA. In DLN, DC numbers increased further 48 and 72 hours following exposure to both chemicals, such that values for DNCB- and TMA-treated mice were no longer significantly different. Flow cytometric analyses of lymph node cells revealed no difference between DNCB- and TMA-activated DC with respect to the expression of Ia, CD80, CD86, CD54, or CD8α. Although to date there has been no clear distinction between the phenotypic characteristics of DC isolated from DNCB- and TMA- sensitized mice, it is possible that the early kinetic differences in the accumulation of DC in DLN may ultimately influence the quality of response induced by these chemicals.

850 EVALUATION OF PROTEIN ALLERGENIC POTENTIAL IN MICE : INTER-LABORATORY COMPARISONS.

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There is a growing interest in the development of methods for the evaluation of the allergenic potential of novel proteins. One approach is the measurement of specific IgE antibody production stimulated by systemic (intraperitoneal; ip) exposure of BALB/c strain mice. In the current investigations, inter-laboratory comparisons have been performed of the ability of various food proteins to induce IgE antibody. Female BALB/c strain mice (n = 5) were exposed to 0.1% peanut agglutinin, an allergenic constituent of peanuts, to 2% ovalbumin (OVA), a major allergenic constituent of hens' egg, or to a protein considered to lack significant allergenicity, potato agglutinin (5%). These doses were selected based on previous experience to induce similar levels of immunogenicity (specific IgG). Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay. Two independent experiments were conducted in each laboratory. Administration of peanut agglutinin and OVA each stimulated marked IgE antibody responses in every experiment; titers ranging from 1 in 32 and 1 in 64, and 1 in 8 and 1 in 32, respectively, detected in both laboratories. In contrast, exposure to potato agglutinin failed to induce vigorous IgE production, with no detectable IgE (negative with neat serum) or titers of 1 (positive with neat serum only) recorded. These data demonstrate that the induction of IgE antibody by food proteins of differing allergenic potential is a relatively robust phenomenon and transferable between laboratories. Furthermore, these results suggest that the measurement of antibody (IgE) responses in BALB/c mice may allow discrimination between allergens and those materials that apparently lack allergenicity

851 NONRADIOISOTOPIC MEASUREMENT OF LYMPHOCYTE PROLIFERATION.

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The murine local lymph node assay (LLNA) is a method for the prospective identification of chemical contact allergens. The current validated protocol assesses lymphocyte proliferation induced in the draining lymph node by in situ incorporation of radiolabelled thymidine. We have explored the possibility of using an alternative non-radioisotopic marker of cell division, the membrane-permeant cytoplasmic dye carboxyfluoresein succinimidyl ester (CSFE). When the cells divide, the CSFE-labelled cytoplasmic proteins are distributed equally between the daughter cells, thus the number of divisions each cell has undergone can be tracked. BALB/c strain mice were exposed topically to various concentrations of the contact allergen 2, 4-dinitrochlorobenzene (DNCB) or to the non-sensitizing skin irritant methyl salicylate (MS). Five days later, lymph node cells (LNC) were labelled with CSFE, cultured for 96 h, then incubated with fluorescently labelled anti-CD4 (T helper) and -CD8 (T cytotoxic) cell antibodies and proliferating CD4+ and CD8+ cells analyzed by flow cytometry. In LNC populations derived from vehicle-treated animals, less than 2% of either cell population had undergone one cell division or more. Topical exposure to MS (2.5% to 20%) did not increase the numbers of proliferating cells. Exposure to DNCB, however, resulted in a marked increase in the number of cells undergoing division, with between 5% and 9% of CD4+ cells and 9% to 16% of CD8+ cells proliferating in response to treatment with 0.25% and 0.5% DNCB, respectively. Cells derived from animals exposed to 0.1% DNCB also showed increased cell division in the CD8+ cell fraction. The threshold concentration for induction of proliferation by DNCB in the standard LLNA is approximately 0.05%, indicating that this method is somewhat less sensitive than is in situ incorporation of radiolabelled thymidine. However, these preliminary data suggest that this method may be applied to provide an alternative nonradioisotopic endpoint for the LLNA, particularly for the identification of potent contact allergens.

852 EVALUATION OF PROTEIN ALLERGENIC POTENTIAL IN MICE : DOSE RESPONSES.

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There is a growing need for the development of approaches for the characterization of the allergenic potential of proteins. Although immunogenicity is a common property of foreign proteins, few are significant dietary allergens with the capacity to provoke IgE antibody production and immediate type hypersensitivity responses. We have examined the induction of IgG and IgE antibody expression following systemic (intraperitoneal; ip) exposure of BALB/c strain mice. Mice were exposed to a range of concentrations (0.2% to 10%) of ovalbumin (OVA), a major allergenic constituent of hens' egg, or to the milk allergen bovine serum albumin (BSA), and to materials considered to lack allergenicity; a crude potato protein extract and a purified potato protein, potato agglutinin. Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay and specific IgG antibody measured by enzyme-linked immunosorbant assay. Each of the 4 proteins induced IgG antibody responses at all doses tested, although there was some variation with respect to vigor of IgG responses. Marked differences in the capacity of these proteins to induce IgE responses was observed, with high titer IgE antibody provoked by OVA and BSA over the dose ranges examined whereas the potato proteins stimulated low titer IgE antibody at the highest dose (10%) only. Significant differences in the number of IgE responder animals were observed also. Importantly, differences in IgE antibody production were observed against a background of equivalent immunogenicity (IgG antibody responses). Thus, ip administration of OVA and potato agglutinin (0.5% to 10%) induced equivalent levels of IgG antibody but substantial (and statistically significant) differences in both the number of IgE responder mice and IgE titer were recorded. These data suggest that the measurement of antibody (IgE) responses in BALB/c mice may allow discrimination between allergens and those materials that apparently lack allergenicity

853 THE 90 KDA HEAT SHOCK PROTEIN ASSOCIATES WITH PPARα AND DIFFERENTIALLY REGULATES TRANSCRIPTIONAL ACTIVITY COMPARED WITH PPARβ OR PPARγ

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The peroxisome proliferator-activated receptor α (PPARα), which belongs to the Type II steroid hormone receptor superfamily, may exist in a multimeric complex. One of the possible components of the PPAR complex is the 90 kDa heat shock protein (hsp90). In order to examine whether PPARα is associated with hsp90, liver cytosol from a C57BL/6N mouse was fractionated using a sucrose gradient. Co-migration of PPARα with hsp90 was observed. To further investigate the association between hsp90 and PPARα, hsp90 from C57BL/6N mouse liver cytosol was immunoprecipitated. A concomitant depletion of PPARα in hsp90-depleted mouse liver cytosol was detected. To test whether all three PPAR subtypes can bind to hsp90, a series of hsp90-GST fusion proteins were incubated with *in vitro* translated PPAR subtypes and GST pull-down assays were performed. The results indicate that both *in vitro* translated rat and mouse PPARα bind to the middle domain

of the hsp90, while mPPARβ or mPPARγ bind to a lesser extent. To further investigate the differential binding of PPARα to hsp90, mammalian 2 hybrid assay was conducted. While PPARα demonstrated a strong interaction with hsp90, the association between PPARβ and hsp90 was weak. In this assay, PPARγ was unable to interact with hsp90. In order to test the possible functional involvement of hsp90 in PPAR complexes, both COS-1 and HepG2 cells were co-transfected with PPARα and TPR domain of serine phosphatase 5. Co-transfection of TPR with PPARα resulted in an increased PPRE-driven reporter activity. Transcriptional activity of other isoforms of PPAR is unaffected by TPR co-transfection. The co-transfection of cells with the Hsc70-interacting protein (CHIP), a TPR containing E3 ubiquitin ligase that binds hsp90, increased PPARα transcriptional activity. Taken together, these results suggest that hsp90 is associated with PPARα as a transcriptional repressor. (Supported by NIH ES07799)

854 EVIDENCE THAT PPARα IS COMPLEXED WITH THE 90 KDA HEAT SHOCK PROTEIN AND THE HEPATITIS VIRUS B X-ASSOCIATED PROTEIN 2 (XAP2).

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The perioxisome proliferator-activated receptor α(PPARα) is a ligand inducible transcription factor, which belongs to the nuclear receptor superfamily. PPARα mediates the carcinogenic effects of certain peroxisome proliferators in rodents. In addition, this receptor plays a fundamental role in regulating energy homeostasis *via* control of lipid metabolism. To study the possible role of chaperone proteins in the regulation of PPARα activity, a monoclonal antibody (mAb) was made against PPARα and designated as 3B6/PPAR. The specificity of mAb 3B6/PPAR in recognizing PPARα was tested in immunoprecipitations using *in vitro* translated PPAR subtypes. The mAb 3B6/PPAR recognized PPARα, failed to bind to PPAR β or PPARγ, and is efficient in both immunoprecipitating and visualizing the receptor on protein blots. The immunoprecipitation of PPARα in mouse liver cytosol using mAb 3B6/PPAR has resulted in the detection of two co-immunoprecipitated proteins, which are heat shock protein 90 (hsp90) and the hepatitis B virus X-associated protein 2 (XAP2). Complex formation between XAP2 and PPARα/FLAG was also demonstrated in an *in vitro* translation binding assay. Transient expression of XAP2 co-expressed with PPARα resulted in down regulation of a peroxisome proliferator response element (PPRE)-driven reporter gene activity. In mammalian one-hybrid assays XAP2 inhibited PPARα-driven transcriptional activity in the presence of ligand. Taken together, these results indicate that PPARα is in a complex with hsp90 and XAP2, and XAP2 appears to function as a repressor. This is the first demonstration that PPARα is stably associated with other proteins in tissue extracts and the first nuclear receptor shown to functionally interact with XAP2. (Supported by NIH ES07799)

855 PPARα-DEPENDENT REGULATION OF TUMOR SUPPRESSORS P19 AND C-ABL BY PEROXISOME PROLIFERATORS AND PHORBOL ESTERS.

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Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor responsible for the carcinogenic effects of peroxisome proliferators (PPs) in rodents. In addition to being activated by ligands such as Wy-14, 643, PPARα activity may be affected by phosphorylation events. Wy-14, 643 treatment of the rat hepatoma (FaO) cell line induces changes in the phosphorylation of Protein Kinase C (PKC), MAPK, and other kinases. Also, phorbol esters affect the transcriptional activity of PPARα in reporter assays. Both observations suggest a significant interaction between PKC and PPARα signaling. To investigate this crosstalk, we used liver cell lines generated from wild-type (wt) and PPARα-knockout animals (null). Although both cell types respond normally to phorbol ester (PMA)-induced mitogenesis, only wt cells respond to Wy-14, 643-induced mitogenesis. Gene expression arrays were used to find candidate genes differentially regulated by combinations of PMA and Wy-14, 643 treatment. A tumor suppressor, p19, was expressed constitutively higher in wt cells, but was repressed by Wy treatment only in that cell type. Both Wy and PMA treatment repressed the tumor suppressor c-Abl in wt cells, though neither had substantial effects in the null cells. Quantitative RT-PCR confirmed a substantially higher constitutive expression of c-Abl in wt cells. The regulation of these cell cycle genes in a PPARα-dependent manner helps to explain the carcinogenic effect of PPs in murine model systems. In addition, the fact that PMA regulates some gene expression in a PPARα-dependent manner suggests a significant amount of cross-talk with PKC signaling pathways. (Supported by NIH DK49009).

856 THE ROLE OF LIGAND INDUCED CONFORMATION CHANGES IN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR α COREGULATOR RECRUITMENT.

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The peroxisome proliferator activated receptor α (PPARα) is a member of the nuclear receptor family and regulates genes that are involved in the metabolism of fatty acids in response to peroxisome proliferaters (PPs). In addition, numerous fatty acids and their metabolites are ligands for PPARα. PPs and endogenous ligands for PPARα have a wide variety of effects ranging from carcinogenesis to inhibition of cancer, often in a chemical-specific manner. One possible explanation for this varity of effect is specific conformational changes in the receptor induced by the ligand, similar to what has been observed with estrogen receptor (ER) and selective ER modulators (SERMs). In these studies, examination of these conformation changes was performed using cell culture based and *in vitro* based systems. The results indicate unique conformation changes in response to PPs with differing overall function. The possibility that these conformational changes will effect other aspects of the PP signaling cascade such as recruitment of coregulators is also being examined.

857 TRANS-ACTIVATION OF PXR BY PHTHALATE MONOESTERS.

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Phthalate esters, widely used as plasticizers in the manufacture of products made of polyvinyl chloride, are hepatocarcinogenic and induce reproductive/developmental toxicities in rodents. Recent studies demonstrate high human urinary levels of several phthalates, including monoethyl phthalate, monobenzyl phthalate (MBzP) and monobutyl phthalate (MBuP), raising the question of whether human environmental exposure to these chemicals is associated with adverse health effects. The present study investigates the effects of phthalates on the nuclear pregnane X receptor (PXR). PXR mediates the induction of CYP3A enzymes involved in the hydroxylation of steroids, bile acids and other lipophilic chemicals. PXR is activated by certain endogenous steroids (pregnanes, estrogens), as well as by endocrine disruptor chemicals, which interfere with the synthesis, secretion, transport, binding or elimination of hormones. Recently, several missense mutations in the ligand-binding domain of PXR leading to variant PXR proteins have been identified in human populations, suggesting these variants could contribute to individual differences in responsiveness to PXR-activating chemicals. To assay the effects of phthalate monoesters on PXR-mediated transcription, HepG2 cells were transiently transfected with mouse PXR and with wild-type or variant human PXR in a reporter gene assay. Treatment with mono-2-ethylhexyl phthalate (MEHP) increased the transcriptional activity of both mouse and human PXR with EC_{50} values of 3.6 and 4.4 μM, respectively. MEHP activated the human PXR variant A370T 5-fold, with an EC_{50} value of 2.7 μM. By contrast, the human PXR variant D163G was totally unresponsive to MEHP (500 μM). The transcriptional activity of mouse and human PXR (wild-type and variants A370T and V140M) was also activated 3-fold by MBzP. Thus, PXR-mediated transcription can be stimulated by phthalate monoesters, which could lead to disruption of endocrine function by altering PXR-regulated steroid hormone metabolism, with potential adverse health effects in exposed humans. [Supported in part by NIH/NIEHS grants ES07381 (DJW) and F32 ES11105 (CHH)].

858 EEECT OF CAR AND PXR ACTIVATORS ON TESTOSTERONE CLEARANCE IN CASTRATED MALE SPRAGUE-DAWLEY RATS.

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Cytochromes P450 (CYP) 3A1 and 2B1 are regulated by the nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) and catalyze the hydroxylation of testosterone. CAR and PXR are promiscuous receptors for structurally diverse chemical ligands, including some environmental compounds. Potent induction of CYP 3A1 and 2B1 by the activators of CAR and PXR may affect testosterone homeostasis. The aim of the current study was to test the hypothesis that induction of CYP 3A1 and 2B1 by CAR and PXR activators increases the rate of clearance and alters the metabolism of testosterone. Castrated male Sprague-Dawley rats were exposed to DDE or phenobarbital (PB), CAR and PXR activators, or pregnenolone 16-α carbonitrile (PCN), a PXR activator, for 7 days prior to administration of exogenous C^{14}-labeled testosterone. Urine and feces were collected to determine whether chemical-induced changes in testosterone metabolism were reflected in the rate of testosterone clearance. Overall clearance of

testosterone was determined by the recovery of radioactivity in urine and fecal samples, and testosterone metabolites were analyzed by LC/MS. Exposure to DDE, PB, or PCN did not alter the overall rate of testosterone clearance. However, the amount of unmetabolized testosterone recovered was significantly lower in rats treated with DDE, PB, or PCN than in control rats. Unmetabolized urinary testosterone in DDE-, PB-, and PCN-treated rats was 28%, 38%, and 14% of controls, respectively. PCN exposure resulted in a decrease in radioactivity in the urine and an increase in the feces, suggesting treatment-mediated alteration in phase II conjugation. This study demonstrates a chemical-induced alteration in metabolism of testosterone, not of overall clearance, and suggests a treatment-induced change in the rate of metabolic inactivation of biologically active testosterone.

859 THE E3 UBIQUITIN LIGASE CARBOXYL TERMINUS OF HSC70-INTERACTING PROTEIN (CHIP) CAN MEDIATE HUMAN ARYL HYDROCARBON RECEPTOR PROTEIN TURNOVER.

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Regulation of transcription factor levels relies often on protein degradation mechanisms. This report presents evidence that the human aryl hydrocarbon receptor (hAhR) and the 90KDa heat shock protein (hsp90) levels can be reduced dramatically by transient expression of the E3 ligase carboxyl terminus of hsc70-interacting protein (CHIP) in COS-1 cells. However, CHIP expression had no effect on the co-chaperones p23 and hepatitis B virus X associated protein-2 (XAP2) protein levels. A deletion construct of CHIP lacking its three tandem tetratricopeptide repeats did not affect hAhR levels while a construct lacking its University-box domain had minimal effects on the hAhR. Furthermore, CHIP reduces the amount of hAhR competent for transactivation, as determined in cell-based reporter assays. Immunoprecipitates of myc-tagged CHIP revealed the presence of hAhR and hsp90, suggesting that they associate directly in cells. XAP2 is known to enhance AhR levels when it is transiently expressed in COS-1 cells, while p23 is thought to modulate AhR ligand responsiveness. However, expression of XAP2 and p23 failed to inhibit CHIP-mediated hAhR turnover. In contrast, the heat-shock-protein organizing protein (HOP) was able to counteract the effects of CHIP on the hAhR, suggesting that a balance between HOP and CHIP protein levels may dictate whether the hAhR is degraded or not. Future studies may help determine whether the observed downregulation of the hAhR is in fact due to the ability of CHIP to target the hAhR for destruction directly or a consequential effect of CHIP-induced hsp90 degradation. It remains unknown whether CHIP plays a role in the ligand-induced downregulation of the AhR. (Supported by NIH, ES04869).

860 ESTABLISHMENT AND CHARACTERIZATION OF SIMIAN VIRUS 40 IMMORTALIZED AHR-NULL MOUSE HEPATOCYTES AND THEIR USE TO ASSESS THE ROLE OF THE AH RECEPTOR IN GENE REGULATION.

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor that has been identified as a primary cellular target for 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. There is a need for suitable models to gain insight into the AhR-signaling pathway. One potentially useful cell culture system is the establishment of immortalized Ahr-null hepatocytes from null (Ahr-/-) mice. Hepatocytes from Ahr-null (AhRKO) and wild-type (AhRWT) neonatal mice were immortalized by infecting with Simian virus 40 harboring temperature sensitive large T antigen gene. The AhRKO and AhRWT cell cultures grow at the permissive temperature of 34oC and at 37oC, however, at 39oC cellular growth declines after 24 h. Both AhRKO and AhRWT equally expressed albumin as well as proteins that participate in the AhR pathway such as Ah receptor nuclear translocator, 90 kDa heat-shock protein and hepatitis B virus X-associated protein 2. However, there was a complete absence of the AhR in AhRKO cells. RT-PCR studies revealed induction of CYP1A1 (200-fold) and CYP1B1 (8-fold) mRNA by TCDD in wild-type but not in the Ahr-null cell line. Both cell lines expressed CYP1B1, although the constitutive level in wild-type was higher than the null-cells. AhRKO cells also exhibited high transient transfection efficiency. Therefore utilization of this Ahr-null cell line with restored wild-type and mutant AhR signaling will provide a valuable tool for the elucidation of the mechanisms that underlie AhR mediated gene regulation. In addition, this study establishes the usefulness of immortalization of primary cells with SV40 virus from gene disrupted mice. AhRWT and AhRKO cells are being utilized in DNA microarray experiments to identify genes whose expression is altered due to TCDD-induced AhR signalling as well as the constitutive activity of the AhR.

861 ADRENERGIC RECEPTOR CROSSTALK DURING DEVELOPMENT: ADVERSE EFFECT OF TOCOLYTICS?

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Alpha-2 adrenergic receptors (A2ARs) are overexpressed throughout the fetus and are thought to play a role in cell proliferation. In most tissues, A2ARs decrease with the development of synaptic connections, coincident with the fall in mitotic activity. We evaluated whether the maturation of neural connections is responsible for the ontogenetic decline in A2ARs in the developing liver of newborn rats. Animals were sympathectomized on postnatal day (PN) 1 with 6-hydroxydopamine; A2AR expression (binding of 3H-rauwolscine) was decreased on PN6 but elevated on PN15. These results indicated that neural stimulation modulates the ontogenetic expression of A2ARs, with a positive trophic relationship early in development, followed by suppression of A2ARs once innervation becomes fully functional. The developing liver also contains high concentrations of beta-2 ARs (B2ARs), so we next determined if these receptors participated in the trophic effect. Animals were treated with terbutaline, a B2AR agonist that is commonly used to arrest preterm labor, using a regimen (10 mg/kg s.c. daily for four days) that produces biologic effects similar to those seen when used for maternal tocolysis. Terbutaline given on PN2-5 produced a significant decrement in A2AR receptors, and similar deficits were obtained with treatment on PN11-14, the period of rapid onset of sympathetic function. Isoproterenol, a mixed B1/B2-agonist, also elicited a decrease in hepatic A2ARs. Given the overexpression of A2ARs in many fetal or neonatal tissues, and their relationship to cell proliferation, the effects seen here may contribute to adverse effects of tocolytics. (Supported by NIH HD09713)

862 ADULT TO CHILD EXTRAPOLATION OF THE PHARMACOKINETICS OF ANAESTHETICS USING AGE-SPECIFIC PHYSIOLOGICAL MODELS.

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The extrapolation of exposure concentrations from adults to children is a challenge to health risk assessors dealing with environmental contaminants. In this context, the data available and lessons learnt regarding the inhalation pharmacokinetics of anaesthetics might be useful. These data can be used to improve our understanding of adult-child extrapolation of exposure concentrations. The objectives of the present study were to develop a physiologically-based pharmacokinetic (PBPK) model for nitrous oxide and halothane in adults, and to apply this model for predicting the kinetics of these anaesthetics in children using age-specific physiological parameters. The PBPK model used in this study contained age-specific values of alveolar ventilation rate, cardiac output, tissue blood flow rates and tissue volumes, and facilitated the simulation of the ratio of exhaled to inhaled quantity (QE/QI) of anaesthetics during exposure. The PBPK model simulations in adults were consistent with the experimental data, which suggested a QE/QI value of 0.55 for halothane and 0.94 for nitrous oxide. Assuming the tissue:blood and blood:air partition coefficients to be the same in adults and children, the simulations for children (halothane: 2 months old; nitrous oxide: 3 years old) were obtained. The PBPK model simulations and experimental data indicate that, following a 20-min exposure to 5000 ppm, the QE/QI value in children is 0.75 for halothane and 0.96 for nitrous oxide. In general, for the anaesthetics investigated in this study, the QE/QI approached 1 in children and the adult-child difference in QE/QI could be explained by adult-child differences in alveolar ventilation rate and liver blood flow rate. These results suggest that the adult-child extrapolation factor for poorly metabolized anaesthetics and atmospheric contaminants is likely to be within a factor of two.

863 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF 1-PROPANOL (CAS # 71-23-8) IN HUMANS AND RATS.

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Physiologically based pharmacokinetic (PBPK) modeling is potentially a useful tool in minimization of animal experimentation mandated under various regulatory initiatives. A method that holds promise is the development of a "metabolic series approach" for developing toxicological information on compounds linked *via* steps in a metabolic pathway. Our effort to describe pharmacokinetic data for propyl series of compounds (propyl acetate, 1-propanol, propionaldehyde, and propionic acid)

in rats and humans has focused on 1-propanol. The existing human data consist of blood time courses of 1-propanol following iv injection in the presence and absence of ethanol, and for coingestion of 1-propanol and ethanol. Blood:air and tissue:air partition coefficients of 1-propanol are also available from the literature. These data were adequate for development, parameter fitting, and validation of a model for 1-propanol by the oral route or iv injection. The model structure includes metabolism of 1-propanol in the liver and in perfused tissues other than fat and urinary filtration of 1-propanol. The model predictions of the human data are generally very good to excellent. Sensitivity analysis indicates that the data are adequate to determine metabolic parameters. Preliminary models of 1-propanol inhalation and ingestion were developed, permitting sensitivity analysis and application of the rat model in experimental design.

864 MODELING THE INFLUENCE OF ALCOHOL DEHYDROGENASE GENETIC POLYMORPHISMS IN ETHANOL DISPOSITION IN HUMANS.

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The family of cytosolic enzymes known as alcohol dehydrogenase (EC 1.1.1.1) catalyze the reversible oxidation of ethanol to acetaldehyde, with corresponding reduction of NAD^+ to NADH. Alcohol dehydrogenase in humans exists as multiple forms, including class and isozyme multiplicity, as well as allelic variations within several different isozyme forms. In the current study a continuous system model based on the Theorell-Chance mechanism for each isoform of alcohol dehydrogenase was incorporated into a physiologically based pharmacokinetic model for ethanol in humans. Models for the C_1 and C_2 homodimers, and the B_1 homodimer included the previously reported negative cooperativity and substrate inhibition, respectively, while heterodimers were modeled as the sum of the activity of the respective monomers. Association of dimers from monomers was assumed to occur randomly, and expression of various isoforms within a specific genotype were assumed to be equal. Simulations indicated that alcohol dehydrogenase genotype contributed significantly to the overall disposition of ethanol. For example, at an ethanol dose of 591 mg/kg, the elimination rate constant for ethanol was found to be 1.4275 mmol/L/h for a genotype of $B_3B_3C_1C_1$, while the same constant for the genotype $B_3B_3C_2C_2$ was 7.7803 mmol/L/h. These studies suggest that metabolic differences in alcohol dehydrogenase genetic polymorphisms are important contributors to the variability of ethanol disposition in a population, and that such variability can be described with biologically based models useful for risk assessment. (Supported by a grant from The American Chemistry Council).

865 ESTIMATING INTERINDIVIDUAL VARIATION IN PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING PARAMETERS FOR DICHLOROMETHANE METABOLISM IN HUMAN VOLUNTEERS.

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The toxicokinetics of dichloromethane (DCM) are well understood in both laboratory animals and humans. DCM is metabolized by two enzyme systems: 1) mixed function oxidases, which follow Michaelis-Menten kinetics (Vmax, Km); and 2) glutathione-S-transferases, which can be described with a first-order rate constant (KFC). The latter pathway has been implicated as a key determinant of cancer risk in animals. This work includes a more detailed analysis of some previously published human kinetics data for DCM (DiVincenzo and Kaplan). In this study, a group of 14 volunteers (11 men, 3 women) were exposed to 1 of 4 concentrations of DCM (50, 100, 150, or 200 ppm) for 7.5 hours. Individual time-course data were collected for up to 40 hours post exposure for the following: 1) DCM concentrations in blood; 2) DCM concentrations in exhaled breath; 3) carboxyhemoglobin levels in blood; and 4) and carbon monoxide concentrations in exhaled breath. Variation in the tissue and exhaled breath concentrations was relatively small at the end of the exposure period (CV=0.02-0.44). Predictions for the time-course data were made using a validated PBPK model for DCM in humans. Individual values for body weight were used. Volume of adipose tissue was estimated based upon a consideration of age, height, and sex of the individual. Optimal fits for model predictions were obtained by adjusting Vmax and KFC for each individual, while all other values were held constant. Parameter distributions for Vmax and KFC were derived from the optimized parameter values generated from the individual data sets. The resulting distributions were compared graphically to several alternative distributions, including distributions from 1) OSHA, used in establishing a permissible exposure limit for DCM; 2) an expert elicitation effort; and 3) the published literature. The potential impact of these data on human health risk assessment for DCM is discussed.

866 PBPK MODEL ANALYSIS OF INTRATHYROIDAL IODIDE TRANSPORT IN HUMANS.

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An improved physiologically-based pharmacokinetic (PBPK) model for perchlorate (ClO4-) -induced inhibition of iodide (I-) uptake in adult humans was used to explore the influence of endogenous iodide on intersubject variability in radioiodide uptake measurements (RAIU) in the thyroid. The improved model thyroid includes four subcompartments for the stroma, follicle, colloid and bound iodide (ie., thyroid hormones). Both passive diffusion and active uptake were described between the stroma and follicular epithelium and between the follicular epithelium and colloid. First order rate constants, estimated from the literature, were used to simulate iodide organification and its secretion (thyroid hormones) to systemic circulation. Follicular maximum capacities, Vmax(s), were estimated from visual fits of thyroid RAIU measurements in human volunteers (Greer et al., 2002) and varied across subjects over an order of magnitude. However Vmax(s) did not vary within subjects (between RAIUs taken pre and post ClO4- exposure). Several factors are known to affect Vmax. Thyroid stimulating hormone (TSH), released during low levels of circulating thyroid hormone, increases sodium iodide symporters (NIS), thereby increasing the Vmax. Dietary iodide also affects Vmax, by altering intrathyroid levels of inorganic iodide. All subjects used had normal functioning thyroids and TSH levels (1.99+/-0.88 uU/mL). Even during two weeks of low perchlorate exposure (0.007 to 0.5 mg/kg/d), there was no observed effect on individual TSH and Vmax values. Therefore, dietary iodide was suspected to be the major contributor to individual variability in Vmax(s). Because alimentary restrictions were not implemented in the study, dietary iodide was estimated from predictions of endogenous serum and urinary iodine measurements. An inverse relationship was found between Vmax and both intrathyroidal iodide levels and dietary iodide.

867 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF SPECIES-SPECIFIC EFFECTS OF PLASMA BINDING OF TRICHLOROACETIC ACID FROM TRICHLOROETHYLENE IN MICE, RATS, AND HUMANS.

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Recently, PBPK models have been used in the assessment of liver cancer risk from trichloroethylene (TCE) exposure, particularly to aid in the extrapolation of rodent bioassay data to human risk at environmental levels. The models were used to determine a range of human risks based on equivalent mouse exposures, using total plasma and liver area under the curve (AUC) for the metabolites trichloroacetic acid (TCA) and dichloroacetic acid (DCA) as dose metrics. Previous studies showed a significant difference in the ability of human and rodent plasma to bind TCA. From these results, we developed a PBPK model for TCE incorporating the plasma binding of TCA in mice, rats, and humans. This model included a Michaelis Menton expression within the blood compartment to determine free concentration of TCA in terms of total concentration. The expression for binding was governed by the parameters P (plasma albumin concentration), N (number of binding sites per protein molecule), and Kd (binding equilibrium dissociation constant). Species-specific physiological and metabolic parameters were used in the PBPK model. The proportion of free TCA in blood available for uptake into tissues was much less in humans than in rats or mice. Binding of TCA to proteins in human plasma dramatically affected the renal elimination rate compared to the rodents, explaining the long residence time of TCA in human blood. Our findings indicate that free TCA, as opposed to total TCA in the blood, is a more appropriate dose metric for extrapolation of TCE dosimetry from rodents to humans. Our model provides a quantitative framework to examine tissue uptake of free TCA over a wide range of TCE exposures. (This research was supported by DOE Cooperative Agreement # DE-FC02-02CH11109).

868 DEVELOPMENT OF A PBPK MODEL FOR PROPYLENE GLYCOL MONOMETHYL ETHER AND ITS ACETATE FOR RATS, MICE AND HUMANS.

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1-Methoxy-2-propanol (propylene glycol monomethyl ether, PGME) is the most widely used propylene glycol ether solvent. Commercial PGME consists of >99% 1-methoxy-2-propanol with less than 1% 2-methoxy-1-propanol. The major isomer of PGME is metabolized to either glucuronide or sulphate conjugates (minor) or propylene glycol (major). The major toxic effects of PGME in animals studies

includes sedation, very slight alpha 2u globulin-based nephropathy (male rats only) and hepatomegally at high exposures (typically >1000 ppm). Sedation in animal studies usually resolves within a few exposures to 3000 ppm (the highest concentration used in subchronic and chronic inhalation studies) due to the induction of metabolizing enzymes. Partition coefficients were determined and data from a variety of pharmacokinetic and mechanistic studies were incorporated into a PBPK model for PGME and its acetate in rats, mice and humans. Published controlled exposure and workplace biomonitoring studies were included for comparisons of internal dosimetry between laboratory animals and humans. PGME Acetate has a very short half-life (hydrolyzed to PGME) in humans (~36 min in blood and liver homogenates) and even shorter in rats (~15 min in blood and 30 min in liver homogenates). Thus, very little PGME Acetate is found *in vivo*. Regardless of the source for PGME (either PGME or its acetate), rats were predicted to have a higher Cmax and AUC for PGME in blood than humans, especially at concentrations >100 ppm. This would indicate that the major systemic effects of PGME would be expected to be less severe in humans than rats at comparable exposures.

869 COMPARISON OF THE DERMAL AND INHALATION ROUTES OF EXPOSURE ON THE ABSORPTION OF TOLUENE IN HUMAN VOLUNTEERS.

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Toluene can be present as a contaminant in both well and surface water. An assessment of the dermal contribution to total toluene uptake is useful for understanding human exposures. To evaluate the significance of these exposures, the dermal absorption of toluene was assessed in human volunteers using exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Human volunteers were submerged in warm tap water to neck level in a stainless steel hydrotherapy tub containing an initial concentration of 0.5 mg/L toluene. Volunteers were provided breathing air to eliminate inhalation exposures, and exhaled breath was continually analyzed before, during, and post exposure to track the absorption of toluene in real time. A PBPK model was used to estimate the dermal permeability coefficient (Kp) to describe the exhaled breath data from n=6 volunteers. An average Kp value of 0.012 +/- 0.007 cm/hr was found to provide a good fit to all data sets. Volunteers also participated in a second study, in which the subject was allowed to breathe the room air during immersion, thus both dermal and inhalation exposures occurred. Exhaled breath analyses revealed that concurrent inhalation of volatilized toluene resulted in a transient 50% increase in peak exhaled breath levels compared to those observed in dermal only studies. For perspective, the total intake of toluene associated with oral consumption of 2 liters of water containing toluene at bath water concentrations were estimated to be more than 30 times greater than the dermal contribution due to bathing. (Supported by NIEHS grant 1-P42-ES10338-01).

870 INCORPORATION OF AGE-, GENDER-, AND SPECIES-SPECIFIC DATA ON THE METABOLISM, PROTEIN BINDING AND RENAL CLEARANCE OF 2-BUTOXYETHANOL AND ITS METABOLITE, BUTOXYACETIC ACID, INTO A PBPK MODEL.

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2-Butoxyethanol (BE) is the most widely used glycol ether solvent. BE's major metabolite, butoxyacetic acid (BAA), causes hemolysis with significant species differences in sensitivity. Several PBPK models have been developed over the past two decades to describe the disposition of BE and BAA in male rats and humans to refine health risk assessments. More recent efforts by Lee et al. (1999) to model the kinetics of BE in male and female F344 and B6C3F1 mice from the NTP chronic inhalation studies required the use of several assumptions to extrapolate model parameters from earlier PBPK models developed for young male rats. To replace these assumptions, a series of studies were conducted to determine the impact of age, gender and species on the metabolism of BE, and the tissue partitioning, renal acid transport and plasma protein binding of BAA. The Lee et al. PBPK model was updated and expanded to include metabolism of BAA by mice and humans and the salivary excretion of BE and BAA (mice) which may contribute to the forestomach irritation observed in the NTP study. The revised model was validated against several recent inhalation, oral gavage and intraperitoneal injection kinetic studies in rats and mice as well as the NTP chronic inhalation study. Peak blood concentrations of BAA have been used in several human health risk assessments that focused upon hemolysis. The revised model predicted that peak blood concentrations of BAA are greatest in female rats followed by female mice, male mice, male rats then humans at 6-hr inhalation exposure concentrations exceeding 100 ppm. At lower concentrations (i.e. <50 ppm), mice are predicted to have the lowest peak blood concentrations of BAA. The revised model serves as a quantitative tool for integrating an extensive pharmacokinetic and mechanistic database into a format that can readily be used to compare internal dosimetry across dose, route of exposure and species.

871 DEVELOPMENT OF A PRELIMINARY PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR 1, 2-DIETHYLBENZENE IN THE F344 RAT.

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1, 2-Diethylbenzene (1, 2-DEB) s a moderately volatile, colorless liquid used as an intermediate in the production of divinylbenzene, as a heat transfer fluid, and is found in gasoline, kerosene, and fuel oils. Environmental release of 1, 2-DEB may occur at sites where it is produced or used; 1, 2-DEB is listed as a contaminant found in drinking water supplies in some US cities. The most probable route of human exposure to 1, 2-DEB is by inhalation or dermal contact, mainly in occupational settings where the commercial grade mixture is used as a solvent. To better understand the kinetics of 1, 2-DEB, a preliminary physiologically based pharmacokinetic (PBPK) model to describe the absorption, distribution, metabolism and elimination of 1, 2-DEB in rats was developed. Partition coefficients were experimentally determined in rat tissues and blood samples using an *in vitro* vial equilibration technique. Both saturable Michaelis-Menten metabolic rate constants Km (affinity) and Vmax (capacity), and a first-order (Kfo) metabolic constant were derived from the optimization of a series of *in vivo* gas uptake curves conducted at various initial chamber concentrations. Pretreatment with pyrazole, an inhibitor of oxidative microsomal metabolism, did not impact the slope of the gas uptake curve, indicating that metabolism was not affected. The completed PBPK model was evaluated against real-time exhaled breath data collected from rats receiving an intraperitoneal (IP) injection of 1, 2-DEB. Exhaled breath profiles from animals treated with 1, 2-DEB by IP injection were evaluated and the absorption rate (Ka) determined using the PBPK model. Development of a PBPK model for 1, 2-DEB in rats is the first stop toward future extrapolations to understand the kinetics in humans. (Supported by NIEHS grant 1-P42-ES10338-01).

872 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THE STUDY OF 2-METHYLIMIDAZOLE KINETICS.

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In support of the National Toxicology Program (NTP) bioassay for 2-Methylimidazole (2-MI), a model was developed to enhance the understanding of 2-MI kinetics. Earlier studies have identified thyroid as a possible target for 2-MI so the model included diffusion-limited delivery of 2-MI to a thyroid compartment, as well as diffusion-limited compartments representing muscle, skin, adipose, brain, liver, GI, kidney, and other aggregated tissues. The model included the administration of 2-MI orally and by IV. Elimination of 2-MI was from the feces, urine, or metabolism in the liver. Parameters not available from the literature were estimated with maximum likelihood methods by fitting the model predictions to data. Published data included 2-MI concentrations over time in tissues (adipose, brain, kidney, liver, muscle, skin, thyroid, venous blood) following oral and IV administration to male F344 rats, while the NTP toxicokinetic data included blood concentrations over time for IV and oral administration to F344 rats and B6C3F1 mice of both sexes. The model was able to adequately simulate the data across administration routes, species, and sexes. The model was used to evaluate, with formal statistical tests, differences in absorption, metabolism, and elimination rates between male and female F344 rats and B6C3F1 mice. These data and PBPK model permit a better mechanistic understanding of the relationship between exposure and target tissue dosimetry that will enhance the risk assessment process for 2-MI.

873 A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR GAVAGE AND I.V. ADMINISTRATION OF METHYLEUGENOL IN F344/N RATS AND B6C3F1 MICE.

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Methyleugenol is a flavoring and perfuming agent naturally found in nutmeg, mace, and foods and spices. Due to the structural similarity of methyleugenol to other known carcinogens, including safrole, isosafrole, and estragole, it has been tested for toxicity and carcinogenicity by the National Toxicology Program (NTP).

It was concluded that methyleugenol is toxic and carcinogenic in male and female rats and mice. As part of this toxicity testing, the NTP conducted single-dose intravenous and oral gavage toxicokinetic studies of methyleugenol in male and female rats and mice. A diffusion limited physiologically based pharmacokinetic (PBPK) model mathematically representing the absorption, distribution, metabolism, and elimination of methyleugenol in rats and mice was developed to quantitatively describe the process involved in methyleugenol toxicokinetics. The differential equations used in this model describe mass transfer of methyleugenol between tissue compartments and have parameters representing physiological quantities and chemical-specific parameters. The physiological parameters were chosen from literature values. The chemical-specific parameters were estimated from the plasma concentration time course data following intravenous and gavage administration of methyleugenol to the rodents. Absorption rates, metabolism rates, and permeability constants were estimated separately for rats and mice. Likelihood-based statistical methods were used to test hypotheses regarding the structure of the model and similarities across species. The analysis demonstrates that absorption of methyleugenol in rats and mice is rapid and complete, that the distribution of methyleugenol to tissues is not hindered by capillary permeability, that metabolism of methyleugenol is saturable, and that an extrahepatic site for metabolism is needed for both species.

874 UTILIZATION OF A PBPK MODEL TO PREDICT THE DISTRIBUTION OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN HUMANS DURING CRITICAL WINDOWS OF DEVELOPMENT.

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TCDD is a ubiquitous environmental contaminant that induces a wide spectrum of toxic responses, including developmental toxicity. Some of these developmental effects have different critical windows of sensitivity. The utilization of a PBPK model to predict maternal to fetal transfer of TCDD can be important for improving human health risk assessments for the developmental effects of TCDD. While, PBPK models for TCDD have been published for different species including, rat, mice, fish and humans, no PBPK models have described the distribution of TCDD during pregnancy. The aim of this work was the development of a PBPK model to predict the distribution and accumulation of TCDD between maternal and fetal compartments in humans. Previously validated in the rat, this model consisted of 4 maternal compartments (liver, fat, placenta and, rest of the body) and 1 fetal compartment corresponding to the whole fetus. The model described Ah receptor binding, CYP1A2 induction and binding, and physiological alterations occurring during gestation. The model assumed the distribution of TCDD was diffusion limited for fat and liver. This model focused on oral exposure because dietary exposures represented over 95% of the daily intake of TCDD. Different exposure scenarios were examined including chronic exposure and chronic exposure with intermittent periods of high exposures (i.e. meals containing higher than average concentration of TCDD). This model assumed a background exposure prior to the gestation period. Initial testing of this model provided reasonable fits to human exposure data. This model may be a useful tool for use in risk assessments as well as for understanding basic pharmacokinetic and pharmacodynamic processes during development. Acknowledgments: This project was funded by in part by a cooperative agreement (CR 828790) with NRC, NAS and performed at USEPA Research Triangle Park, NC, USA. (This abstract does not represent USEPA policy).

875 IMPROVED PBPK LACTATION/NEONATAL MODEL FOR PERCHLORATE-INDUCED INHIBITION OF ENDOGENOUS AND RADIOIODIDE UPTAKE IN THE RAT.

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Ongoing modeling efforts have resulted in predictive physiologically based pharmacokinetic (PBPK) models for perchlorate and radioiodide kinetics in the male, pregnant, lactating, fetal and neonatal rat, as well as in the adult human. The models successfully predict acute radioiodide control and inhibition data, and both acute and sub-chronic perchlorate data at several dose levels, and can be used to quantitatively determine estimates of both inter-species and intra-species (age, gender, perinatal) variability. However, to make these rat models more relevant to humans, they were expanded to simultaneously describe the kinetics of endogenous (dietary) iodinde and its incorporation into hormones (thyroxine, triiodothyronine, etc.), together with its effect on perchlorate-induced inhibition. Thyroid and serum compartments are divided into two sub-compartments describing incorporated iodine, which comprises 93% and 13% of the total iodine, respectively, and free iodide. Published total iodine levels were accurately simulated by the model in the maternal and neonatal gastro-intestinal tract and contents, skin, serum, and the maternal mammary gland and milk. By incorporating dietary and hormonal iodide, the models are able to address the physiological changes during development (e.g. increased serum binding) and environmental factors (e.g. iodine deficiency or iodine/hormone supplementation), in addition to the previously described susceptibility of the neonate to inhibition of iodide.

876 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF GENISTEIN IN RATS.

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Phytoestrogens are a class of endocrine-active compounds that occur naturally in some food crops, particularly soy products, with genistein being a significant component of these. High concentrations of genistein occur in tofu and soy infant formula. Genistein is associated with a range of effects from chemoprevention of breast and prostate cancer, cardiovascular disease, and postmenopausal ailments to observed reductions in weight and anogenital distances of rodents at birth. To estimate circulating levels of genistein during experimental health-effects studies, PBPK models for genistein in rats are being developed. A preliminary model was previously developed using published data, although those data rarely distinguished parent genistein from its glucuronide and other conjugates. Measurements of parent genistein and total radiolabel activity (including conjugates) in plasma have now been generated for rats, along with total radiolabel activity in a wide range of tissues. Binding of genistein and its conjugates to rat plasma protein has also been measured. The model is now being updated accordingly. However, the model did not fit the recently obtained data when using parameter values previously estimated for the same rat strain. Data from the literature shows that genistein undergoes enterohepatic circulation, but inclusion of this process without direct observation of biliary excretion rates also creates significant challenges in parameter identification. Despite those challenges, we concluded that a good description of genistein kinetics required two changes from the standard, perfusion-limited PBPK structure: description of transport through the gastrointestinal tissues as diffusion-limited and inclusion of binding of genistein to serum proteins. (Research supported in part by the American Chemistry Council and the Food Standards Agency, UK.)

877 INTERSPECIES PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF GENISTEIN.

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The evaluation of potential human health benefits from genistein, the principle soy isoflavone, often requires initial studies using experimental animals. Of particular value are metabolism and disposition studies that can permit extrapolation of animal blood and tissue measurements to human studies where only blood data are available. Genistein pharmacokinetic data obtained from laboratory animal experiments as well as literature reports of human exposures were simulated with a unique general purpose physiologically based pharmacokinetic (PBPK) model that was capable of simultaneously fitting data for both the active aglycone and inactive conjugated (primarily the glucuronide) forms. Single and multi-dose oral exposure as well as iv and ip injections were simulated using data obtained from mouse, rat, and human experiments. Metabolism and concurrent enterohepatic recirculation complicated the kinetics and prolonged the elimination of genistein in all three species. The interconversion between the aglycone and conjugate required the two PBPK models to be bidirectionally linked. Statistical and visually satisfactory fits were obtained for 10 sets of mouse data, four sets of rat data, and 19 sets of human data. Despite the extensive conjugation of circulating genistein, tissue levels of the aglycone after multi-dosing attained concentrations in all species that have been demonstrated to activate estrogen receptors α and β. This estrogen agonist activity is the likely basis for many of the putative beneficial effects and potential adverse effects of genistein and soy consumption

878 AGE-RELATED DIFFERENCES IN HEART RATE, BUT NOT BODY TEMPERATURE IN RATS PERFORMING OPERANT TASKS AT EQUIVALENT TRIAL RATES IN AIR AND WHILE INHALING TOLUENE.

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A physiologically-based pharmacokinetic (PBPK) model is being developed to estimate the dosimetry of inhaled toluene in rats under experimental conditions requiring varying degrees of physical activity. Heart rate was used as a surrogate index

to adjust the model input parameters for cardiac output and ventilation rate in active rats. Telemetered heart rate (HR) and core body temperature (Tco) were collected during performance of two operant tasks in young adult (7-8 mo, n=12) and old rats (20 mo, n=6) trained either on a signal detection task or a simple lever pressing task. HR and Tco were determined in 1-hr tests at rest and while performing the task at 4, 7, 10 and 13 trials per minute (tpm). HR increased by ~10% above sedentary rates at all trial rates in the old rats, whereas the HR of young rats increased from ~15% to 25% above sedentary rates, as a direct function of trial rate. Tco rose by 1.0 deg C during the first 10 min of performing the tasks in young and old rats under all conditions. Young rats were also exposed to toluene (0 and 2000 ppm) while performing either task at 5 and 10 tpm. Toluene elevated HR and attenuated the increase in Tco during performance of the tasks at both trial rates. These data indicate that activity-dependent increases in HR appear to be dampened in aged rats, but changes in Tco are not. Changes in activity level during exposure to volatile organic chemicals should be accounted for in the dosimetry of the chemical; in addition, the physiological effects of the chemical itself may impact its dosimetry. (This abstract does not necessarily reflect EPA policy.)

879 A PHYSIOLOGICAL PHARMACOKINETIC MODEL BASED ON CLEARANCE AND VOLUME OF DISTRIBUTION.

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Clearance terms have been applied to describe certain physiological functions of organs such as kidney and liver. These organs remove chemical from the blood stream by metabolism or filtration. Clearance (CL) of chemicals in systemic circulation is expressed in units of blood volume per unit time. Volume of distribution (Vd), expressed in L blood, represents the apparent volume in which the chemical is distributed. Classical compartmental models frequently describe the pharmacokinetics of chemicals using Vd, CL and an elimination constant calculated as CL/Vd. These parameters are not directly used in physiologically-based pharmacokinetic (PBPK) descriptions. The aim of this study was to recast a conventional PBPK model in terms of clearances and effective volumes of distribution in tissues. In this study, the rate of change in the amount of chemical in tissues was computed as the difference between the input to tissue per unit time (mg/hr) and the product of the amount in tissue (mg) and the elimination constant (/hr). Whereas chemical input to tissue was calculated as influx clearance (L blood/hr) x arterial blood concentration (mg/L blood), the elimination constant was calculated as the ratio of tissue clearance (L blood/hr) to the effective volume of tissue distribution (L blood). Tissue clearance corresponded to the blood flow rate of tissues except liver for which intrinsic metabolic clearance capacity was also taken into account. In implementing the above modeling approach, there was no need to calculate the venous blood concentration exiting tissues (Cvt) for solving the mass balance differential equations. Further, the basic form of equations used in this study resembles that used in non-physiological pharmacokinetic models. The PBPK model based on CL and Vd was validated by simulating blood concentration of styrene following a 6-hr inhalation exposure to 80 ppm or 600 ppm. The simulations obtained were identical to that of the conventional PBPK model. The present study facilitates a better understanding of CL and Vd concepts as they relate to PBPK models.

880 A NOVEL APPROACH FOR PBPK MODELING OF METABOLIC INTERACTIONS IN CHEMICAL MIXTURES.

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Changes in tissue dose and toxicity during mixed exposures frequently occur due to metabolic interactions, which alter the hepatic extraction ratio (E) of mixture components. If the change in E during mixed exposure is known, then change in tissue dose of each component in a mixture can be predicted. The aim of this study was to develop an approach for calculating the change in E to facilitate the prediction of altered pharmacokinetics and tissue dose of chemicals during mixed exposures. The methodology involved the calculation of E based on the total exposure dose and pharmacokinetic equivalence of mixture components. The E value was then specified in an individual chemical PBPK model to simulate pharmacokinetics and tissue dose during mixed exposure. The proposed methodology was applied to model a mixture of three hydrocarbons, namely, toluene (TOL), m-xylene (XYL) and ethylbenzene (EBZ). The change in E value of these chemicals during mixed exposure to 100 ppm each was quantified by considering the mixture as the combination of different doses of a single chemical (on the basis of pharmacokinetic equivalence). In this study, the mixture was expressed in terms of TOL and TOL-equivalent doses of XYL and EBZ in order to determine the E value of TOL in the mixture. This method was then repeated for all components of the mixture. The E value of TOL, XYL and EBZ decreased between individual and mixed exposures from 0.30 to 0.05, 0.28 to 0.04 and 0.14 to 0.04, respectively. The calculated E values were identical to those obtained using an interaction-based PBPK model. Further, the use of these E values in individual chemical PBPK model provided simulations that were identical to those obtained using a mixture PBPK model. These results suggest that, when metabolic inhibition is the interaction mechanism, the change in E and pharmacokinetics of chemicals occurring during mixed exposures can be predicted with the knowledge of the total dose of the mixture and pharmacokinetic equivalence of components.

881 LOW-DOSE VALIDATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR TRICHLOROETHYLENE IN THE RAT.

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Trichloroethylene (TCE) is a volatile liquid used as a degreasing agent and is a common environmental pollutant. In 2001, the EPA published a draft risk assessment for TCE that incorporates dosimetry predictions of physiologically based pharmacokinetic (PBPK) models. The current modeling effort represents a lower dose validation of a PBPK model used for dosimetry predictions in the draft risk assessment. The pharmacokinetics of TCE in male Sprague-Dawley (SD) rats were characterized: (a) during inhalation exposure to 50 ppm TCE, (b) following gavage administration of 8 mg/kg TCE; and (c) following intraarterial injection of 8 mg/kg TCE. Blood and tissues (including liver, kidney, fat, muscle, heart, spleen, GI tract, brain) were collected at selected time-points from 5 minutes up to 24 hours post initial exposure. A PBPK model for TCE in rats had been developed previously (Fisher et al., 1991). Strain-specific physiological parameter values for the SD rat were used in the current work. Metabolism rates have been estimated from gas uptake studies in the Fisher-344 (F-344) (Andersen et al., 1987) and Long Evans (LE) (Simmons et al., 2002) rats, resulting in estimates of Vmaxc of 11.0 and 7.34 mg/hr/kg, respectively. The inhalation, gavage and intraarterial time-course data were better described by the LE rat specific value than the F-344 value. Blood and muscle concentrations were adequately predicted by the model. Concentrations of TCE in the fat were modestly overpredicted by the model, while concentrations in the liver were slightly underpredicted for inhalation, gavage and intraarterial exposures. To date, PBPK model validation for TCE in rat tissues has not been performed at inhalation doses lower than 200 ppm. This low dose validation increases our confidence about the use of the rat PBPK model for extrapolation to environmentally relevant doses. (Supported by DOE Coop. Agreement DE-FC02-02CH11109)

882 PBPK MODELING OF THE METABOLIC INTERACTIONS OF CARBON TETRACHLORIDE AND TETRACHLOROETHYLENE IN B6C3F1 MICE.

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Potential exists for widespread human exposure to low levels of carbon tetrachloride (CT) and tetrachloroethylene (TET). Both of these chlorinated hydrocarbons are metabolized by the cytochrome P450 system. The objective of this research was to examine the metabolic interactions between CT and TET using a physiologically based pharmacokinetic (PBPK) model in male B6C3F1 mice. CT was assumed to inhibit its own metabolism (suicide inhibition) directly by the formation of metabolically derived free radicals (CCl3 and OOCCl3) and indirectly by the formation of free radical products from lipid peroxidation. A CT PBPK mouse model was configured to describe suicide inhibition and competitive inhibition with TET. A TET model was configured to describe the metabolic formation of trichloroacetic acid (TCA), a cytochrome P450 mediated metabolite, with metabolism altered by competitive inhibition with CT and loss in metabolic capacity by CT induced suicide inhibition. Metabolic constants for CT were 1.0 mg/kg/hr for Vmaxc and 0.3 for Km (mg/L) and for TET (based in TCA production), 6.0 mg/kg/hr and 3.0 mg/L, respectively. The rate of enzyme loss (suicide inhibition, Vmaxloss) was describe as: Vmaxloss (mg/hr) = -KD * (RAM*RAM), where KD (hr/kg) is a second order rate constant, and RAM (mg/hr) is the Michaelis Menten description of the rate of metabolism of CT. CT caused dose-related depletion in available metabolic capacity for conversion of TET to TCA in animals orally dosed with 1 to 100 mg/kg CT followed one hour later by an oral bolus of 100 mg/kg of TET. Using a KD value of 450 hr/kg successfully described TCA serum levels in mice dosed with 1 to 100 mg/kg of CT. This project demonstrates the utility of PBPK modeling to better understand the metabolic and potential toxic consequences of chemical mixtures. (support by ATSDR #00001663931 and SERDP CU1073)

883 USE OF SENSITIVITY ANALYSIS ON A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR CHLOROFORM IN RATS TO DETERMINE AGE-RELATED TOXICITY.

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Chloroform (CHCl3) is a disinfection byproduct for which several PBPK models are available. Since PBPK models account for physiological differences, they are well-suited to predict differences due to aging. We used a five compartment PBPK constant inhalation model for CHCl3 (Evans et al., 2002) to examine age differences and variation in input parameters. Model simulations were performed in MatLab running on Red Hat Linux. We calculated and compared sensitivity coefficients (SCs) for three model input parameters and four model outputs over three age groups (- 2, 3, and 24 months) and three chamber concentrations, 10, 100 and 500 ppm for 6 hours. We examined the effect of changing Vmax on rate of amount of CHCl3 metabolized (RAML) and amount of CHCl3 in liver (AL), cardiac output (QCC) on venous CHCl3 blood concentration (CV), and fat:blood partition coefficient (PCfat) on amount of CHCl3 in fat (AF). At 10 ppm CHCl3, changes in the 4 output parameters examined (RAML, AL, CV, and AF) were consistently much less than the changes applied to the input parameters. In contrast, at 500 ppm CHCl3, SCs for CV were greater than 1 for all 3 ages. At 500 ppm, the SCs for AF were greater than 1 for adult and old rats, but not young rats. At 500 ppm, the Scs for RAML approached 1, and for AL were much less than 1. Changes in Vmax produced opposite changes in RAML and AL. Increasing Vmax increased RAML and decreased AL. As the absolute magnitude of a SC serves as a measure of the impact of a change in a given input parameter on a model output of interest, SC results can be used to gauge the usefulness of further refinement of selected input parameters. Where SCs are large, increased parameter accuracy ma be needed; uniformly small SCs may indicate further refinement is not needed. This is an example of how modeling can suggest a reduction in the number of animals used. (This abstract does not necessarily reflect EPA policy.)

884 DOSE-RESPONSE ANALYSIS OF BETA-CHLOROPRENE INDUCED CARCINOGENICITY USING PBTK MODELING.

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Beta-chloroprene (2-chloro-1, 3-butadiene, CD), used in the synthesis of polychloroprene, caused significant incidences of several tumor types in B6C3F1 mice and Fischer rats but not in Wistar rats or Syrian hamsters from two separate inhalation bioassays. This project investigated the relevance of bioassay tumor findings to potential human health risk. CD metabolism involves oxidation by cytochrome P450 to two epoxides metabolites, (1-chloroethenyl)oxirane (1-CEO) and (2-chloro-2-ethenyl)oxirane (instability of the latter metabolite precluded quantification for this project). A physiologically-based toxicokinetic (PBTK) model was developed that describes total CD metabolism and 1-CEO detoxification. Key project steps included identification of the plausible mode of action, completing quantification of tissue partition coefficients for the stable 1-CEO, finalizing *in vitro* parameters of CD and 1-CEO metabolism, refining the PBTK model structure, comparing the model with *in vivo* experimental gas uptake data, and selecting model-based values of rodent tissue dose for predicting a corresponding exposure concentration in humans. The most useful tissue dosimetric proved to be the total amount of CD metabolized daily by the lung; greater amounts of CD metabolism occurred in mouse lung compared with the other species. The internal dosimetrics and rodent lung tumor bioassay data were used to estimate an internal lung dose associated with 10% extra tumor incidence. Based on the human version of the PBTK model, the equivalent tissue dose in humans for 10% extra risk would be expected to occur from continuous exposure to 23 ppm CD. Concentrations associated with discontinuous occupational exposures such as 8 or 12 hr shift work were 90 and 100 ppm, respectively. This project demonstrates the applicability of PBTK modeling to quantitative risk assessment.

885 EVALUATING A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR USE IN RISK ASSESSMENT.

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Physiologically-based pharmacokinetic (PBPK) models are increasingly being used in evaluation of the effects of chemical exposures on human health. Due to the increased use of PBPK models, it is becoming more evident in the biological modeling community that a universal set of criteria should be used to evaluate the quality of a model before it is used in a human health risk assessment. The objective of this study was to develop a set of important criteria that can be used to evaluate PBPK models. We present the criteria here and note that this is not an exhaustive list but one that can be used as a starting point for further discussion. Increased confidence in PBPK model predictions of human health risks can be attained if the following criteria are satisfied: 1) the model must be biologically plausible (i.e. known physiological and biochemical properties of a chemical must be accounted for in the model and the appropriate mathematical descriptions should be used); 2) the computer algorithms describing the mathematical equations in the model should be implemented properly; 3) a consistent set of parameters should be used to reproduce available experimental data, including data that were not used to estimate the parameters; and 4) the model should exhibit the appropriate sensitivity to changes in each parameter value. In our study, we used an existing PBPK model for isopropanol (Clewell et al., Toxicol Sciences, 63:160-172, 2001, Gentry et al., Regul Toxicol Pharmacol, in press) as a case study. This model has been proposed as a tool to perform the route-to-route and cross-species extrapolations, among other things, needed to evaluate the potential human health effects of isopropanol, based on studies in rats of systemic, developmental, reproductive, and neurobehavioral toxicities. The isopropanol model largely meets the above criteria and will be used here to illustrate the issues involved in evaluating the quality of a model. (This abstract does not reflect USEPA policy.)

886 USE OF PHYSICO-CHEMICAL PROPERTIES AND *IN VITRO*-DERIVED DATA IN PHYSIOLOGICALLY-BASED BIOKINETIC MODELING: MINIMUM DATA REQUIREMENTS.

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Physiologically-based biokinetic (PBBK) modeling is a useful tool in determining target tissue dose metrics. This information can play a role in the assessment of a compound's hazard or risk. Prerequisites are the availability of data on physico-chemical properties, basic knowledge on the mode of action and hence the target tissues, as well as the availability of the physiological parameters needed to build the PBBK model. Data requirements for the use of models will differ, depending on their complexity, which in turn is depending on the necessity to obtain an approximation rather than a detailed target tissue dose metric. An analysis was made of the requirements for data sets on the physico-chemical properties of chemicals. A determining factor appears to be the hydrophobicity of the compound. Highly water-soluble compounds will distribute over the body water and a first estimate of the volume of distribution will therefore be the body water volume, which is approximately 0.67*body volume. For more hydrophobic compounds it will be necessary to obtain blood-tissue partition coefficients. Approximations of these can be made, e.g. on the basis of partitioning between water and octanol. This allows a quantification of the partition coefficients in quantitative property-property relationships (QPPRs), describing the relationship between e.g. logPow and partitioning between blood and tissues. For both water-soluble and lipophilic compounds protein binding will have to be taken into account for a more accurate PBBK modeling of target tissue dose metrics. Absorption *via* the skin or in the g.i. tract and partitioning to a number of target tissues will involve active transport systems, that may either enhance or diminish the target tissue dose metric. It can be concluded that, depending on the precision of the required data, approximate as well as more precise PBBK models can be based on physico-chemical properties of compounds. Other parameters, e.g. protein binding and active barrier passage may be determined in *in vitro* models.

887 PHYSIOLOGICAL PARAMETERS IN DEVELOPING RATS AND MICE.

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The utility of PBPK models for addressing issues related to children's risk, and estimating tissue doses in young animals, is limited by the lack of published information on point estimates and population distributions of age-related changes in key physiological parameters. To address this issue, we have reviewed the published literature and compiled a database of physiological parameters in developing rats and mice related to tissue volumes, intake (food and water intake, alveolar ventilation), and flow rates (e.g., cardiac output, GFR). Data were collected for the early postnatal stages (birth through postnatal day 60), with some data through day 90 when time-series data were available. Chemical-specific parameters, such as partition co-

efficients and metabolic rate constants, were not included in the current work. To maximize the utility of the database for PBPK modeling, all data were associated with body weight, as well as animal age. To aid in prioritizing the large number of studies identified, emphasis was placed on studies providing data at multiple time points (or data for a given time point covering multiple endpoints). However, for data-poor parameters (e.g., GFR), all relevant data were entered into the database. The database was used to identify data gaps by age, sex, species, and strain. For example, few data are available for the mouse, compared with the rat, and more data are available on tissue volume than flow rates. The results of this analysis will be useful in (1) identifying key areas where additional data are needed in order to develop PBPK models for young animals; and (2) identifying parameters and parameter distributions for developing PBPK models for estimating tissue dose in neonates and young animals.

888 AN INTEGRATED MODEL OF LIFE STAGE-SPECIFIC CHANGES IN PHYSIOLOGICAL PARAMETERS OF FEMALE RATS.

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The pharmacokinetics and tissue dose of chemicals may vary from one life stage to another as a result of changes in physiology, biochemical processes and tissue composition. Some data and physiological models of specific life stages exist in the literature. The objective of this study was to interconnect the existing life-stage specific models to produce an integrated description of temporal changes in physiological parameters in female rats. The methodology involved the review of published literature to gather data on the rat physiological parameters such as body weight, organ weights, cardiac output and tissue blood flows for the different life stages and the development of regression equations for calculation of the age-dependent physiologic parameters. The following were the specific tissues for which weights and blood flows were investigated: brain, liver, adipose tissues, slowly perfused tissues, richly perfused tissues, mammary gland and placenta. The data and existing models for the following life-stages were obtained: gestation, lactation, embryo-fetal growth, nursing pup and post-lactational growth. For most life-stages, tissue-specific physiological parameters were found except for the embryo-fetus stage. The linkage of the five stages together needed some adjustments so as to have a real smooth continuum from the embryo-fetus to nursing pup to growing rat to the gestating dam and lactating dam. The data sources and regression equations identified in this study should be useful for constructing pharmacokinetic models to simulate the uptake and disposition of chemicals during prenatal and postnatal development.

889 EVALUATION OF TWO IMMUNOASSAYS FOR ANALYSIS OF METHAMPHETAMINE CONTAMINATION ON INDOOR SURFACES.

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Illegal synthesis of methamphetamine is a major law enforcement and public health problem in California, where more than 2000 clandestine laboratories have been identified in each of the last three years. Methamphetamine is frequently produced in houses, apartments and mobile homes, and may result in widespread contamination of interior surfaces. Given the cost and delay associated with conventional analytical procedures, assessment of residual methamphetamine contamination is usually based solely on visual evidence, i.e., staining. To address the need for better assessment methods, two immunoassay methods, originally developed for detection of illicit drugs in biological fluids, were modified for detection of surface contamination. A microplate enzyme immunoassay (EIA) and a portable immunoassay utilizing a hand held reader were evaluated. In the validation study, standard solutions of illegally synthesized methamphetamine were prepared, applied to glass plates and allowed to dry. The plates were sampled using absorbent tabs, diluted in buffer, and analyzed using the microplate EIA, portable immunoassay, and GC/MS. Extraction (sampling) efficiency, precision, method bias, method detection limit, and quantitation range were determined. Both immunoassays are rapid and relatively inexpensive assessment tools that can be readily adopted by health officials to identify surfaces in former clandestine methamphetamine laboratories that require remediation and/or additional investigation.

890 AN ANALYTICAL METHOD FOR MEASURING DERMAL EXPOSURE TO HEXAMETHYLENE DIISOCYANATE.

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Occupational exposure to diisocyanates such as hexamethylene diisocyanate (HDI) is well documented as a cause of respiratory sensitization and occupational asthma. However, inhalation exposure may account for only part of the total body dose. Recent animal studies suggest that dermal exposure to diisocyanates is linked to respiratory sensitization; hence there is a need for dermal exposure assessment methods applicable in the workplace. We are developing a method to detect and quantitate dermal exposure to HDI. NIOSH Method 5521: "Isocyanates, Monomeric", originally developed for air sampling, was modified for use with our non-invasive dermal tape-stripping technique, whereby 2.5 by 4 cm rectangles of adhesive tape are applied to exposed skin surfaces, then removed, taking with them the outer layer of keratinocytes and any substances deposited thereon. The tape rectangles are immersed in toluene/1-(2-methoxyphenyl)piperazine solution; diisocyanates are recovered (after reaction with 1-(2-methoxyphenyl)piperazine) as their urea derivatives, and following extraction are ultimately detected and quantitated in that form by liquid chromatography-ion-trap mass spectrometry with electrospray ionization, operated in the positive mode. Compounds were identified by retention times and mass spectra. For quantitation of HDI, the protonated molecular ion of the urea derivative (HDIU; m/z 553.7) was monitored. Limits of detection and of quantitation were 1 pmol and 10 pmol injected, respectively. The urea derivative of octamethylene diisocyanate (ODIU) served as internal standard (1000 pmol per injection), and was detected as its protonated molecular ion (m/z 581.7). Response of HDIU relative to ODIU was linear from 0.01 to 1 mol/mol injected. Triplicate samples were reproducible within 10%. HDI was recovered at variable but detectable levels from paint droplets deposited onto tape, to mimic spray-painting operations. Supported by NIEHS P30-ES10126.

891 PERCHLOROETHYLENE EXPOSURE ASSESSMENT FOR GOVERNMENT INSPECTORS AT DRY-CLEANING FACILITIES.

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This study assessed occupational exposure to perchloroethylene (PERC) among government air quality inspectors at 16 dry-cleaning facilities selected to represent plausible high-end exposures based on historical air quality violation records. Inspectors normally assess airborne PERC levels using a photoionization detector (PID). They follow specific inspection procedures starting with measurements outdoors, at the facility entrance/lobby, in the equipment areas, and at specific locations around each piece of equipment (for leak detection at doors, seals, etc.). In addition to recording these usual measurements at each facility, each of the four inspectors who volunteered for the study was fitted with two personal air monitoring devices: a full-shift badge monitor (approx. 8 hrs.) and an active sorbent tube monitor (battery pump, on during inspections only, < 4 hrs.). Each of the personal samples demonstrated that time-weighted average $\pm$ std.dev. exposures for full-shift (1.3 ± 0.8 ppm, n = 8) and facility-only measurements (3.9 ± 3.7 ppm, n = 4, composite of 4 facilities each) were below occupational exposure limits. The exposure measurements (personal monitors and PID recordings) and inspector rankings of facility PERC exposures on a scale of 1-4 were also assessed for time-weighted exposure trends. Linear regression of full-shift and facility-only PERC levels showed direct correlation with an index of peak PID-minutes, but inverse correlations with entrance PID-minutes and inspector rank-minutes. Inspector rank-minutes was also directly correlated with entrance PID-minutes. These trends are possibly attributable to the observation that inspectors spent less time in completing their inspections in process areas of the facilities with higher ambient indoor PERC levels. The study suggests that trained inspectors utilize PID measurements and work habits to limit their own exposure to PERC at dry cleaning facilities with higher ambient indoor air levels.

892 AUTOMOBILE BRAKE MAINTENANCE AND AIRBORNE CHRYSOTILE FIBER EXPOSURES.

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The use, in the past and to a lesser extent today, of chrysotile asbestos causes health concerns among professional mechanics. Therefore, we conducted 4 separate tests in order to evaluate an auto mechanic's exposure to airborne chrysotile fibers while

performing routine brake maintenance. Four nearly identical Chevrolet Impalas from mid-1960s having 4 wheel drum braking systems were used. Each automobile was fitted with new replacement chrysotile-containing brake shoes and then driven over a predetermined public road course for about 1, 400 miles. Then, each car was separately brought into a repair facility; the brakes removed and replaced with new chrysotile-containing shoes. The test conditions, methods, and tools were as commonly used during the 1960s. The mechanic was experienced in brake maintenance, having worked in the automobile repair profession beginning in the 1960s. Effects of 3 independent variables, e.g., filing, sanding, and arc grinding of the replacement brake shoe elements, were tested. During each test, personal and area air samples were collected and analyzed for the presence of fibers, chrysotile fibers, total dust, and respirable dust. The results indicate airborne chrysotile fiber exposures for each test remained at or below currently applicable OSHA Permissible Exposure Limit of 0.1 fiber/cm^3, 8-h time-weighted average.

893 ARSENIC DRINKING WATER EXPOSURE AND URINARY EXCRETION AMONG ADULTS IN THE YAQUI VALLEY, SONORA, MEXICO.

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The drinking water standard As has been set at 10 ppb based primarily an epidemiology studies in Taiwan. However in the Southwest US and Northern Mexico, drinking water exposure to arsenic is primarily to Hispanic populations. Thus, this study was conducted in the Yaqui Valley, Sonora, Mexico, to explore arsenic exposure and urinary arsenic excretion in this Hispanic population. Forty-four adults residents from four towns (Esperanza, Cocorit, Pueblo Yaqui, and Col. Allende) who drank tube-well water for up to 23 years were examined. Arsenic concentrations in the wells were determined by ICP-MS as 43.3, 19.3, 9.7, and 5.5 μg As/l respectively. Urinary mean concentrations of the sum of As (III), As (V), MMA (V) and DMA(V) were between 79.7 and 31.2 μg/L. The average of arsenic urinary excretion only weakly correlated with the arsenic in drinking water. In contrast to previous studies we found low excretion of DMA V, (56-58 %, of total urinary As) and slight increase in inorganic arsenic As III and As V, 30-33 %, except in Cocorit. Genetic polymorphisms in As methylating enzymes may be responsible, for the different excretion of urinary arsenic metabolites, but further research is needed to identify genetic polymorphisms and altered gene expression in this population (NIEHS 04940).

894 DRINKING WATER ARSENIC EXPOSURE IS ASSOCIATED WITH DECREASED DNA REPAIR GENE EXPRESSION.

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Arsenic is well established as a human carcinogen, but its precise mechanism of action remains unknown. Arsenic does not directly damage DNA, but may act as a carcinogen through inhibition of DNA repair mechanisms, leading indirectly to increased mutations from other DNA damaging agents. The molecular mechanism underlying arsenic inhibition of nucleotide excision repair after UV irradiation is unknown, but could be due to decreased expression of critical genes involved in nucleotide excision repair of damaged DNA. This hypothesis was tested by isolating mRNA from cryopreserved lymphocytes taken from individuals enrolled in a population based case-control study investigating arsenic exposure and cancer risk in New Hampshire. Arsenic levels were determined in toenail clippings using instrumental neutron activation analysis and drinking water samples using high resolution ICP-MS with hydride generation. We previously measured expression of nucleotide excision repair genes by RT-PCR using mRNA from 16 individuals. In a linear regression analysis, toenail and drinking water arsenic levels were inversely correlated with expression of critical members of the nucleotide excision repair complex including, ERCC1, XPF, and XPB, but not XPG. Nucleotide excision repair gene expression was re-examined in mRNA isolated from 54 individuals by real-time PCR using Taqman primers and probes. Levels of nucleotide excision repair proteins in the lymphocytes were also compared by immunoblotting on a subset. Our findings, based on human exposure to arsenic in a US population, show an association between biomarkers of arsenic exposure and expression of DNA repair genes. These results are consistent with the hypothesis that inhibition of DNA repair capacity by arsenic in drinking water is a potential mechanism by which arsenic can contribute to increased cancer risk (supported by NIEHS ES07373, NCI CA57494).

895 HAZ-MAP: OCCUPATIONAL HEALTH INFORMATION FOR THE PUBLIC.

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Haz-Map is an occupational toxicology database designed primarily for health and safety professionals, but also for consumers seeking information about the health effects of exposure to chemicals at work. It links jobs and hazardous tasks with occupational diseases and their symptoms. This relational database of chemicals, jobs, and diseases is part of the TOXNET system of databases on toxicology and environmental health provided by the National Library of Medicine (NLM) on the internet. The approximately 1000 chemicals and biological agents in the database are related to industrial processes and other activities such as hobbies. The linkage indicates the potential for exposure to the agents. The 180 occupational diseases and their symtpoms are associated with hazardous job tasks. This association indicates an increased risk for significant exposure and subsequent disease. NLM developed the Haz-Map Web interface which is accessible to the public *via* the internet. Main features of the Haz-Map Web interface include: text search capability, browsing by hierarchical categories, alphabetical listing of entries in all tables, searching hazardous agents by adverse effects, searching diseases by jobs and findings, and launching searches to NLM's TOXNET system of databases from Haz-Map. Users can easily find definitions of technical terms by clicking hyperlinks to the glossary. The purpose of the poster is to introduce users to the contents and sources of Haz-Map, and to demonstrate the features and navigation of this new occupational medicine resource.

896 HOUSEHOLD EXPOSURE TO PHTHALATES, PESTICIDES, ALKYLPHENOLS, PBDES, AND OTHER ENDOCRINE ACTIVE COMPOUNDS.

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In order to characterize exposures to chemicals of interest for research on breast cancer and other hormonally mediated health outcomes, residential air and dust samples were analyzed for 93 target compounds that 1) have been identified as animal mammary carcinogens or endocrine active chemicals and 2) are used in commercial or consumer products. In data from 120 homes sampled on Cape Cod, MA, 64 of 93 target compounds were detected in indoor air and 72 of 92 were detected in house dust. Chemicals present at highest concentrations in air samples include: diethyl phthalate (DEP) (range 130-4, 300 ng/m3), dibutyl phthalate (DBP) (52-1, 100 ng/m3), o-phenyl phenol (oPP) (12-970 ng/m3), and nonylphenol (NP) (21-420 ng/m3). These concentrations are 2-3 orders of magnitude higher than indoor air concentrations of, for example, the ubiquitous PAH pyrene; suggesting that these chemicals may be important indoor air contaminants. Most abundant compounds in dust include DEHP (range 16.7-7, 700 microgram/g) and several other phthalates. Also commonly detected in dust were NP, OP and their ethoxylates, polybrominated diphenyl ethers, bisphenol A, benzo-a-pyrene, methyl paraben, and several pesticides. Pesticides detected in at least 50% of the homes include: oPP, permethrin, piperonyl butoxide, methoxychlor, DDT, pentachlorophenol, and chlordane. On average, 21 target compounds were detected in each indoor air sample and 28 in each dust sample. For 31 of the chemicals detected, no risk-based criteria are available for evaluating potential health effects, and risk based criteria that are available for the remaining chemicals generally do not consider their endocrine activity. This study identifies endocrine active chemicals that are widespread in indoor environments, making them priorities for future research and regulatory evaluation, for example Tier 2 testing as recommended by EDSTAC. It also provides new exposure assessment tools for the study of hormonally mediated health outcomes.

897 DIOXIN LEVELS IN HUMANS AND FOOD FROM AGENT ORANGE SPRAYED AND NON-SPRAYED AREAS OF LAOS AND VIETNAM COMPARED TO THE USA, GERMANY AND CANADA.

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Laos and Vietnam were countries sprayed with Agent Orange herbicide between 1962 and 1971. Agent Orange was contaminated with TCDD. High levels of TCDD have been reported in sprayed areas of Vietnam in human milk, up to 1,

850 ppt lipid, blood, up to 413 ppt, fish, up to 850 ppt, and soil, up to 1, 100, 000 ppt. Most Vietnam samples were lower. Dioxin studies in Laos have not previously been conducted. We report here the first dioxin measurements from Laos, in non-sprayed Vientiane and in sprayed Sepone City. Blood, human milk, and food were sampled and analyzed. Unlike Vietnam, levels of TCDD were always low in human milk, blood, and in food, from sprayed and non-sprayed areas examined. The highest blood TCDD was 4 ppt in a 55 year-old man from Sepone. Other values were not detectable or very low. Food was lower or similar in dioxin levels to American and European food. Exported Vietnamese food purchased in the USA was also very low in dioxins. Although generally lower, there was some overlap in dioxin levels in Laotian and exported Vietnamese food compared to US and European food. We speculate as to why Laotian human tissue and food levels in sprayed areas did not show elevation of TCDD: The persons sampled were relatively young and born after spraying ended. The defoliant sprayed may not have been Agent Orange, or if Agent Orange, may have been washed away over the years during yearly flooding. Further collaborative research in various locations in Laos is planned.

898 USING COTTON T-SHIRTS AS A SURROGATE FOR CHILDREN'S HOME EXPOSURE FROM FLEA CONTROL COLLARS CONTAINING CHLORPYRIFOS.

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Twelve family units were chosen to determine the levels of insecticide that could be transferred to children from a flea control collar from a pet dog. One child (3 to 12 years) from each household wore t-shirts 5 different days before the application of the collar and for 5 consecutive days between days 14 and 20-post collar application (5 pre and 5 post). The dogs were petted to determine available transferable residues on days 14 and 20 using white cotton gloves. Commercially available flea control collars containing the organophosphate insecticide chlorpyrifos (8%) were applied to dogs as per package directions. Transferable residues were quantified by petting the dog for 5 minutes in 3 areas (neck with collar, neck, and back) and yielded averaged transferable residues of 136-974 µg, 68-622 µg, and 1-22 µg, respectively. The averaged t-shirt (100 in^2 front chest area) samples were 0.29 µg for the pre-application and 0.74 µg for the post-application. Most samples ranged from <2.5 pg to 2 µg with one sample at 12 µg (the 5th post-application sample of family 105 - 105post5). The remaining 105post5 shirt was analyzed for 9 additional regional sections and yielded residues of: 9.95, 7.84, 9.29, 4.99, 5.72, 6.80, 3.88, 7.17, and 4.85 µg. Since the other post-application t-shirts (1st, 2nd, 3rd, and 4th) for 105 were 0.69, 0.99, 0.76, and 0.62 µg, the 12 µg could be from another source. The average post application residue decreased to 0.52 µg without the 105post5 sample. The 109 household gave the next highest samples (0.78, 2.87, 2.44, 1.77, and 0.65 µg; post 1-5 respectively). The remaining 109post2 whole shirt yielded residues of: 0.99, 0.65, 0.66, 0.36, 0.72, 0.99, 1.38, 0.72, and 0.63 µg. The 109 household also gave the highest pre-application (1-5) samples (0.5, 1.94, 0.24, 2.02, and 0.33 µg, respectively). Introduction of flea control collars containing chlorpyrifos into homes may increase amounts of transferable residues, but variability is high. (Supported by EPA R-828017).

899 BIOMARKER MEASUREMENTS IN A FISH-EATING POPULATION EXPOSED TO ORGANOCHLORINES.

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The Lower North Shore region of the Gulf of St. Lawrence (Canada) is home to a fish-eating population that displays an unusually high body burden of several organochlorines, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans. In order to evaluate health risks due to this exposure, we recruited 40 participants for the assessment of biomarkers of exposure and effects. Concentrations of 14 PCB congeners and dioxin-like compounds (DLCs) in plasma were measured by high-resolution gas chromatography and the CALUX assay, respectively. Morning urine samples were collected for D-glucaric acid and porphyrin analyses. Cotinine concentration was also determined in urine samples to document smoking habits. The caffeine breath test (CBT) was performed on a subset of participants. The mean concentration for the sum of PCBs in plasma lipids was 2.9 mg/kg, with values ranging from 0.3 to 5.9 mg/kg (median=2.8 mg/kg). Plasma lipid concentrations of DLCs averaged 159 ng TEQ/kg and ranged from 55 to 469 ng/kg (median=133 ng/kg). Neither total DLCs nor total PCBs were correlated to biomarkers of effects. The rate of caffeine 3-N demethylation (CBT), which reflects liver CYP1A2 activity, was positively correlated to urinary cotinine concentration (Spearman's R=0.59, p=0.005, N=21) and negatively correlated to the PCB 118/PCB 153 congener ratio in plasma (R=-0.50, p=0.021, N=21). Urinary coproporhyrin concentrations were also negatively correlated to the PCB 118/PCB 153 congener ratio (R=-0.35, p=0.03, N=40). Hence, despite the relatively high body burden of PCBs and DLCs in this population, only smoking had a significant influence on biomarkers of effects. The negative correlation observed between caffeine N-3 demethylation and the PCB 118/PCB 153 congener ratio likely reflects an increased biotransformation rate of the mono-ortho PCB congener 118 in individuals with high CYP1A2 activity due to smoking.

900 CONCENTRATIONS OF PERSISTENT ORGANIC POLLUTANTS IN HUMAN MILK FROM NUNAVIK (ARCTIC QUEBEC, CANADA): CHANGES OBSERVED FROM 1989 TO 2001.

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Over the last decades, declining concentrations of organochlorines and increasing concentrations of polybrominated diphenyl ethers (PBDEs) have been reported in human milk collected worldwide. The objective of this study was to determine if similar changes could be observed in the Inuit population of Nunavik, which is particularly exposed to persistent organic pollutants through their traditional diet. Concentrations of 5 chlorinated pesticides, polychlorinated biphenyls (PCBs, 12 congeners), polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) were determined in milk samples collected in this population between 1996-2000 (n = 46 to 55). These data were compared with those obtained in a human milk survey carried out in 1990 in the same region (n = 12 to 43). A similar comparison was carried out for PBDEs using 10 archived samples from each group. Concentrations were expressed on a lipid basis. TEQs were computed for dioxin-like compounds (DLCs: PCB congeners # 77, 126 and 169, PCDDs and PCDFs). Geometric means and confidence intervals ($CI_{95\%}$) were computed for concentrations of all contaminants (when detected) and compared (t-tests). Our results showed an 86% decrease ($p<0.0001$) in human milk concentrations of DLCs, from 43.26 ppt TEQs in 1990 ($CI_{95\%}$[34.27-54.60]) to 6.09 in 2000 ($CI_{95\%}$[4.07-9.12]). TEQs attributable to PCBs, PCDDs and PCDFs showed significant decreases of 90%, 87% and 76%, respectively. Ortho-substituted PCB congeners and organochlorine pesticides showed decreases averaging 66%. However, concentrations of PBDEs (sum of 9 congeners) showed a 70% increase ($p<0.0001$), rising from 5500 ppt in 1990 ($CI_{95\%}$[3240-9320]) to 9320 ppt in 2000 ($CI_{95\%}$[6570-13210]). The decline in organochlorine concentrations and the increase in PBDE concentrations in human milk from Nunavik are both consistent with results from other studies carried out worldwide.

901 ELEVATED BLOOD LEAD FROM GUNSHOT UP TO ONE YEAR AFTER INJURY.

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There are no systematic studies of prevalence or antecedents of elevated blood lead concentration (BPb) in gunshot victims despite a century of published case studies. We studied patients with retained lead bullets for 12 months. We measured BPb (via AAS) from admission to one year, bone lead (KXRF) in the first six weeks and again at one year, patient and injury characteristics. Prevalence of BPb ≥20 µg/dl at mean of 0.3, 3, 18, 94, 188 and 363 days was 0.3, 2.0, 6.8, 9.8, 5.0 and 3.1%, respectively. Logit regression for BPb ≥20 µg/dl at 90 days after injury showed odds ratios (OR) (95% CI) of 6.5 (1.8-23.4) for patients shot in head, 3.1 (1.2-8.3) for patients shot in femur and 13.3 (4.4-40.3) for patients with bone fracture in torso. Every natural log increase in age was associated with an OR of 4.7 (1.2-17.9) and an OR of 1.6 (1.3-2.1) for every natural log increase in number of retained fragments. The logit model correctly predicted outcome in 79% of cases. In mixed model analysis for repeated measures over the first three months BPb increased 31% for every ln unit in number of retained fragments and increased 51% for every common log unit of days after injury. The effect of number of fragments on BPb increased significantly with time after injury; the effect of number of fragments on BPb was 28% greater in patients with bone fracture in torso than in those without such fracture. BPb increases with time in a majority of gunshot patients up to 90 days after injury. ~10% of patients have BPb approaching or exceeding CDC action limits at 90 days and a few patients show sustained elevations or increasing BPb out to 12 months after injury. These patients may go on to develop frank lead toxicity.

902 USEPA'S RESPONSE ACTIVITIES TO THE WORLD TRADE CENTER DISASTER.

A. Galizia. *USEPA, Edison, NJ.* Sponsor: S. Gavett.

The United States Environmental Protection Agency (USEPA) as well as other Federal, State and local agencies, responded promptly to the unprecedented attack on and subsequent collapse of the World Trade Center (WTC)towers. The USEPA's

response to address potential harm to human health and the environment associated with this catastrophic event included command, operational and monitoring activities. Command activities were coordinated by the Federal Emergency Management Agency (FEMA), with participation by Region 2 and other regional and national offices. Operational activities included dust suppression by HEPA vacuuming and power washing, set up of vehicle, personnel and landfill wash stations; distribution of appropriate protective equipment for the rescue workers; development of a database system and a website for the monitoring data; outreach to public officials, the public and community groups. The monitoring effort consisted of sampling at an extensive network of air monitoring stations, as well as targeted water and bulk dust sampling. The air monitoring network was established to address potential effects of demolition construction materials (PM, Asbestos, lead) and products of incomplete combustion (dioxins, VOCs, PCBs, PAHs). Much of the data from this monitoring effort can be obtained from the USEPA website, www.epa.gov under Response to September 11th. Results from the monitoring done at the World Trade Center and nearby surrounding areas show that: (1)there was no real impact to ambient, discharge and drinking water due to the WTC disaster; (2)there were some exceedences of benchmark air standards for the contaminants of concern, but they generally occurred soon after September 11. For example, a single PM2.5 exceedence of the Air Quality Index of 40 ug/m3 was reported in the days that follow September 11. Also, asbestos was found to exceed the AHERA standard of 70 s/mm2 in less than 1% of the samples taken in lower Manhattan. For lead, a few early samples were above the National Ambient Air Quality Standard (NAAQS) of 1.5ug/m3 but were well below the OSHA Permissible Exposure Limit (PEL) of 50 ug/m3.

903 PREDICTING RISK OF HOSPITAL ADMISSIONS USING AN AIR POLLUTION MODEL.

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A set of environmental indicators was constructed into a model in an attempt to predict respiratory-related hospital admissions. This model was subsequently tested on the sixty-seven counties in Florida. Government databases were reviewed to access the 1996-1999 estimates of environmental releases for the State of Florida by county. The environmental indicators selected included: carbon monoxide (CO), nitrogen dioxide (NO2), particulate matter less than 10 microns (PM10), particulate matter less than 2.5 microns (PM2.5), sulfur dioxide, volatile organic compounds, ammonia, and total industrial releases. In addition, a socio-economic indicator, an estimate of the percentage of adult smokers and the median age were obtained for each county. The second set of data was comprised of the Florida Agency for Health Care Administration's hospital discharge diagnosis data for each county in 1996-1999, for four respiratory diagnoses. Those admissions with the International Classification of Diseases (ICD-9) codes 506-519 encompassed respiratory conditions due to chemical and fumes, pneumonitis, emphysema, pleurisy, pneumothorax, and lung abscess. The ICD-9 codes 480-486 included diagnoses of pneumonia and influenza. The Chronic Obstructive Pulmonary Disease (COPD) category was represented by the ICD-9 codes 490-496. The diagnosis of asthma was extracted from the dataset by ICD-9 code 493. GB-Stat V8.0 and SAS were used to analyze the data. For these four years, the full model explained 92% to 95% of these admissions. Carbon monoxide was consistently one of the top five predictors, followed by PM2.5, PM10, hazardous air pollutants (HAP) and NO2. This model appears to be a valid tool for predicting respiratory-related hospital admissions in Florida.

904 BLOOD HOMOCYSTEINE (HCY) LEVELS IN NON-SMOKERS EXPOSED TO ENVIRONMENTAL TOBACCO SMOKE (ETS) AND THE EFFECT OF POLYMORPHISMS IN THE GLUTATHIONE-S-TRANSFERASE M (GSTM), GLUTATHIONE-S-TRANSFERASE T (GSTT) AND NAD(P)H QUINONE OXIDOREDUCTASE (NQO1) GENES.

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Exposure to ETS may be a risk factor for cardiovascular disease (CVD). HCY is an independent risk factor for CVD whose mechanism of toxicity is thought to result from endothelial damage through oxidative processes. GST and NQO1 are Phase II detoxifying enzymes whose activities can prevent oxidative damage. As part of the Nevada Environmental Tobacco Smoke and Health Study funded by NIEHS, non-smokers, confirmed by serum cotinine, occupationally exposed to ETS and not living with a smoker were tested for plasma HCY levels as part of their baseline analysis and blood drawn with informed consent. Plasma HCY levels were assessed by HPLC with fluorescence detection at 385 nm excitation and 575 nm emission. GST polymorphisms were assessed in DNA from whole blood by multiplex PCR followed by separation on an agarose gel. NQO1 polymorphism (C-T transversion at 609 bp) was assessed with PCR followed by Hinf1 restriction endonuclease digest and separated by agarose gel electrophoresis. Preliminary findings based upon the analysis of 36 subjects indicates no relationship between plasma HCY levels and polymorphisms for any of the genes. Interestingly however, overall plasma HCY levels for males in the study population was 10.6 ± 2.7 μM and 9.6 ± 2.0 μM for females. These values are 13% and 23% higher than previously reported normal values for men and women respectively. A significant difference ($p<0.05$) in HCY levels between Caucasians and non-Caucasians was observed, with a value 2.6 μM higher in the Caucasian population. These preliminary results suggest that non-smokers exposed to workplace ETS exposure may have higher plasma HCY levels than the general population. These studies were funded in part by PHS NIEHS grant ESO0520 and the Fund for a Healthy Nevada.

905 SERUM OXIDIZED LDL LEVELS ARE INCREASED IN NONSMOKERS EXPOSED TO ENVIRONMENTAL TOBACCO SMOKE (ETS) AND ASSOCIATED WITH GENETIC POLYMORPHISMS IN GST-T, GST-M, AND NQO1.

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Exposure to ETS may be a risk factor for cardiovascular disease (CVD). Oxidized LDL (OxLDL) is considered to be an independent risk factor for CVD. In this study we looked at whether workplace exposure to ETS in non-smokers had an effect on serum OxLDL levels. Genetic polymorphisms to Phase II detoxification enzymes such as the glutathione-S-transferases and NAD(P)H quinine oxidoreductases (NQO1) could impact the extent to which ETS effects OxLDL levels. We tested whether genetic polymorphisms in glutathione-S-transferase-μ(GST-μ), glutathione-S-transferase-θ(GST-θ) or NQO1 affected OxLDL levels in these non-smoker, ETS exposed subjects. Blood samples were obtained from subjects in the Nevada ETS and Health Study. EIA was used to quantitatively measure serum autoantibodies against OxLDLs. Genetic polymorphisms were identified using DNA extracted from whole blood. GST- μ and GST- θ were qualitatively identified with multiplex PCR followed by agarose gel electrophoresis for null or wild type genotypes. A single nucleotide polymorphism of NQO1 was assessed with PCR and subsequent restriction digest and agarose gel electrophoresis. Preliminary findings suggest that our cohort of ETS exposed non-smokers had OxLDL values higher than previously reported OxLDL values for non-smokers. When stratifying these results for the GST and NQO1 polymorphisms, no difference was seen between the groups except for the group displaying the polymorphism in both GST-μ and GST-θ. These subjects had a significantly higher oxidized LDL ($p<.031$) than the other groups. These results suggest that non-smoker workplace exposure to ETS increases serum oxidized LDL and that the presence of a polymorphism in both GST-μ and GST-θ significantly increases serum oxidized LDLs and therefore may increase the risk for cardiovascular disease. This study was funded in part by PHS, NIEHS grant ESO9520 and the Trust Fund for a Healthy Nevada.

906 ASSOCIATION BETWEEN URINARY PORPHYRINS, MERCURY, SYMPTOMS, AND MOOD.

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Potential associations between urinary porphyrins and urinary mercury (Hg) (0-10 ug/l) with 68 self reported symptoms and 6 mood scales was conducted among 81 male dentists (DDs) and 97 female dental assistants (DAs) in Washington State. Symptoms were categorized as current (today), recent (past 3 months) and chronic (> one year). The Profile on Mood State measured 6 mood scales. Pooled copro-, precopro, and penta-carboxyl urinary porphyrins and mercury were measured at the time of the symptom and mood survey. Crude correlations were used to identify a set of potential determinants ($p<.05$) that were later tested in multiple regression models. Differences between sexes were sufficient to support separate analyses. Pooled copro-, precopro-, and penta-porphyrins were strongly associated (Beta =0.42 or 0.49) with urinary mercury at levels about 0-10 ug/l. Urinary Hg was associated with reported paresthesias among DDs (chronic only) and DAs (all 3 temporal categories), as well as with reports of chronic symptoms of muscular weakness and coordination among DDs. Urinary porphyrins were also associated with chronic muscular symptoms among DDs. Among DAs, urinary porphyrins were associated with recent and chronic symptoms involving memory and paresthesias,

as well as current anxiety and chronic skin symptoms. With respect to mood, urinary Hg was associated with mood among DAs while urinary porphyrins were associated with several dimensions of mood among DDs, however, these associations were in an unexpected direction. A number of self reported medical conditions and medication for these symptoms appeared as potential confounders, being associated with both porphyrin and Hg measures of exposure and the symptom and mood outcomes. This was particularly true for women with immune or endocrine conditions. In this study, porphyrin excretion levels were more closely associated with the outcomes generally attributed to Hg exposure, especially memory, and may be an important alternative measure of the effects of Hg exposure. Supported by ES04696 and ES07033.

907 ASSOCIATION OF TAMOXIFEN (TAM) AND TAM METABOLITE CONCENTRATIONS WITH SELF-REPORTED SIDE EFFECTS OF TAM IN WOMEN WITH BREAST CANCER.

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The positive effects of tamoxifen (TAM) on breast cancer recurrence and survival as well as on overall mortality has led to its use as the predominant adjuvant therapy among women with breast cancer. However, the association of TAM intake with several undesirable side effects has been reported in numerous studies. This analysis was carried out to assess whether the concentrations of TAM or TAM metabolites, N-desmethyltamoxifen (N-DMT) and 4-hydroxytamoxifen (4-OHT), were associated with self-reported side effects of TAM in women diagnosed with breast cancer. Participants were 76 post-menopausal breast cancer patients who had been taking TAM for at least 30 days. Each participant completed a questionnaire that was used to ascertain whether she experienced certain specific symptoms while taking TAM. In addition, each woman provided a blood sample that was used to measure plasma concentrations of TAM, N-DMT, and 4-OHT by high pressure liquid chromatography. Results of the analysis showed that women who reported experiencing visual problems or fever had significantly higher levels of both TAM and N-DMT compared to those women who reported experiencing no visual problems or fever. In addition, women who reported experiencing nausea or migraines had significantly higher levels of N-DMT than women who did not experience these side effects. 4-OHT levels were not significantly associated with any TAM-related symptoms. The results of this study suggest that the self-reported occurrence of certain symptoms during TAM treatment is related to TAM metabolism. Future studies should assess subgroups of women with specific TAM and TAM metabolite profiles to determine whether alternate, equally effective therapies would decrease their risk of experiencing certain undesirable side effects. Supported by DOD Grant DAMD17-00-1-0321 and the University of Maryland, Maryland Statewide Health Network.

908 DIETARY EXPOSURE ASSESSMENT OF CADMIUM CLOSE TO THE CURRENT PROVISIONAL TOLERABLE WEEKLY INTAKE AND ITS EFFECTS ON RENAL BIOMARKERS AMONG FEMALE FARMERS IN JAPAN.

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Cadmium (Cd) is reported to induce renal tubular damage at higher oral exposures. However, few epidemiological studies with precise assessment of dietary Cd exposure have been undertaken to evaluate the effects on health of Cd in food. The present epidemiological study was undertaken to evaluate whether sustained low-dose dietary Cd exposure may increase prevalence of renal dysfunction among 1310 female farmers of 5 districts in Japan who consumed their own rice with various levels of Cd contamination. The geometric means of Cd concentrations in the rice (R-Cd) collected from study participants were 0.022, 0.061, 0.054, 0.113, and 0.154 microg/g in district A, B, C, D, and E, respectively. The estimates revealed that 0.5 - 2.5% of the participants in district A were exposed to a higher Cd dose than the current Provisional Tolerable Weekly Intake (PTWI), i.e. 4.5 - 20.3% in district B, 6.9 - 22.2% in district C, 24.0 -52.5% in district D, and 35.6 - 66.8% in district E. Creatinine-adjusted urinary Cd (U-Cd) and blood Cd (B-Cd) increased age-dependently, and correlated with the degree of Cd contamination in the districts. Both urinary alpha1-microglobulin and beta2-micoroglobulin, biomarkers of renal tubular dysfunction, showed statistically significant increases in an age-dependent manner in all the districts, but were correlated with neither University-Cd nor B-Cd, nor R-Cd. Multiple regression analysis depicted no significant increase in the prevalence of renal biomarkers in each district after adjustment for age. In conclusion, this study showed that the prevalence of renal tubular dysfunction remains the same among female farmers exposed to life-long dietary Cd close to or above the current PTWI. This research protocol was approved by the Committee on Medical Ethics in Jichi Medical School.

909 ISCHEMIC HEART DISEASE AMONG MINERS.

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Miners are exposed to gases and respirable particles. Some previous studies have observed an increased mortality regarding ischemic heart disease (IHD) among miners and industrial sand workers. Objective: This study is a comparison of male miners with gainfully employed men in Sweden in order to investigate the occurrence of ischemic heart disease (IHD). Methods: Male miners were identified in the Swedish National Census. Miners with the same occupation in 1970 and 1980 were included. The referent group comprised all gainfully employed men with the same occupation in the 1970 and 1980 censuses. The cohort was followed from 1980 until December 31, 1995. All workers were identified and linked to the Cause of Death Register during the period of follow-up. Ischemic heart disease (IHD) was defined as code 410-414 of the International Classification of Diseases (ICD 7 and 8). The Standardized Mortality Ratio (SMR) has been calculated as the ratio between observed and expected numbers of deaths. Result: An increased risk due to IHD was observed among the miners, table 1. Conclusion: The increased risk of IHD is unlikely explained by smoking habits. Smoking habits were surveyed in the male population in 1963 and found to be almost identical between miners and the general male population. Shift work might explain some of the increased risk. Inhalation of air pollutants retained in the lungs will hypothetically create a low grade inflammation associated with an increase in plasma fibrinogen. The high concentration of fibrinogen will increase the likelihood for blood clotting and thereby the risk for myocardial infarction and IHD. Table 1. Standardized Mortality Ratios (SMR) of IHD among miners identified in the 1970 and 1980 Censuses and followed until the end of 1995.

Observed	Expected	SMR	95% confidence limits
88	56.3	1.56	1.25-1.92

910 USE OF THE GLOBAL ASSESSMENT SYSTEM FOR HUMANS IN MILITARY VETERANS TO DISCRIMINATE PERFORMANCE DEFICITS.

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Acute and chronic exposures to neurotoxic compounds can compromise performance capabilities with effects ranging from minor to severe. The Global Assessment System for Humans (GASH) is a comprehensive set of neurobehavioral tests that is being developed to detect performance compromise resulting from exposure to neuroactive compounds and/or stressors. Last year we presented data using the GASH to identify performance deficits in military personnel exposed occupationally to jet fuel as part of our ongoing effort to systematically determine which neurobehavioral tests to include in the GASH. Tests are being selected based on ease of administration and sensitivity to detect and differentiate neurobehavioral deficits. In the current project 3 of the tests from the more comprehensive battery were used with military veterans in out-patient programs for either post-traumatic stress disorder (PTSD) or substance abuse (SA). They were evaluated by a clinical neuropsychologist prior to participation to confirm diagnosis. They were tested for performance on acoustic startle/prepulse inhibition (AS/PPI) with 3 different prepulse latencies (30, 60, & 120) to evaluate the integrity of a brainstem reflex gating mechanism for the response to loud bursts of sound, on eyeblink classical conditioning to assess a low level learning and memory system, and on a number of neuropsychological evaluation tasks from the computer-based Behavioral Assessment and Research System (BARS) where higher cognitive abilities were evaluated in tests of finger tapping, matching to sample, the Oregon dual task procedure, reaction time, symbol-digit matching, and serial digit learning. The results showed that acoustic startle and eyeblink conditioning were the tests that best discriminated between these groups. They demonstrated sufficient sensitivity to distinguish different aspects of neurobehavioral compromise in two separate groups of military veterans, and meet the criteria for inclusion in the final GASH product.

911 RISK FACTORS IN CARBON MONOXIDE POISONING.

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Carbon monoxide (CO) is a toxic by-product of the combustion of fossil fuels. In confined spaces, inefficient combustion sources, such as furnaces, stoves, kerosene heaters and automobiles can generate levels of CO that interrupt oxygen transport throughout the body, potentially resulting in death. CO interferes with oxygen transport primarily by avidly binding to the oxygen carrying pigment hemoglobin, rendering it incapable of its normal function of oxygen delivery to the tissues. CO also binds other iron-containing heme proteins like the cytochrome respiratory pigments in mitochondria, but these are secondary effects in the pathophysiology of CO. Emergency treatment for moderate to severely poisoned patients is primarily achieved through the use of hyperbaric oxygen treatment. At high pressure, sufficient oxygen dissolves in the blood plasma to provide oxygen to the tissues, making the poisoned hemoglobin irrelevant. During hyperbaric oxygen treatment, CO also dissociates from poisoned hemoglobin (carboxyhemoglobin) with a shorter half-time, leading to complete detoxification. In order to better prevent CO toxicity, we sought to understand the causal factors predisposing individuals to CO poisoning. We thus undertook a review of medical records from the Center for Hyperbaric Medicine in the University of Cincinnati Department of Emergency Medicine. We analyzed the records of patients by home address, age, gender, CO source, ethnicity, date of poisoning and educational status. We found that educational status was the strongest predictor of potential for CO poisoning. There was approximately a 50-fold difference in the poisoning rates between those with less than a grade-school education and those having a college degree. Clearly, education is a primary tool for the prevention of CO poisoning. These data illuminate the potential problem that the highest risk subpopulation is likely to have literacy problems, and may not be amenable to traditional public health education efforts.

912 ILLNESSES ASSOCIATED WITH A NOV. 1999 SPRINKLER APPLICATION OF METAM SODIUM IN EARLIMART, CA.

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The pesticidal action of metam sodium (MS) is dependent on breakdown to gaseous methyl isothiocyanate (MITC). MITC and several other MS degradates (MIC, CS_2, H_2S) are ocular and respiratory irritants. On Nov. 13, 1999, an illegal MS sprinkler application to a field ~1/3 mile southeast of Earlimart, CA produced a plume of airborne degradation products that moved off-site over several neighborhoods. This movement was caused by a shift in wind direction and the development of a post-sundown atmospheric inversion layer. Eye or upper respiratory irritation was documented in 81% of 136 self-selected individuals in zone A (0-0.6 miles from the field), 61% of 18 in zone B (0.6-0.82 miles), 50% of 10 in zone C (0.82-1.08 miles), and 60% of 5 in zone D (>1.08 miles). Non-specific symptoms (headache, nausea, dizziness, shortness of breath, abdominal pain, vomiting, weakness) occurred in 61% of the 173 total individuals evaluated. 16% had respiratory complaints, including 2.9% with exacerbated asthma or other lower airway problems. MITC concentrations were estimated with a Gaussian air dispersion model (ISCST3), using data from a local weather station and prior MITC monitoring studies. Estimated 1-hr time weighted average concentrations in zone A fell between 0.5 and 1 ppm, with estimated peak 1-min concentrations as high as 7 ppm. These air concentration estimates and the corresponding symptoms were consistent with the regulatory values developed in DPR's recently released risk assessment of MITC following agricultural applications of MS (critical acute NOEL=220 ppb, based on eye irritation in humans at 800 ppb [REL=22 ppb]; critical subchronic NOEL=100 ppb, based on nasal epithelial atrophy in rats [REL=1 ppb]). This report highlights the potential health concerns surrounding sprinkler applications of MS.

913 COMPARATIVE ACUTE AND COMBINATIVE TOXICITY OF AFLATOXIN B1 AND FUMONISIN B1 IN ANIMALS AND HUMAN CELLS.

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Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) are important food-borne mycotoxins that historically caused a variety of acute animals and human mycotoxicoses. Ubiquitous co-contamination of AFB1 and FB1 in human dietary components has been widely reported and is believed to play an important contributing role in high incidence of primary liver and esophageal cancers in certain areas of world. In this study, acute and combinative toxicity of AFB1 and FB1 were tested in F-344 rats and Mosquitofish (Gambusia affinis). Young male rats were randomly divided into five groups and were orally treated with 10.0, 4.64, 2.15, 1.0, and 0 mg/kg for AFB1 or 46.4, 10.0, 4.64, 2.15, and 0 mg/kg for FB1. The rats were observed for 7-days and lethality and toxic symptoms were recorded. The LD50 for AFB1 was calculated as 2.71 mg/kg with the 95% of confident limit (CI) at 2.00-3.69 mg/kg. No death was observed in FB1 treated groups although toxic symptoms in high dose groups are apparent. Healthy fish (half male and female) were randomly assigned to 6 groups and treated with 4.64, 2.15, 1.0, 0.464, 0.215, and 0 ppm of AFB1 or 10, 4.64, 2.15, 1.0, 0.464, and 0 ppm of FB1. The treated fish were observed for 5-days and death and toxic signs of fish were recorded. The LC50 for AFB1 was calculated to be 681 ppb with 95% CI at 420-800 ppb and LC50 for FB1 was 4.64 ppm with 95% CI at 2.15-10.0 ppm, respectively. Combinative studies used both biotoxins at doses of 1.0, 3/4, 1/2, 3/8, 1/4, and 1/8 LD50 or LC50 in both species. Additive toxic effects were observed in both species. Acute and combinative cytotoxicity of AFB1 and FB1 were also tested in human bronchial epithelial cells (BEAS-2B) and hepatoma cells (HepG2). These results will help for future studies on long-term combinative toxic and health effect of these mycotoxins and their mixtures. (Supported by the Soldier and Biological Chemical Command, US Army).

914 FOOD CONTAMINATION OF FUMONISIN B1 IN HIGH-RISK AREA OF ESOPHAGEAL AND LIVER CANCER.

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Fumonisins (FNs), produced mainly by Fusarium moniliforme, are ubiquitous contaminants of cereal grains around the world. Fumonisin B1 (FB1), the representative toxin, was the causative agent for several animal mycotoxicoses, and has etiologically been linked to human esophageal and liver cancers in certain areas of South Africa and China. To study relationships between exposure to FNs and human cancer risks, we investigate current status of FB1 contamination in food samples in Huaian and Fusui. Both areas have among the highest incidence of esophageal and liver cancers in China. Corn samples were collected from individual households in Haian in December 2001 and from Fusui in May 2002. Corn samples were also collected in Zibo, Shangdong Province, an area with low incidence of both cancers. The enzyme-linked immunosorbent assay (ELISA) and immunoaffinity-HPLC methods were used for analysis of these samples. In corn samples collected from residents of Huaian, FB1 was detectable in 93.3% (70/75) of the samples with the medium at 3.2 ppm (range from 0.1 to 25.5 ppm). FB1 was also detected in 85.2% (52/61) of the Fusui samples with the medium at 0.6 ppm (range from 0.1 to 2.7 ppm). Nineteen out of 41 (46.3%) Zibo samples were also detected for FB1 with the medium at 0.9 ppm ranged from 0.1 to 5.7 ppm. Twenty-four out of 70 (34.3%) positive Huaian samples had level of FB1 larger than 2.0 ppm, as compared with the level been found only in 3/19 (15.8%) Zibo samples. The high contamination rates of FB1 found in food from these areas, along with other previous reports, suggest the possible contributing role of FNs in human esophageal- and hepato-carcinogenesis. (This work was supported by the NCI grant CA94683).

915 ANTIBODIES AGAINST MOLDS AND MYCOTOXINS AFTER EXPOSURE TO TOXIGENIC FUNGI IN A WATER-DAMAGED BUILDING.

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Abstract Epidemiological studies have demonstrated an association between indoor fungal growth in water-damaged buildings and immunological symptoms. In relation to fungal antigens and allergens, there is significant emphasis on IgE mediated allergy. Since exposure to molds can cause IgE and non-IgE mediated allergy in humans, IgG and IgA antibody responses against different molds have been examined only in a few studies. The purpose of this study was to evaluate simultaneous measurements of IgG, IgM, IgA and IgE antibodies, as markers for fungal exposure, against the most common molds and their mycotoxins that have been cultured from water-damaged buildings as markers for fungal exposure. This study compared 40 patients occupationally exposed to molds with 40 control subjects without a history of exposure to indoor molds. Mold-exposed patients showed the highest levels of antibodies against one or more mold species. Most patients with high levels of antibodies against the antigens of molds exhibited elevated IgG, IgM, IgA or IgE antibodies against purified mycotoxins bound to human serum albumin (HSA), as well. This indicated patients' exposure to mold spores and mycotoxins.

These mold and mycotoxin antibodies were also detected in an insignificant percentage of healthy control subjects suggesting population exposure to environmental molds and mycotoxins. These antibodies against molds and mycotoxins are specific, since immune absorption studies demonstrated that only fungal specific antigens and mycotoxins could significantly reduce antibody levels. Detection of high cfu/m3 of molds, which strongly suggests the existence of a reservoir of spores at the time of sampling in the building along with a significant elevation in IgG, IgM or IgA antibodies against molds and mycotoxins, could be used as a marker for fungal exposure.

916 INHIBITION OF CERAMIDE SYNTHASE IN CORN SEEDLINGS INFECTED WITH *FUSARIUM VERTICILLIOIDES* OR EXPOSED DIRECTLY TO FUMONISIN B1 IN SOIL.

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Fumonisins (FB) are water soluble mycotoxins produced by *F. verticillioides*, which is parasitic to corn plants. FBs are inhibitors of ceramide synthase. While FB1 is not known to cause plant disease, it is found in the ear, roots, and stalks of corn. FB1 in corn debris can be leached by rainfall and can move through soils intact. However, a large amount is bound in certain soils. In sandy loam soils, FB1 can be released under acid conditions. These data suggest that FB1 from corn debris, or produced by *F. verticillioides* in the soil, can accumulate in soil and under certain environmental conditions the FB1 could be released and become biologically available. The objectives of this study were to determine: i) can *F. verticillioides* produce FB1 in soil, ii) is the FB1 in soil biologically active, and iii) is FB1 in soil toxic to corn seedlings. Corn seeds were inoculated with spore suspensions from pathogenic or non-pathogenic strains of *F. verticillioides*. Seedlings were grown for 21 days in potting soil. The roots and soil were analyzed for sphingoid bases (a marker of ceramide synthase inhibition) and FB1, respectively. Only seedlings inoculated with the pathogenic strain had elevated levels of sphinganine and the degree of elevation was correlated with the severity of the pathology. FB1 was only detected in soil from the plants inoculated with the pathogenic strain. In a second experiment surface sterilized seeds were planted and watered for six days with of 1, 5, or 10 ppm FB1 and grown for 15 more days. The FB1 in soil and the sphinganine in roots were closely correlated with the FB1 dosage. Reduced root mass was noted at 10 ppm. These results show that under laboratory conditions i) FB1 can be produced by *F. verticillioides* in soil, ii) the soil FB1 taken up by plants is biologically active, and iii) FB1 in soil can have adverse effects on plants. Thus, providing proof in principle that FB1 in soil can cause corn seedling disease.

917 TRICLOSAN AS INHIBITOR OF THE SULFONATION AND GLUCRUONIDATION OF 3-HYDROXY-BENZO(a)PYRENE IN HUMAN LIVER.

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Triclosan (2, 3, 4'-trichloro-2'-hydroxydiphenyl ether) is a very common antibacterial agent used in a number of products as diverse as toothpaste, detergents, and clothing. Due to its similar structure to hydroxylated PCBs, which were recently demonstrated by our laboratory as potent inhibitors of sulfonation and glucuronidation of 3-hydroxy-benzo[a]pyrene (3-OH-BaP) in either fish or human, it is of interest and important that the health effects of triclosan be assessed. In this study, triclosan was shown to inhibit the sulfation and glucuronidation of 3-OH-BaP at low, environmentally relevant, micromolar concentrations (IC50, about 5/*mu*/M) in human liver. Second, kinetic studies suggested that the nature of the 3-OH-BaP sulfonation and glucuronidation inhibited by triclosan is non-competitive, similar to the inhibition of 3-OH-BaP sulfotransferase by hydroxylated PCBs in our previous research. Finally, the sulfonation and glucuronidation of triclosan were investigated. It was shown that triclosan was metabolized to the glucuronide (Vmax: 833 pmol/min/mg protein) more readily than sulfate (Vmax: 100 pmol/min/mg protein), as observed similarly in skin in previous study. These findings reveal that the commonly used, effective bactericide triclosan can create unwanted effects, and specifically may have important consequences for the biotransformation and toxicity of other phenolic xenobiotics.

918 SHORT TERM FEEDING OF 2, 4-HEXADIENAL (HX) ALTERS STOMACH GROWTH IN IMMEDIATE POST-NATAL SPRAGUE-DAWLEY RATS.

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HX (an unsaturated aldehyde formed as the product of lipid peroxidation) is found naturally or as additives in many foods. Long term feeding of HX led to high incidence of forestomach cancers in mice and F344 rats. In F344 rats, short term feeding (10 days) also caused microscopic and macroscopic changes in their forestomach. We did short term feeding of HX to Sprague-Dawley rats to see whether it would change their gastric growth characteristics. HX (200 mg/kg/day) fed to ~26 day-old rats for 5 days led to an increase in total stomach weight (wt). Both fore and glandular stomach showed increases in their respective wts with the forestomach being more pronounced such that the ratio of fore/total stomach wts increased significantly. HX feeding did not affect the wt of the esophagus or kidney but decreased that of the liver. The increase in forestomach wt was dependent on the dose of HX and the duration of feeding. An increase in forestomach wt/100 gm body wt was evident even at 6.25 mg/kg of HX for 5 days but the total stomach wt/100 gm body wt did not attain significant difference from controls till the dose of HX reached 200 mg/kg for 5 days. At 200mg/kg of HX, a moderate increase in forestomach wt was found after 4 days of feeding. Further increase in forestomach wt was observed after 5 days. Changes in forestomach wt and fore/total stomach wt ratio were transient, as they returned to values comparable to controls 4 days after the cessation of HX feeding. HX seems to promote a selective growth in the stomach particularly the forestomach but not in other tissues studied. This suggests that HX targets the stomach, specifically the forestomach. How this growth promotion relates to cancer development in prolonged HX feeding is unknown at present.

919 EXPOSURE TO CARBON MONOXIDE AT ALTITUDE.

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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. Populations at altitude comprise residents, fully adapted to altitude, and short-term visitors or sojourners. It is the sojourner who is at greater risk from exposure to CO at altitude. The most prominent feature of the altitude environment, decreased oxygen tension (hypoxia) in the inspired air causes respiratory changes that affect the dosimetry and potential toxicity of CO. The NAAQS for carbon monoxide (CO) is 9 ppm for 8 hrs and 35 ppm for 1 hour exposure. These values are derived from studies in subjects with exercise-induced angina exposed to CO while exercising at sea level. Kleinman et al (1998) showed that time to onset of angina was reduced by both exposure to CO at sea-level (9%) or to clean-air exposures at high-altitude (11%), compared with clean air at sea level. However, joint exposure to CO at a high altitude reduced the time to onset of angina, relative to clean air, by 18%. Thus, CO presents a more significant threat to cardiac-impaired subjects at altitude. Moreover, emissions of CO from combustion sources are higher at altitude because of the lower oxygen tension in the atmosphere available for combustion. Accordingly, emissions from snowmobiles, older vehicles and vehicles whose emission controls have been altered by tampering, heating and cooking devices are all increased at altitude. CO measurements made along a snowmobiling trail in the Grand Teton National Park, WY were as high as 23 ppm with individual measurements as high as 45 ppm (Snook and Davis, 1997). Ambient CO concentrations in various microenvironments may also be elevated at altitude. Measurements made in diverse shelters (tents, igloos, snow caves) on Denali, AK usually exceeded 35 ppm and reached a maximum of 190 ppm (Turner et al, 1988). Therefore, CO presents a special hazard at altitude because of the altered physiological status of healthy individuals and cardiac-impaired subjects, the pathophysiological status of cardiac impaired subjects and its increased production by combustion sources.

920 MORPHOLOGICAL CHANGES AND METAL ACCUMULATION IN THE LUNG OF AGED DOGS.

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Physiological function of the respiratory system is known to become less effective with advance of age. There have been, however, few pathological reports on the age-related pathological changes of lungs in humans as well as in animals. Reported changes include emphysema, thickening of the alveolar walls by increased volume of collagen and/or elastic fibers. Little is known of the mechanism of these changes. We examined 1) morphological changes and 2) accumulation of the inhaled suspended particulate matters (SPM) with the special emphasis on the metals in the lungs from dogs. Laminin immunohistochemistry and silver staining for the analysis of basement membrane, Prussian blue DAB and post DAB enhancement technique for the detection of iron and azan stain for the fibrosis were carried out as histological examination. Metals were determined using ICP-MS after wet digestion of the tissue samples. Lung and hilar lymph nodes from 7 young animals, ranging in age from 1 week to 4 years, and from 30 old animals, from 10 to 17 years, were used. Fibrosis and attenuated basement membrane were demonstrated in the alveolar walls of aged dog lungs. Intensity of SPM deposition in both lung tissue and hilar lymph nodes increased with age. Signs of tissue damage by the released reactive oxygen species were also shown with the use of 8-OHdG immunohistochemistry. Age-related increase of the level of metals (Fe) known to responsible for the

generation of the oxygen radicals was also demonstrated. Lung and hilar lymph nodes from the aged dogs may be good biological samples for the study of the effect of air pollution on the respiratory system.

921 DIESEL SOOT BINDS IL-8 IN A BIOACTIVE FORM.

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Exposure to air pollution particulate material (PM) has been implicated in health effects, including inflammatory responses in the lungs. To examine the direct effects of a surrogate air pollution PM on alveolar epithelial cells, we exposed monolayers of A549 cells to a wide range of concentrations of diesel PM (DPM: National Institute of Standards and Technology Standard Research Material 2975) and analyzed release of the neutrophil chemoattractant Interleukin-8 (IL-8) from these cells by ELISA. Low doses of DPM increased the concentration of IL-8 detected in the conditioned medium after 24 hours. Higher doses appeared to suppress the response, although this suppression was not related to acute DPM toxicity as determined by release of lactate dehydrogenase. In a cell-free system, incubation of IL-8 with DPM resulted in a loss of immunoreactive IL-8 in the supernatant of the reaction. In contrast, carbon black did not reduce the concentration of IL-8 in the mixture. The DPM-induced loss was only weakly blocked by a large excess of bovine serum albumin (BSA), indicating some specificity of the effect. When human blood neutrophils were exposed to DPM that had been pre-incubated with IL-8, then washed to remove free IL-8, they underwent morphological changes suggestive of orientation toward the particles. This orientation was not observed with either carbon black that had been incubated with IL-8 or with DPM alone. These results suggest that DPM binds IL-8 in a particle- and protein-selective manner, and that the resultant DPM-bound IL-8 is biologically active. Supported by Office of Heavy Vehicle Technology, US Department of Energy.

922 MECHANISMS OF NITROGEN DIOXIDE-MEDIATED CYTOTOXICITY.

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Nitrogen dioxide (NO_2) is a ubiquitous, pollutant gas that produces a broad range of patho-physiological changes in the lung. In our present study, we evaluated the mechanism(s)of NO_2- mediated cytotoxicity, using normal human bronchial epithelial (NHBE) cells as an *in vitro* experimental model. Cellular responses following brief exposure to high levels (45 ppm) of NO_2 in presence or absence of various cytokines, were monitored. At the end of 1, 6, 24 and 48 hours post exposure, levels of pro-inflammatory cytokines such as IL-8, TNF-α, IFN-γ & IL-β, were measured. Generation of NO, sICAM-1 and mICAM-1 were also monitored. Our results have shown a time-dependent increase in NO/nitrite levels (3-5 folds) in exposed NHBE cells. In order to ascertain the source of this high nitrite, iNOS and eNOS protein levels were checked at different time intervals. Increased expression of iNOS and to a smaller extent, of eNOS, were observed at 24 hrs post exposure. Also, lack of complete inhibition by addition of iNOS specific inhibitors indicated that a significant amount of the increased nitrite is formed *via* non-enzymatic reactions. Also, significantly elevated levels of IL-8 and IL-1β were observed during early time intervals(1-6 hr), suggesting the role of proinflammatory mediators in initiating NO_2-mediated cytotoxicity. In order to ascertain the role of cell-cell interactions in NO_2 - mediated cytotoxicity, we also checked for the levels of intercellular adhesion molecules (sICAM-1 & mICAM-1). Our initial experiments indicate an increased expression of both sICAM-1 & mICAM-1 proteins in NO_2 - exposed cells. Significant ICAM-1 induction was observed in presence of TNF-α and IFN-γ, either alone or in combination. N- [3-(amino methyl) benzyl] acetamidine (1400w), a highly specific iNOS inhibitor, inhibited ICAM-1 expression significantly. This suggests an involvement of NO in expression of ICAM-1. In conclusion, we suggest that proinflammatory mediators such as IL-8, IL-1β and NO may play important roles in NO_2 mediated cell injury, either directly or indirectly *via* induction of ICAM-1.

923 PHOTOCHEMCIAL REACTIONS OF URBAN AIR POLLUTION MIXTURES ENHANCE INFLAMMATORY RESPONSES IN LUNG CELLS.

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There has been an increasing interest in examining complex urban air mixtures that realistically mimic urban smog rather than investigating the adverse health effects induced upon exposure to individual known components of urban smog. Smog chambers that has been used to conduct experiments for developing photochemical models for ambient ozone (O3) concentrations, were used to generate photochemical air mixtures. These photochemically altered air pollution mixtures were interfaced with an *in vitro* exposure system to compare the inflammatory effects of complex air pollutant mixtures with and without sunlight driven photochemistry. Briefly, two matched outdoor chambers capable of using real sunlight were utilized to generate two test atmospheres for simultaneous exposures to cultured lung cells. One chamber was used to produce a photochemical active system, which ran from sunrise to sunset, producing O3 and the associated secondary products. A few hours after sunset, NO was added to titrate and remove completely the O3, forming NO2. In the second chamber, an equal amount of NO2 was injected and the same amount of the 55-component hydrocarbon mixture used to setup the photochemical system in the first side. A549 cells, an alveolar type II-like cell line grown on membranous support were exposed to the photochemical mixture or the original NO2/hydrocarbon mixture for 5 hours and analyzed for IL-8 mRNA production 4 h post-exposure. In addition, a variation of this experiment was conducted to compare the photochemical system producing O3 and NO2, with a simple mixture of only the O3 and NO2. Our data suggest that the photochemically altered mixtures which produced secondary products induced an about 2-3 fold greater IL-8 mRNA production than the mixture of NO2 and hydrocarbons or O3. These results indicate that secondary products generated through the photochemical reactions of NOx and hydrocarbons significantly contribute to the inflammatory responses induced by exposure to urban smog.

924 GEOCHEMICAL SOLUBILITY OF ASBESTOS TOXICOLOGICAL STANDARDS IN SIMULATED LUNG FLUIDS.

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Asbestos mineral dust exposure is associated with lung diseases such as asbestosis, malignant mesothelioma, and bronchogenic carcinoma. Within the past decades, *in vitro* and *in vivo* studies have shown asbestos to be cytotoxic and mutagenic, yet the mechanisms responsible have escaped definitive identification. Asbestos-related diseases may be the result of two mechanisms, mechanical and intracellular, based on mineralogy, size, shape, chemistry, elemental speciation, and electral properties of the asbestos particles yet in the literature contradictions for specific asbestiform minerals can be found. Thus, the mechanistic questions may best be answered through increased collaborative research between the geochemistry and health-related communities. One key step in the research process is the mineralogical and geochemical characterization of commonly used asbestos toxicological standards. The USGS has analyzed 5 sets of asbestos standards (5 amosites, 4 anthophyllites, 6 chrysotiles, 5 crocidolites, 4 tremolites) by ICP-MS XRD, SEM, and EDS. The level of toxicity was determined as well. The initial analysis revealed mineralogical, geochemical and toxicological variations between the asbestos standards as well as within the sets of the same nominal asbestos mineral. To further characterize these standards, geochemical solubility studies in simulated lung fluids (SLF) were performed. Elemental solubility determined by ICP-MS analysis following sample incubation with SLF as compared to water revealed additional variations between the standards and within each standard set. Furthermore, SLF and water generated different solubility profiles for each of the individual asbestos standards. These variations between the asbestos standards, or variance within the sets of the same asbestiform mineral, may be responsible for conflicting toxicological results, ultimately inhibiting mechanistic identification. Differences in elemental solubility between the SLF and water indicate that the testing matrix may play a role in the toxicological response.

925 THE CHEMICAL COMPOSITION AND REACTIVITY OF DUSTS DEPOSITED BY THE 9/11/2001, WORLD TRADE CENTER COLLAPSE.

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As part of an environmental study of the WTC area after 9/11/2001 (http://greenwood.cr.usgs.gov/pub/open-file-reports/ofr-01-0429/), we characterized chemical composition and reactivity of dusts deposited in lower Manhattan. We analyzed sweep samples (2 indoor, 15 outdoor) collected within the first 2 weeks after 9/11. Major-element concentrations (Si, S, Mg, Al, Fe, C) of the dust samples reflect the contributions of glass fibers, concrete, gypsum wallboard, window glass, metals, paper, and other materials. Concentrations of some trace metals (Zn, Ba, Pb, Cu, Cr, Mo, Sb, Ti) are higher than in many natural soils, reflecting contributions from paints, lighting, electrical wires, pipes, fire retardant materials, computer equipment, electronics, and other diverse materials. Leach tests of the dust samples using water show the dusts are quite chemically reactive. Alkaline leachate solutions were

produced due to rapid partial dissolution of Ca(OH)2 from concrete particles. Indoor dust samples generated higher pH (11.8-12.4) than outdoor dust samples (8.2-10.4), indicating that the alkalinity of the outdoor dusts can be progressively neutralized by reactions with carbonic acid in rainwater. Although they generate caustic alkalinity, the indoor dusts are not as caustic as common drain cleaner or cement. Leach tests with simulated lung fluids (SLF) produced smaller pH shifts due to the buffering capacity of SLF components. Some metals in the dusts (Si, Al, Cr, Sb, Mo, Ba, Cu, Zn, Co., Ni) are quite soluble in water, and many are even more soluble in SLF due to chelation by chloride, citrate, and glycine. Leach tests with simulated gastric fluids are currently in progress. Information on the materials makeup, chemical composition, and chemical reactivity of the dusts may be useful in interpreting potential origins of some of the health problems that developed in individuals exposed to WTC dust after 9/11, such as WTC cough, bronchial hyperreactivity, and gastroesophageal reflux.

926 POLYCYCLIC AROMATIC HYDROCARBONS AND METALS IN SOILS OF TWO NEW ORLEANS COMMUNITIES.

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We evaluate 16 polycyclic aromatic hydrocarbons-PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz(a)anthracene, Chrysene, Benzo[k]fluoranthene, Benzo[j]fluoranthene, Benzo(a)pyrene, Indeno[1, 2, 3-cd]pyrene, Dibenz[a, h]anthracene, and Benzo[g, h, i]perylene) and 8 metals (Pb, Zn, Cd, Mn, Ni, Cu, Cr, V) in soils of an inner city and an outlying suburban community of New Orleans. Surface (2.5 cm deep) samples were air-dried and sieved (2 mm). Accelerated solvent extraction was used for PAH analysis with gas chromatography-mass spectrometry. Metals were extracted at a 5:1 ratio of 1 mol nitric acid (room temperature) to soil, shaken 2 hours, centrifuged (1000 x g - 15 min.) and filtered for inductively coupled plasma-atomic emission spectrometry analysis. Pearson product moment correlation reveals that urban soils exhibit a correlation coefficient = 0.78, P = 10^{-6}, between PAHs and metals. Totals for PAHs and metals are summarized in Table 1 for the residential locations described above and indicate significant differences. Because children are so sensitive to contaminants, the data pose questions about toxic effects of chemical mixtures to human health in real-world settings. ACKNOWLEDGEMENTS: Metal research funded by ATSDR/MHPF cooperative agreement U50/ATU398948 and PAH research funded by DOD Grant # DSWA01-97-1-0028.

	Suburban Community		Inner City Community	
Percentiles	PAHs µ/kg	Metals mg/kg	PAHs µ/kg	Metals mg/kg
Min	527	72	906	392
10	615	86	1147	545
25	663	112	1880	840
Median	731	183	2927	1323
75	889	218	5164	2036
90	1167	320	7026	4846
Max	3753	559	7285	15498
N	19	19	19	19

927 METALLOTHIONEIN AND GLUTAMATE-CYSTEINE LIGASE GENE EXPRESSION IN METAL-EXPOSED SMALL MAMMALS.

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Preliminary studies of small mammals at the Anaconda Smelter Site (Montana) have shown varying tissue concentrations of arsenic, lead, cadmium, copper, and zinc. This has provided a unique opportunity to study multi-species and multi-dose-responses for gene expression in tissues from wild rodent populations. Glutamate-cysteine ligase (GCL, the initiating enzyme in glutathione synthesis) and metallothionein (MT) levels are known to increase with heavy metal exposures in mammalian tissues. GCL (both the catalytic and modifying subunits) and MT mRNA expression have been studied in laboratory colonies of mice and rats after acute and semi-chronic individual heavy metal exposures. However, few field-based chronic-exposure studies of wild rodent populations have been conducted with these gene expression biomarkers. In this study, GCL and MT mRNA expression levels in the livers of *Peromyscus maniculatus* collected from the Anaconda Smelter site were measured with real-time quantitative PCR (RT-PCR). Post-transcriptional regulation will also be determined by Western blot analysis. Following these analyses, cDNA microarray analyses will be attempted to determine novel biomarkers. Preliminary analyses of RT-PCR data indicate significant correlations between transcriptional gene expression for GCLc and cadmium liver concentrations in both males (R^2=0.5732, p=0.004) and females (R^2=0.4857, p=0.046); and between both GCL subunits and copper concentrations in females only (GCLc R^2=0.6465, p=0.005; GCLm R^2=0.5483, p=0.002). However, no correlation was found for MT and liver concentrations of any of the metals. Interestingly, a correlation has also been found between GCLc and GCLm expression for males (R^2=0.6456, p⟨0.05) and females (R^2=0.5916, p=0.02), suggesting possible co-regulation. Sponsored by NIEHS grants P42ES04696 and P30ES007033.

928 EFFECTS OF CHROMATED COPPER ARSENIC (CCA) TREATED WOOD EFFLUENTS ON RATS.

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CCA is the most commonly used chemical treatment of wood products. Humans are exposed to CCA when they contact treated wood which is used in fencing, playgrounds, decks, porches, utility posts, rafts, sound barriers, and building frames. Exposure can also occur when people contact water runoff from treated wood. CCA is a chemical mixture for which there is limited data, although there are several toxicity reports on the individual components of this mixture. The major objective of this study is to evaluate the effects of the CCA mixture (Type I) on rats. Adult rats were exposed to CCA by oral gavage. Some of these animals received various doses of CCA from wood soaked effluent. Other groups received various doses of a solution of the chemical mixture. Control groups received the vehicle alone. During the exposure, body weights, clinical signs, and feed consumption were monitored. Hematological and clinical chemistries were evaluated. Pathological and toxicological evaluations were conducted on various organs. Also, the mutagenic potential of the CCA mixtures was evaluated. There was a significant decrease in body weight gain among rats exposed to 40 mg/kg/day of CCA mixture. There was a trend toward increased feed consumption during the first and fourth weeks of exposure. Alkaline phosphatase and alanine transaminase were significantly higher than in the control rats. Based on these data the CCA mixture adversely affected rats by oral exposure.

929 UTILIZATION OF A HOMING PIGEON (*COLUMBA LIVIA*) MODEL TO ASSESS THE EFFECTS OF NON-LETHAL EXPOSURES TO MINE WASTES AND PESTICIDES IN MIGRATORY BIRDS.

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Other than the Migratory Bird Treaty Act, which is predicated upon no acceptable losses of migratory birds, there are no regulatory provisions that afford protection for health and survival of migratory bird species. Acute non-lethal exposures to mine wastes and pesticides including cyanide, arsenic, organophosphates and carbamates have been shown to have adverse biochemical effects on avian species, which could affect migration. It is unclear how these biochemical effects might impact these birds at a population level by affecting their ability to migrate. We previously showed that cyanide and arsenic exposure deplete intracellular ATP in these birds. Studies reported by this laboratory also suggested that homing pigeons could serve as a model to assess the impact of cyanide exposure on migratory birds. Preliminary results suggested an increase in flight time following non-lethal oral doses of cyanide. We have expanded these studies to demonstrate that this non-lethal exposure to cyanide results in a dose-dependent increase in flight time. The data also show that flight time increases with distance traveled. These studies are currently being expanded to test this homing pigeon model on arsenic and the acetylcholinesterase-inhibiting pesticides diazanon, methidathion, and aldicarb. Mitochondrial respiration, tissue ATP levels, and cholinesterase-inhibiting activity will provide information on biochemical effects as they are related to flight times. The purpose of these expanded studies will be to show the utility of this homing pigeon model for testing the biological impact of non-lethal exposure to various anthropometrically generated xenobiotic compounds on migratory birds. These studies were funded in part by USDA Hatch Grant NEV 00727.

930 REGIONAL, NOT TROPHIC, FORAGING PATTERNS SUBSTANTIATE DIFFERENCES IN CONTAMINANT LEVELS BETWEEN TWO NORTH PACIFIC ALBATROSS SPECIES.

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Seabirds, due to their high trophic level and wide foraging ranges, are good indicators of contamination in the marine environment. We tested the hypothesis that seabird contaminant body burden is determined by 1) the trophic level at which they feed, and/or 2) the location in which they forage, by measuring contaminant loads (sum PCBs, sum DDTs) as well as carbon and nitrogen stable isotopes ($\delta^{15}N$, $\delta^{13}C$) in two closely related seabirds: Black-footed (*Phoebastria nigripes*) (BFAL) and Laysan (*P. immutabilis*) (LAAL) Albatross. Blood samples (~6mls) were collected from adult birds on Sand Island, Midway Atoll in May of 2000 and 2001. Samples were analyzed for PCBs and DDTs by gas chromatography. $\delta^{15}N$ and $\delta^{13}C$ values were measured by mass spectrometry. PCB and DDT levels for BFAL were 3 to 4-fold higher than for LAAL (PCBs and DDTs (ng/mL ± SD) for BFAL (n=25) = 161 ± 91 and 127 ± 146; for LAAL (n=16) = 46 ± 17 and 30 ± 15 respectively; $p \leq 0.001$, Mann-Whitney University). $\delta^{15}N$ values, used to ascertain trophic position, were marginally not significantly different between species (p=0.057, t-test, n=31 BFAL, 17 LAAL), however, $\delta^{13}C$ values, used to discriminate foraging areas, were highly significantly different ($p \leq 0.001$, t-test, n=31 BFAL, 17 LAAL). The $\delta^{15}N$ and $\delta^{13}C$ values suggest that differences observed in contaminant levels between BFAL and LAAL are driven primarily by differences in contaminant loads associated with different feeding locations, and less by trophic position. Furthermore, DDE levels for LAAL and BFAL show a 2- to 3-fold increase compared to levels reported from 1992-93 (Auman et al., 1997). When combined with albatross satellite foraging data and the observation that levels of contaminants, especially DDE, may not be diminishing over time in the environment, the results of this study help to elucidate regions of high marine contamination.

931 ESTROGEN RECEPTOR ALPHA, BUT NOT BETA OR GAMMA EXPRESSION IS INDUCED BY ESTRADIOL AND XENOESTROGENS IN FISH LIVER TISSUE.

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Estrogen receptors (ER) are nuclear transcription factors that control the expression of numerous genes involved in the reproductive process. We have obtained the entire coding regions of three distinct ER isotypes, alpha, beta, and gamma from Largemouth Bass (*Micropterus salmoides*) (LMB). The three ERs are most highly conserved in the DNA binding domain and show the most diversity in the trans-activation domains. ERs beta and gamma however share more sequence identity overall in comparison to ER alpha. The discovery of these multiple ER isotypes raises questions as to whether these receptors regulate gene expression similarly in response to 17β-estradiol (E2). One such gene that is induced *via* the ER pathway is vitellogenin (vtg). In order to determine the normal expression patterns of the ER isotypes and vtg during reproduction we measured mRNA levels by real-time PCR in females from October through April. The data show that maximal ER alpha induction correlates with peak vtg mRNA and plasma levels of vtg and E2. There was no correlation between the levels of vtg and ERs beta and gamma suggesting that the ER isotypes are not regulated in the same manner by E2. Male oviparous species do not normally produce vtg but the synthesis of this gene can be induced by E2. When male LMB were injected (ip) with E2 (2.5 mg/kg), ER alpha and vtg mRNAs were induced, with no significant increase in ER beta and gamma mRNAs. Similarly, exposure of LMB to nonylphenol (NP) produced the same response. We also investigated the induction of the ERs over time following a single injection of NP (50 mg/kg). ER alpha and vtg expression increased at 24 hours, peaked at 48 hours, and returned to basal levels by day 7 while no significant change in the expression levels of ERs beta and gamma occurred. This data indicates that NP affects ER mediated gene expression in a similar manner to E2 by inducing only the ER alpha isotype.

932 RELATIVE BINDING AFFINITY OF ENDOCRINE DISRUPTING CHEMICALS TO ESTROGEN RECEPTOR IN TWO SPECIES OF FRESHWATER FISH.

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The USEPA has been mandated to screen industrial chemicals and pesticides for potential endocrine activity. This study was designed to assess the plausibility of making inter-species extrapolations of affinity of chemicals to bind fish estrogen receptors (ER). Fifteen chemicals previously tested for binding affinity to rainbow trout liver ER (rtER) were tested for affinity to fathead minnow ER (fhmER). Liver tissue from 20 (male) to 50 (female) fish was the source of cytosolic ER. Binding affinity was measured by incubating liver cytosol (255 µl) with a saturating concentration (5nM) of ^{3}H-estradiol (^{3}H-E2) and a range of test chemical concentrations for 20hr at 4°C. Bound chemical was determined as the amount of ^{3}H-E2 displaced from the ER. RBA was calculated as: (IC_{50} of E2/IC_{50} of chemical)*100 (E2 = 100%). Chemicals tested included estradiol (E2), estrone (E1), estriol (E3), ethinylestradiol (EE), diethystilbestrol (DES), methylestradiol (MeE2), genistein (GEN), tamoxifen (TAM), testosterone (T), methyltestosterone (MT), α-trenbolone (ATren), β-trenbolone (BTren), p-tert-octylphenol (PTOP), p-nonylphenol (PNP) and methoxychlor (MXC). DES, EE, MeE2 and E1 bound with high affinity to the fhmER, having RBAs of 590, 170, 70 and 30%, respectively. E3, TAM and GEN yielded RBAs of 5, 4 and 1, respectively. The alkylphenols PNP and PTOP had weak affinity for the fhmER, with RBAs of 0.1 and 0.01, respectively. The androgens ATren (RBA = 0.002) and BTren (RBA = 0.02) fully displaced ^{3}H-E2, whereas T, MT, and MXC only partially displaced ^{3}H-E2;thus RBAs could not be calculated. This weak affinity of T, MT and MXC for the fhmER was also seen with the rtER. Comparison of binding affinities of the chemicals tested for fhmER and rtER shows that the rank order of RBAs are nearly identical across species. This abstract does not necessarily reflect EPA policy.

933 EFFECTS OF ATRAZINE ON REPRODUCTIVE SUCCESS IN THE MARINE FISH, CUNNER (Tautogolabrus adspersus).

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Atrazine, the most widely used herbicide in the world, leaches into ground water and surface runoff after agricultural and forestry applications. It has been detected in concentrations in the ppb range in ground water, surface waters, rivers, streams, and precipitation. Atrazine has also been reported to have endocrine disrupting activity, and is theorized to affect steroidogenesis in vertebrates by inducing aromatase, the enzyme complex that converts testosterone to estradiol. We tested the hypothesis that exposure to atrazine will affect reproductive success in a marine fish species, cunner (*Tautogolabrus adspersus*). During a one week pre-exposure period, cunner in spawning condition were monitored daily to determine egg production, egg fertility, and egg viability. Atrazine, mixed into a slow release matrix, was then implanted subcutaneously into each fish below the dorsal fin. Treatments were control (matrix only) and two nominal concentrations of atrazine, 0.5 and 5 mg/kg (500 and 5000 ppb, respectively). Each treatment consisted of four replicate tanks containing up to two male and three female fish. Eggs were collected daily and evaluated within 24 hours to determine total number, number fertile, and number developing normally (viable). At termination of the experiment, sperm motility was checked in males and blood was drawn from all fish. Fish were dissected to determine gonad and liver weights. Brain, gonad, liver and adipose samples were flash frozen and archived for later analysis. Results showed no significant differences between treatments or between pre- and post-exposure fish in daily female egg production, percent fertile eggs, and percent viable eggs. All males produced milt and had motile sperm. Likewise, there were no significant differences between treatments in average gonadosomatic and hepatosomatic indexes of female or male fish. These results indicate that this type of atrazine exposure does not affect the reproductive success of mature cunner.

934 LABORATORY FISH GONADAL SEX DIFFERENTIATION: A COMPARISON OF THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*), SHEEPSHEAD MINNOW (*CYPRINODON VARIGATUS*) AND ZEBRA FISH (*DANIO RERIO*).

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Due to their close interactions with the aquatic environment fish are regarded as a sentinal species for monitoring environmental endocrine disruption. Exposure of fish to endocrine modulating chemicals (EMCs) during the period of gonad development leads to skewing of the population sex distribution, intersex fish (i.e ovotestes) and abnormalities in the gonadal ducts. The study was performed to assess the maturity of the gonads at 30, 60 and 90 days post hatch to determine the optimal time-point for gonadal histopathological evaluation. 20 fish per species per time point were sectioned using methods developed in our laboratory that allowed evaluation of all gonadal zones and associated duct structures. Slides were evaluated histologically. Results: In all three species, the female gonad developed earlier than

the male, with ovarian follicles identifiable in a proportion of fish by 30 days and all females by 60 days post hatch. Male gonads were identifiable in some fish by 60 days, and in all male fish, in each of the three species, by day 90. Although gonadal sex was easily determined by day 90, reproductive maturity (as indicated by mature spermatid populations) was not detectable in fathead minnows at this time. Unlike some laboratory populations of zebra fish where males pass through an intersex phase during gonadal differntiation, the male fish we studied differentiated directly to the male phenotype, with no intersex phase. Sheepshead minnows, unlike the other species that have paired gonads, have a single central gonad. In conclusion by 90 days it is possible to definitively determine the gonadal sex of all three species allowing detection of EMCs affecting gonadal sex differentiation. This evaluation would not be possible at earlier time-points, as a proportion of the fish have indeterminate gonads. The technical processing allowed evaluation of the EMC sensitive accessory ducts.

935 ENDOCRINE DISRUPTION AND PERSISTENT BIOACCUMULATIVE CONTAMINANTS IN COLUMBIA RIVER WHITE STURGEON.

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Columbia River white sturgeon reproduction may be impaired due to loss of habitat and contaminant exposure. This is a report on research from a 3-year study looking at endocrine disruption and reproduction in Columbia River white sturgeon. Sturgeon were collected from the Columbia River estuary, and pools above Bonneville, The Dalles, and John Day dams. Condition factor (CF) and gonadosomatic index (GSI) were measured for all fish. Blood plasma samples were analyzed for 17β-estradiol, testosterone (T), and 11-ketotestosterone (KT), using RIA. Plasma vitellogenin (Vtg) was analyzed using ELISA. Livers and gonads were examined histologically and analyzed *via* GC-ECD for chlorinated pesticides and PCBs. Muscle tissue was analyzed for mercury using CVAA. The putative cytochrome P450 3A was measured in hepatic microsomes by western blotting. p, p'-DDE was the predominant contaminant found in gonad and liver tissues. Mercury was detected in all muscle samples, with >13% of the sample concentrations exceeding 0.5 ppm. CF was lower and incidence of histological gonad and liver abnormalities were higher in Bonneville fish versus fish from other sites. Liver and gonad p, p'-DDE was significantly higher in Bonneville fish. Male plasma T and KT levels and GSI were negatively correlated with liver p, p'-DDE. Hepatic cytochrome P450 3A in males was positively correlated with p, p'-DDE and negatively correlated with plasma androgens. Plasma Vtg was significantly higher in John Day and The Dalles males compared to fish from other sites. These data indicate that endocrine disruption is occurring in Columbia River white sturgeon, which may be contributing to poor reproductive success.

936 THE EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON ORNITHINE DECARBOXYLASE (ODC) INDUCTION IN THE GONAD OF THE EASTERN OYSTER (*CRASSOSTREA virginica*).

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The naturally occurring polyamines putrescine, spermidine and spermine are present in all living cells, prokaryotes, eukaryotes, plants and animals. Ornithine decarboxylase (ODC) is the key enzyme of polyamine synthesis. The physiological activity of ODC is associated with cell proliferation. ODC is a highly inducible enzyme regulated by hormones, growth factors, xenobiotics carcinogens and tumor promoters. The environmental contaminant 2, 3, 7, 8-TCDD has been shown to be a tumor promoter as well as, cause adverse reproductive effects in several human and animals models. The effects of TCDD on ODC and tissue polyamine levels are controversial. Determination of TCDD induced changes in tissue polyamine levels may explain some of the degenerative processes seen in TCDD- treated animals. The rational for this study is based on the well documented reproductive toxicity of TCDD and the importance of ODC in regulating polyamine levels essential for cell growth and differentiation. Oysters (N=30) were adductor muscle injected with 10 pptr 2, 3, 7, 8-TCDD at day 1 and day 14. Oyster gonadal tissues (N=20) were sampled on day 30 for ODC activity and polyamine levels. In this study 2, 3, 7, 8-TCDD was shown to decrease ODC levels in the gonadal tissue of the eastern oyster (*Crassostrea virginica*) during gametogenesis. TCDD treated oysters showed a 10 fold decrease in gonadal ODC induction compared to control groups. Putrescine levels also showed a 10 fold decrease compared to control polyamine levels. Histologically, TCDD has been shown to cause delayed gonadal development and decreased egg size in the eastern oyster. Also, TCDD has been shown in egg fertilization assays to cause decreased egg viability and ultimately a decrease in egg fertilization in the oyster. Studies are on going to determine if 2, 3, 7, 8-TCDD exposure during gametogenesis interferes with normal reproductive development by altering the level of ODC induction resulting in delayed gonadal development, decreased egg viability, and egg fertilization.

937 MERCURY IN THE ENVIRONMENT OF NORTHWEST ALABAMA.

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According to the USEPA (1998), approximately 51 tons of mercury (Hg) are emitted nationwide each year from coal-burning power plants. A large coal-burning power plant is located on the Tennessee River in the Shoals area of northwest Alabama. Catfish from local lakes and ponds were collected and analyzed for Hg. The appearance of Hg at similar levels in fish collected from widely separated sites in a local environment supports atmospheric deposition as a source. We analyzed wood from two trees for the presence of Hg. Tree ring dating could provide a means to examine Hg release over time. Blue catfish were harvested from lakes Pickwick and Wilson on the Tennessee River. Channel catfish were collected from six local ponds. The power plant is situated on the banks of Pickwick Lake. The ponds are located within a 30 km radius of Pickwick Lake. The wood samples, a red hickory and a shortleaf pine, were collected approximately 10 km from the power plant. Mercury concentrations in fish and wood were determined using USEPA Method 245.1, Cold Vapor Atomic Absorption. Mercury concentrations in fish from each location are reported as the mean in ug/g dry weight. Catfish from Lake Pickwick had muscle Hg concentrations of 0.03 and liver concentrations of 0.35 (n=6). Wilson Lake fish had muscle Hg concentrations of 0.10 and liver concentrations of 0.98 (n=13). Catfish collected from five ponds had mean muscle concentrations of 0.09 and liver concentrations of 0.67 (n=13). The sixth pond was constructed in 1999 and stocked with fingerling catfish in 2000. Two channel catfish were collected from this pond in April, 2002. They had Hg concentrations in muscle of 0.10 and 0.09 ug/g, and in liver of 0.95 and 0.65 ug/g. Wood collected from the outer circumference of the pine tree had a Hg concentration of 12.4 ng/g. Wood from the outer circumference of the hickory had a concentration of 7.4 ng/g. Atmospheric deposition of Hg generated by local coal-burning facilities ofers a plausible explanation for the Hg contamination observed in this study.

938 MERCURY DISTRIBUTION IN SEDIMENTS AND UPTAKE INTO THE AQUATIC FOOD WEB AT COTTAGE GROVE RESERVOIR, OREGON.

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The Cottage Grove Reservoir in Lane County, Oregon has been impacted by a point source (Black Butte Mine), where mercury mining and processing has occurred within its watershed. To identify the extent of impact, total mercury concentrations [Hg-T] in sediments of the reservoir, tributary streams, and representative food web species were measured in 2002. The concentrations measured in 2002 were qualitatively compared to 1995 [Hg-T] to determine if [Hg-T] in sediments and tributaries have changed over time and if parallel changes have occurred in the food web. Sediment cores from both time periods were collected from the deepest areas of the reservoir. [Hg-T] from both sampling events increased from the surface to the bottom of the core, suggesting a gradual reduction in mercury loading since mercury production halted. Average [Hg-T] ranged from 1.4 mg/kg at the surface to a maximum of 3.7 mg/kg located 30 cm below the surface. [Hg-T] in transect samples collected from the center of the reservoir gradually increase from the inlet (0.7 mg/kg) to the dam (3.6 mg/kg). Transect and tributary samples collected during 1995 are spatially similar and concentrations trends generally agree with those collected in 2002. Mercury concentrations in largemouth bass (up to 2 mg/kg) are of concern for human health, and fish consumption advisories are posted at the Reservoir. Mercury concentrations were measured in organisms representing three trophic levels including benthic invertebrates, omnivorous amphibians/fish, and carnivorous fish. Average [Hg-T] measured in benthic organisms ranged from <0.02 mg/kg in snails to ~0.05 mg/kg in chironomids; average [Hg-T] measured in omnivorous amphibians and fish ranged from <0.02 mg/kg in bullfrog tadpoles to ~ 0.04 mg/kg in fingerling brown bullhead; and [Hg-T] were near or exceeded 1 mg/kg wet weight in largemouth bass (Micropterus salmonides). Food web results indicate substantial mercury biomagnification to higher tropic levels.

939 1, 1-DICHLOROETHYLENE-INDUCED MITOCHONDRIAL PERMEABILITY TRANSITION IN MURINE LIVER.

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1, 1-Dichloroethylene (DCE) produces hepatotoxicity in mice. Our studies suggested that functional alterations in mitochondria initiate the toxic response. Here we provide evidence that DCE-mediated mitochondrial perturbations are a result of permeability transition pore (PTP) opening, an event that can result in both apoptotic and/or necrotic cell death. Liver mitochondria were isolated from 10 min to 12 h after DCE (125 mg/kg) treatment. Glutamate (complex I)- and succinate (complex II)-supported mitochondrial respiration was assessed by measurement of state-3 (ADP-stimulated) and state-4 (resting) rates of oxygen consumption, and respiratory control ratios (RCR: state-3/state-4) calculated. State-3 respiration rates and RCRs for glutamate-supported respiration were significantly decreased at 20 min after DCE treatment, and those for succinate-supported respiration at 90 min; however, all respiratory parameters returned to control levels by 6 h. A transient decrease in the mitochondrial membrane potential (Delta Psi(M)), measured spectrophotometrically, was concurrent with DCE-induced mitochondrial dysfunction. Additionally, Western blotting showed greater immunoreactivity for cytochrome c in cytosolic fractions from mice treated with DCE (125 and 225 mg/kg) than in control animals. All of these mitochondrial alterations were inhibited by pretreatment with cyclosporin A (CsA; 100 mg/kg), a specific inhibitor of the mitochondrial PTP, suggesting that DCE-mediated mitochondrial changes were the result of PTP opening. Liver toxicity, as assessed by serum alanine aminotransferase activity, was also temporally evaluated. Significant elevations observed as early as 1h after DCE treatment were inhibited by CsA. Collectively, these data suggest that DCE produces apoptotic cell death by inducing a transient mitochondrial permeability transition that causes loss of mitochondrial membrane potential and release of cytochrome c into the cytosol. (Supported by Grant MOP 11706 from the Canadian Institutes of Health Research).

940 MECHANISM OF PERFLUORINATED CARBOXYLIC ACID INDUCED MITOCHONDRIONOPATHY *IN VITRO*.

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Perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and substituted perfluorooctanesulfonamides are widely used as surfactants on fabrics and papers, as anti-corrosion agents and fire retardants, as well as numerous other commercial applications. The sulfonamides include perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamidoacetate (FOSAA), N-ethylperfluorooctanesulfonamide (N-Et-FOSA), 2-(N-ethylperfluorooctanesulfonamido) ethyl alcohol (N-Et-FOSE), and N-ethylperfluorooctanesulfonamidoacetate (N-Et-FOSAA). Their broad application, global distribution and environmental persistence has generated interest in the metabolic and potentially toxic effects of these compounds. We have previously reported that perfluorinated carboxylic acids interfere with mitochondrial bioenergetics by inducing the mitochondrial permeability transition (MPT). The purpose of this investigation was to more thoroughly characterize the role of the MPT in the mechanism of toxicity associated with perfluorinated carboxylic acids. The carboxylated sulfonamides FOSAA and N-Et-FOSAA induced calcium-dependent mitochondrial swelling, inhibited uncoupled respiration, and induced ROS generation, all of which were inhibited by cyclosporin-A. Adding exogenous cytochrome c also restored uncoupled respiration. PFOA induced the MPT, however ROS generation was not detectable at the concentration tested. The non-carboxylated compounds PFOS, FOSA, N-Et-FOSA and N-Et-FOSE did not induce the MPT or ROS generation, nor did they inhibit uncoupled respiration. We conclude that induction of the MPT by perfluorinated carboxylic acids results in the release of cytochrome c and inhibition of respiration, leading to ROS generation, which may ultimately be responsible for any cytotoxicity associated with these compounds. (Supported by a grant from the 3M Company)

941 MITOCHONDRIAL-MEDIATED CELL KILLING BY BILE ACIDS.

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Cholestasis results from hepatocyte dysfunction due to the accumulation of bile acids in the cell. Ursodeoxycholate acid (UDCA) is used worldwide for treatment of chronic liver disease, but its therapeutic benefit remains to be substantiated. In the present study we determined if UDCA alters bile acid-induced cytotoxicity, and investigated the role of the mitochondrial permeability transition (MPT) in the mechanism of pathogenesis. Viability of Wistar rat hepatocytes in primary culture was measured by LDH leakage after exposure to bile acids alone or in combination. Mitochondrial membrane potential and ATP content were also evaluated. The percentage of cell killing increased in a time- and dose-dependent manner in cells exposed to unconjugated bile acids. Tauro- and glyco-conjugates of chenodeoxycholate (CDCA) and UDCA were less toxic than the corresponding unconjugated forms. Although relatively non-toxic, UDCA caused synergistic cell killing by lithocholate, CDCA, glyco-CDCA and tauro-CDCA. Furthermore, UDCA potentiated the mitochondrial depolarization, induction of the MPT, and ATP depletion caused by CDCA. Fructose maintained ATP levels and prevented bile acid-induced cell killing. Cyclosporine A (CyA), a potent inhibitor of the MPT, substantially reduced CDCA-induced cytotoxicity. Our results demonstrate that at cholestatic concentrations, cell death correlates with the degree of lipophilicity of individual bile acids. Additionally, induction of the MPT and impairment of cellular ATP generation appears to be associated with the final pathway leading to cell death after exposure to CDCA. In view of the wide implication of the MPT in chemical induced cell injury, these data draw into question the potential benefit of prescribing UDCA to treat cholestatic liver disease. (Supported by PRAXIS XXI/21454/99, FCT and the Luso-American Foundation)

942 ROLE OF MITOCHONDRIAL DYSFUNCTION IN COMBINED BILE ACID-INDUCED APOPTOSIS.

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Chenodeoxycholic acid (CDCA), a hydrophobic bile acid, is toxic to hepatocytes by mechanisms involving mitochondrial dysfunction. It has been previously observed that ursodeoxycholate (UDCA) induces apoptosis in hepatocellular carcinoma cells and potentiates CDCA-induced necrosis in primary cultured hepatocytes due to induction of the mitochondrial permeability transition (MPT). Since UDCA has been used in the treatment for cholestatic liver disease in humans, the goal of this investigation was to evaluate whether CDCA-induced apoptosis is prevented by UDCA or tauroursodeoxycholic acid (TUDC) and to characterize the involvement of mitochondria in the process. Due to the interconnection between cell proliferation and programmed cell death, we also investigated the effects of bile acids on cell cycle. Cultured human HepG2 cells were treated in a dose- and time-dependent protocol in order to establish a low exposure to CDCA that causes apoptosis but not necrosis. Low dose CDCA induced a transient G1 and S phase block determined by flow cytometry. As a result, cell proliferation was prevented. UDCA or TUDC had no effect. CDCA-induced apoptosis, as determined by fluorescence microscopy of Hoescht 33342-stained nuclei, was strongly potentiated by UDCA, but not TUDC. Additionally, after exposure to UDCA plus CDCA, the cell membrane was permeable to fluorescent dyes, indicating that the combination caused both apoptosis and necrosis. The results suggest that UDCA potentiates CDCA cytotoxicity through both apoptotic and oncolytic cell death pathways, both of which may be initiated at the level of potentiation of induction of the MPT. (Supported by PRAXIS XXI/21454/99, FCT and the Luso-American Foundation)

943 GALACTOSAMINE INDUCED ONCOTIC NECROSIS AND CASPASE-DEPENDENT APOPTOSIS IN RAT LIVER.

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Galactosamine (Gal)-induced liver injury in rats is a well-recognized animal model resembling human viral hepatitis. Hepatocellular apoptosis and oncosis have been shown to occur after Gal treatment. However, it is yet unclear whether these two modes of cell death are sequential events or independent of each other. Female Sprague-Dawley rats received 500 mg/kg Gal and were observed for 3, 6 or 24 h. There was a time-dependent increase in plasma alanine transaminase (ALT) activities (24 h: 430 ± 122 University/L). Procaspase-3 processing and elevated levels of caspase-3 enzyme activity in the liver accompanied this. There was a 35-fold increase in the number of apoptotic hepatocytes after Gal (24 h: 5.4 ± 1.0 %) compared to untreated controls (0.14%). In addition to the apoptotic cells, cells with oncotic morphology were identified (24 h: 6.9 ± 0.8 %). Treatment with the pancaspase inhibitor IDN-7314 (2 doses of 10 mg/kg) or pretreatment with uridine (1 g/kg), reduced all parameters of apoptosis to baseline. However, there was no significant change in plasma ALT levels and oncotic necrosis at 6 h and only a 50% reduction at 24 h with the pancaspase inhibitor. Uridine, on the other hand, pre-

vented the increase in plasma ALT levels and reduced the number of apoptotic and oncotic cells by >80%. When animals were treated with a combination of Gal and Salmonella enteriditis endotoxin (0.1 mg/kg) (Gal/ET) for 6 h, plasma ALT levels, hepatic and plasma caspase-3 activities, and the number of cells undergoing apoptosis and oncosis, were higher compared to animals treated with Gal alone. Again, the pancaspase inhibitor reduced the parameters of apoptosis to control levels. However, plasma ALT levels and oncotic necrosis were only reduced by 50% and 30%, respectively. Conclusions: Galactosamine-induced hepatocellular apoptosis in rats is caspase-dependent. Although some of the apoptotic cells may undergo secondary necrosis, a significant number of hepatocytes die through oncotic necrosis as an independent mechanism of cell death.

944 MITOCHONDRIAL AUTOPHAGY DURING REMODELING OF CULTURED HEPATOCYTES.

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BACKGROUND. Cultured hepatocytes de-differentiate, a phenomenon usually interpreted in terms of altered and suppressed gene expression. However, cellular remodeling (changes in cellular structure) also occurs during de-differentiation. **AIM.** To determine the role of autophagy and the mitochondrial permeability transition (MPT) in remodeling of cultured hepatocytes. **METHODS.** Isolated rat hepatocytes were cultured in Waymouth's medium. Mitochondria and acidic organelles (lysosomes and autophagosomes)were counted by confocal microscopy from the first to the fifth day of culture after labeling with 200 nM tetramethylrhodamine methylester and 150 nM LysoTracker Red (LTR), respectively. A LTR fluorescence assay was also used to quantify acidic organelle proliferation. To visualize movement of mitochondria into lysosomes, hepatocytes were co-loaded with 250 nM MitoTracker Green (MTG) followed by 150 nM LTR. **RESULTS.** Mitochondrial number decrease 55± 5% from 1000.4 ± 49 mitochondria per hepatocyte to 454 ± 52 ($p<0.0001$, n=30 cells) from the first to the fifth day of culture. Simultaneously, the number of acidic organelles increased per hepatocyte from 60 ± 6 to 204 ± 12 from the first to the third culture day, and decreased to 168 ± 18 on the fifth day ($p<0.0001$). The changes of LTR-labeled organelles observed by confocal microscopy correlated closely with measurements of total LTR fluorescence. Based on this assay, LTR fluorescence increased 250 ± 8% from the first to the third day of culture. CsA and NIM 811 (5 μM)), inhibitors of the MPT, blocked acidic organelle proliferation in the third day by 100 ±7 and 84 ± 10%. Tacrolimus (5 μM) an immunosuppressant that does not inhibit the MPT, was without effect. Confocal microscopy of hepatocytes on the first and second day of culture revealed movement of MTG-mitochondria into LTR-acid organelles as mitochondria disappeared from the cells. **CONCLUSIONS.** During remodeling of cultured hepatocytes, mitochondria undergo accelerated autophagy. This mitochondrial autophagy involves the MPT.

945 S-ADENOSYLMETHIONINE PROTECTS AGAINST ACUTE ALCOHOL INDUCED HEPATOTOXICITY.

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Alcoholic liver disease is a major medical complication of alcohol abuse and a common liver disease in the western countries. Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the development of alcoholic liver disease.Adenosylmethionine (SAMe) is a key intermediate in the hepatic transsulfuration pathway and serves as a precursor for glutathione (GSH) as well as the methyl donor in most transmethylation reactions. Although SAMe has beneficial effects on many hepatic disorders; the effects of SAMe on acute alcohol-induced liver injury are still unknown. In present study, we investigated effect and mechanism of SAMe on liver injury induced by acute alcohol administration. Our results showed that acute ethanol administration (5g/kg BW) caused prominent microvesicular steatosis with mild necrosis and an elevation of serum enzyme (ALT) activity. In association with the hepatocyte injury, acute alcohol administration induced marked decreases in both hepatic SAMe and mitochondrial GSH levels along with lipid peroxidation and mitochondrial dysfunction. SAMe (50 mg/ kg BW) treatment significantly attenuated the liver injury. Pretreatment with SAMe prevented hepatic SAMe and mitochondrial GSH depletion as well as mitochondrial dysfunction following acute alcohol exposure. These results demonstrated that SAMe protects liver from acute alcohol induced injury by preventing mitochondrial GSH depletion and mitochondrial dysfunction. (Supported by VA, NIH R01 AA01762, NIH R01 AA 10496).

946 PPAR-α ACTIVATION IS ESSENTIAL FOR DIABETES-INDUCED RESISTANCE AGAINST ACETAMINOPHEN HEPATOTOXICITY.

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We recently reported a critical role for nuclear receptor, PPAR-α, in conferring resistance to acetaminophen (APAP) hepatotoxicity in diabetic (DB) mice. The present work examines the mechanisms mediating this resistance. WT-DB mice treated with APAP (600 mg/kg, LD_{100} in WT non-DB mice), showed only 30% mortality and 50% less liver injury (ALT and histopathology). In contrast, diabetic-PPAR-$\alpha^{-/-}$ mice were not protected from APAP toxicity. Pulse labeling studies and PCNA histochemical staining after injury showed earlier and robust tissue repair in WT-DB mice, but not in the PPAR-$\alpha^{-/-}$DB mice. Hepatic CYP2E1 and 1A2 protein, and APAP selective covalent binding to 58, 56 and 44 KDa acetaminophen binding proteins were similar among DB and non-DB mice. Using c-DNA microarrays we analyzed the expression of 588 genes at 12 h after APAP treatment in non-DB and DB, WT and PPAR-$\alpha^{-/-}$ mice. Hierarchical clustering (Cluster™) of genes revealed six groups of genes, with correlations among genes from similar functional families. WT-DB mice had significantly muted expression of Gadd45, Gadd153, EGR-1 and heme oxygenase-1 compared to WT non-DB mice after APAP treatment consistent with lower liver injury. WT-DB mice showed significantly greater increases in heat shock proteins 105, 25 and 70 after APAP, compared to PPAR-$\alpha^{-/-}$DB cohorts. Remarkable changes were observed in cell-cycle regulators in WT-DB selectively. Among others, WT-DB, unlike the PPAR-$\alpha^{-/-}$ DB mice revealed 10-fold increase in cyclin D1 gene expression, consistent with earlier and higher DNA synthesis. Microarray data were confirmed *via* real-time PCR analysis of 15 genes. These findings suggest enhanced p38 MAPK/cyclin D1 signaling as mechanisms underlying upregulated liver tissue repair *via* PPAR-α activation in diabetes.

947 UROPORPHYRIA CAUSED BY ETHANOL IN Hfe(-/-) MICE; ROLE OF HEPATIC IRON ACCUMULATION.

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Two major risk factors for the development of porphyria cutanea tarda (PCT) are alcohol consumption and homozygosity for the C282Y mutation in the hereditary hemochromatosis gene (HFE). To develop an animal model, Hfe knockout and wild-type mice were treated continuously with 10 % ethanol in their drinking water. By 4 months, uroporphyrin (URO) was detected in the urine. At 6 to 7 months, hepatic URO was increased and hepatic uroporphyrinogen decarboxylase (UROD) activity was decreased. Untreated Hfe(-/-) mice, or wild-type mice treated with or without ethanol, did not display any of these biochemical changes. The ethanol treatment increased hepatic non-heme iron and hepatic 5-aminolevulinate synthase activity in Hfe(-/-), but not wild-type mice. The increases in non-heme iron in Hfe(-/-) mice were associated with diffuse increases in iron staining of parenchymal cells, but without evidence of significant liver injury. Wild-type mice treated with both high doses of iron dextran and ethanol also became uroporphyric. In conclusion, this study indicates that the uroporphyrinogenic effect of ethanol is mediated by its effects on hepatic iron metabolism and is associated with increases in hepatic 5-aminolevulinate synthase. Ethanol-treated Hfe(-/-) mice appear to be an excellent model for studies of alcohol-induced PCT. This work was supported by funds from the Department of Veterans Affairs and by grants from the National Institutes of Health ES06263 (PRS) and AG14731 (GG).

948 CARBAMOYL PHOSPHATE SYNTHETASE I: CHRONIC ETHANOL EXPOSURE AFFECTS ENZYME ACTIVITY IN HEPATOCYTES VIA A 4-HYDROXYNONENAL MECHANISM.

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Chronic consumption of ethanol has been demonstrated to elicit lipid peroxidation, resulting in electrophilic aldehyde by-products such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). These aldehydes have gained notoriety due to their long-lived ability to form adducts with proteins and nucleic acids. Although

the temporal relationship between protein-adduct formation and oxidative stress has been established, the specific proteins targeted for adduction and the resulting biomolecular perturbations have yet to be expounded. The objective of this study was to isolate and identify the proteins adducted by 4-HNE and MDA, and to evaluate the consequences that adduction impose on the normal biochemistry of hepatocytes. The novel research presented here used immunoprecipitation and western blot analysis to identify carbamoyl phosphate synthetase I (CPS-I) as a protein targeted for adduction by both 4-HNE and MDA. In animals that exhibited hepatic steatosis, CPS-I activity was decreased by as much as 33% below control levels resulting in an increase in serum ammonia levels. Although *in vivo* experiments using rats in a chronic ethanol feeding model demonstrated that both aldehydes form adducts whith CPS-I, assays using control hepatocytes treated with 4-HNE or MDA at 100μM or 1mM concentrations indicated that the inhibition of enzyme activity is associated with 4-HNE, but not MDA. Additionally, 4-HNE treatment of primary cultured hepatocytes lead to a decrease in CPS-I protein concentration, whereas MDA treatment of identical cells had no effect. To our knowledge, this is the first report that indicates the rate-limiting enzyme of the urea cycle is adducted by the ethanol-induced, lipid peroxidation products, 4-HNE and MDA, and that only 4-HNE decreases enzyme activity and concentration. (Supported by NIH/AA 09300)

949 SUBSTRATE MODIFICATION BY 4-HYDROXYNONENAL MODULATES DEGRADATION BY THE 26S PROTEASOME.

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Alcoholic liver disease (ALD) is associated with the accumulation of undegraded proteins within hepatocytes. A pathway commonly employed by mammalian cells to degrade proteins involves polyubiquitination and ensuing degradation by the 26S multicatalytic protease complex (26S proteasome). Therefore, modulation of this pathway is a likely mechanism explaining altered degradation of proteins. Also associated with ALD are lipid peroxidation and the ensuing production of reactive aldehydes such as 4-hydroxynonenal (4-HNE). To test the hypothesis that 4-HNE modulates 26S proteasomal degradation through substrate modification, an *in vitro* system consisting of rabbit reticulocyte lysate (RRL) fortified with an ATP-regenerating system has been employed to monitor degradation of 4-HNE-modified alcohol dehydrogenase (ADH). This enzyme was selected as a model protein due to the likelihood it is a target for adduction because of its involvement in the metabolism of 4-HNE. Exposure of ADH to concentrations of 4-HNE ranging from 10μM to 100μM resulted in varying degrees of covalent adduct formation. The rate of RRL-mediated disappearance of ADH modified by 10μM 4-HNE was increased by approximately 30 percent over the unmodified control, while exposure to 100μM 4-HNE inhibited the degradation of ADH. Involvement of the 26S proteasome was verified using the specific proteasome inhibitor MG-132 and a non-hydrolyzable ATP analogue, both of which prevented ADH degradation. Finally, enzymatic digest of modified ADH followed by mass spectral analysis has facilitated identification of several sites of 4-HNE adduct formation. The data presented here describe a concentration-dependent ability of 4-HNE to modulate protein degradation by the 26S proteasome through substrate modification, and suggest that aldehydic products of lipid peroxidation may be involved in dysregulation of protein degradation associated with alcohol-induced liver injury. Support for this work was provided by NIH/AA09300.

950 WHY DOES INJURY PROGRESS EVEN AFTER TOXICANT IS GONE? A NOVEL MECHANISM.

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Liver injury induced by hepatotoxicants such as acetaminophen and CCl_4, progresses long after the chemical is eliminated from the body. We tested the hypothesis that necrotic cells dying from mechanism based events release hydrolytic enzymes, which attack the surrounding healthy cells causing progression of injury. Here we provide evidence that calpain, a cysteine protease released from dying cells, mediates progression of toxicant-inflicted injury. Therapeutic injection of a calpain inhibitor N-CBZ-VAL-PHE- methyl ester (CBZ; 60 mg/kg) 1 h after the toxicant, protects 80 % of male SW mice and 75 % of male S-D rats treated with lethal doses of acetaminophen (600 mg/kg, ip) and CCl_4 (3 ml/kg, ip), respectively. CBZ does not affect CYP2E1 (Western blot and p-nitrophenol hydroxylase), the primary bioactivating enzyme, ruling out the possibility of decreased bioactivation of these hepatotoxicants. Extracellular leakage of calpain, assessed by immunohistochemistry in liver and calpain activity in plasma was substantially lower in the necrotic area after CBZ treatment. Treatment with E64, a cell impermeable inhibitor of calpain, also reduced CCl_4-induced liver injury in S-D rats. Calpain-mediated damage of surrounding cells assessed by degradation of cytoskeletal protein α-fodrin in CCl_4-treated rat livers was substantial while it was nearly abolished in CBZ treated rats. Calpain was able to induce cell death in a Ca^{2+}-dependent manner in freshly isolated hepatocytes, which was completely blocked in the presence of CBZ (100 nm, 500 nm, and 1 μm). Hepatocytes incubated with plasma of CCl_4-treated rats (collected at 12 h when most of the CCl_4 is eliminated) induced extensive cell death, which was prevented by CBZ. These data provide substantial evidence for a novel mechanism involving calpain released from dying cells after CCl_4 and acetaminophen treatment mediates the progression of liver injury. (Supported by Kitty DeGree Endowment and LBRSP).

951 STUDIES ON THE SUSCEPTIBILITY OF TRANSPORT DEFICIENT (TR⁻) HYPERBILIRUBINEMIC RATS TO ACETAMINOPHEN HEPATOTOXICITY.

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Biliary excretion of several conjugated metabolites of APAP is significantly reduced in transport deficient (TR^-) hyperbilirubinemic rats. They also have impaired biliary excretion of many organic anions and increased retention of hepatic glutathione (GSH) due to mutational deficiency of the canalicular transporter Mrp2. In these experiments, the susceptibility of TR^- rats to APAP hepatotoxicity was investigated. For this purpose, weight-matched normal male Wistar and TR^- rats were fasted overnight and treated with 1g APAP/kg, ip. Controls received vehicle only (0.2% Arabic gum). Liver toxicity was assessed 24 hrs later by plasma SDH activity and histopathology. Normal Wistar rats treated with APAP showed significant elevation in plasma SDH activity at 24 hrs. However, administration of the same dose of APAP did not increase plasma SDH activity in TR^- rats. Histopathology confirmed these findings. As expected, hepatic non-protein sulfhydryl (NPSH) content was significantly lower in normal rats treated with APAP when compared to vehicle controls. In contrast, vehicle treated TR^- rats had approximately 57% higher hepatic NPSH content than vehicle treated normal rats. No changes in NPSH content were detected at 24 hrs after APAP treatment in TR^- rats when compared to vehicle controls. To investigate the importance of higher hepatic NPSH content in TR^- rats, fasted normal and TR^- rats received buthionine sulfoximine (BSO, 0.89g/kg) three hrs before APAP (1g/kg, ip). Liver injury was determined 24 hrs after APAP. Treatment with BSO by itself decreased hepatic NPSH in TR^- rats to levels similar to those in vehicle treated normal rats. TR^- rats treated with BSO and APAP showed dramatic increases in liver injury, suggesting that increased hepatic GSH content in TR^- rats contributes to their resistance to APAP hepatotoxicity (Supported by NIH grant ES10093).

952 INCREASED HEPATOTOXICITY OF ACETAMINOPHEN IN HSP 70I KNOCKOUT MICE.

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The cellular stress response is characterized, in part, by the highly regulated expression of a set of proteins (heat shock proteins or Hsps). Interest in Hsps stems from numerous reports that correlate induction of Hsps to cytoprotection. For example, previous studies have found that mice subjected to transient hyperthermia (elevating all Hsps) were more resistant to acetaminophen hepatotoxicity than control mice. In these studies, the inducible form of Hsp70 (Hsp70i) was strongly upregulated in liver in response to both acetaminophen and hyperthermia. In an effort to assess the influence of Hsp70i on hepatotoxicity, acetaminophen was administered to Hsp70i knockout mice and controls. Lack of Hsp70i response in liver was verified by challenging knockout mice with hyperthermia or an acutely toxic dose of acetaminophen. Knockout animals were found to be similar to the control strain in metabolic parameters significant to acetaminophen bioactivation (CYP2E1, GST-t, and glutathione levels). Dose response studies found that the hsp70i knockout mice were more susceptible to acetaminophen induced hepatotoxicity than controls as evidenced by elevated serum ALT levels 12, 24 and 48 hours after dosing. Pretreatment with transient hyperthermia to produce a general upregulation of Hsps resulted in decreased acetaminophen hepatotoxicity in both the knockout and control strains. Residual toxicity from acetaminophen in the thermally-pretreated knockouts appeared to be greater than controls, although the differences were not statistically significant. These experiments provide evidence that hsp70i induction is partially responsible for the cytoprotective effects of transient hyperthermia. Furthermore, it suggests that endogenous levels of hsp70i produced at the time of hepatotoxic insult are cytoprotective and limit the extent of the APAP lesion. Supported by ES 07213.

953 PROTECTIVE ROLE OF KUPFFER CELLS IN ACETAMINOPHEN-INDUCED HEPATIC INJURY IN MICE.

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Hepatic injury induced by various toxic agents including acetaminophen (APAP) has been attributed to the production of pro-inflammatory cytokines and other mediators by resident Kupffer cells (KC) within the liver. However, evidence from our laboratory has demonstrated that hepato-protective factors, such as interleukin (IL)-10 and cyclooxygenase-derived mediators, are also upregulated in response to hepatic damage to help protect against exacerbated injury, and KC have been suggested to be a source of these modulatory factors. In other models, KC also serve important regulatory functions in pathophysiological states of the liver. Therefore, we re-evaluated the role of KC in a murine model of APAP-induced liver injury using liposome-entrapped clodronate (liposome/clodronate) as an effective KC-depleting agent. We show that in contrast to pretreatment of mice with a widely used macrophage inhibitor, GdCl3, which did not deplete KC but moderately protected against APAP-induced hepatotoxicity as reported previously, the intravenous injection of liposome/clodronate caused nearly complete elimination of KC and significantly increased susceptibility to APAP-induced liver injury as compared with mice pretreated with empty liposomes. This increased susceptibility was apparently unrelated to the metabolism of APAP since liposome/clodronate pretreatment did not alter APAP-protein adduct levels. Instead, KC depletion by liposome/clodronate led to significant decreases in the levels of hepatic mRNA expression of several hepato-regulatory cytokines and mediators, including IL-6, IL-10, IL-18 binding protein and complement 1q, suggesting that KC are a significant source for production of these mediators in this model. Our findings indicate that, in addition to their pro-toxicant activities, KC can also have an important protective function in the liver through the production of a variety of modulatory factors which may counteract inflammatory responses and/or stimulate liver regeneration.

954 INVESTIGATING THE POLYGENIC CONTROL OF SUSCEPTIBILITY TO DRUG-INDUCED LIVER DISEASE (DILD) USING VARIED STRAINS OF MICE.

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DILD causes significant morbidity and mortality and impairs drug development. Currently, no known criteria can predict whether a drug will cause DILD or what risk factors make an individual susceptible. Although we have discovered in mouse studies that the disruption of key regulatory factors (COX2, IL6, IL10) dramatically increased susceptibility, no single factor seems to be absolute. In order to better understand this polygenicity, we now report a genomic approach aimed at uncovering combinations of inherent differences that might more aptly mimic situations seen in patients. Susceptibility differences to the well-known analgesic/antipyretic drug, acetaminophen (APAP), were investigated using 8 common strains of mice. The rank order of liver damage following APAP treatment was C57Bl/6>C3H/HeOu>>129S6/SvEv=BALB/c>C3H/He>ICR=DBA/2>>>SJL. Lethality mimicked these rankings with noteworthy differences that included delayed lethality of C57Bl/6, ~40% deaths of ICR, and a lack of any deaths in DBA and SJL mice. Metabolic analyses of these 4 strains showed no differences in total APAP-protein adducts or CYP2E1 levels, but did reveal significantly lower levels of specific adducts in SJL mice. Using a microarray platform to monitor 36K genes and ESTs, hepatic gene expression time profiles were obtained for C57Bl/6, DBA, ICR and SJL strains in the absence and presence of APAP toxicity. The expression of >2000 known genes and ESTs including many inflammatory and stress responsive regulators, showed unique positive and negative correlations with toxicity suggesting possible mechanistic functions. A confirmed increase of heat shock proteins 40 and 70 in resistant SJL mice represents one example. Further delineation of the factors that correlate with differential toxicity profiles and analysis of ongoing F1 cross studies should facilitate our understanding of DILD and begin to uncover candidates whose altered expression might predict susceptibility.

955 SCAVENGING PEROXYNITRITE WITH GLUTATHIONE ENHANCES SURVIVAL AND PROMOTES REGENERATION AFTER ACETAMINOPHEN OVERDOSE IN MICE: ROLE OF IL-6.

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We recently showed that intravenous injection of glutathione (GSH) at 1.5 or 2 h after acetaminophen (AAP) treatment leads to the accelerated recovery of mitochondrial glutathione levels, which prevented AAP-induced liver injury at 6 h (Knight et al, J Pharmacol Exp Therap 303: in press, 2002). The enhanced cellular GSH content scavenged peroxynitrite but did not prevent mitochondrial dysfunction. Thus, the objective of this investigation was to evaluate the potential long-term benefits of GSH treatment. A dose of 300 mg/kg AAP induced centrilobular necrosis and increase of plasma alanine aminotransferase (ALT) activities (24 h: 3680 ± 320 University/L) in fasted C3Heb/FeJ mice. Only 56% of the animals survived for 24 h and only 5% were alive at 48 h. Although the animals were fed 9 h after AAP treatment, hepatic GSH levels were still suppressed (7.5 ± 1.2 μmol/g liver) compared to controls (19.7 ± 2.6). Glutathione disulfide (GSSG) levels were elevated (337 ± 26 nmol/g) compared to controls (74 ± 3). Treatment with GSH at 1.5 h after AAP attenuated liver injury at 24 h (1240 ± 70 University/L) and at 48 h (65 ± 10 University/L). All animals survived for 48 h. The hepatic GSH content recovered completely but an oxidant stress was still present (GSSG: 252 ± 26 nmol/g). Cyclin D1 and PCNA protein expression, as indicators of cell cycle activity and regeneration, were not detectable in controls or after AAP alone (6 to 24 h). However, in animals treated with AAP+GSH, a substantial increase in cyclin D1 (12 to 48 h) and PCNA levels (24 to 48 h) was observed by western blotting and immunohistochemistry (PCNA). Plasma IL-6 concentrations were elevated 3-5 fold in response to AAP alone (12 to 24 h). Treatment with GSH prevented IL-6 formation. Our data suggest that scavenging peroxynitrite by GSH provides a long-term hepatoprotection against AAP overdose and improves survival despite the continued mitochondrial oxidant stress. Cell cycle activation and regeneration further facilitate recovery. The regeneration response is independent of IL-6.

956 MECHANISM OF ENHANCED CCL_4-INDUCED HEPATOTOXICITY IN TYPE 2 DIABETES: ROLE OF TISSUE REPAIR.

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The objective of this study was to investigate the mechanism of increased CCl_4 hepatotoxicity in type 2 diabetic rats. Type 2 diabetes was induced in male S D rats by feeding high fat diet (20% fat, 24% protein, 54% carbohydrate) and injecting streptozotocin (45 mg/kg, ip) on day 14. On day 24, blood glucose increased threefold (450 ± 66 mg/dl) with normoinsulinemia, high glycated hemoglobin, elevated triglyceride levels, and insulin resistance as measured by oral glucose tolerance test (5 mg/kg, po) confirmed type 2 diabetes. Administration of a normally nonlethal dose of CCl_4 (2 ml/kg, ip) yielded 100% mortality in the diabetic (DB) rats. A time course study with this dose of CCl_4 revealed substantially higher liver injury in DB rats as assessed by ALT and liver histopathology. Death in DB rats was due to hepatic failure as indicated by hyperammonemia and hyperbilirubinemia. Lack of any change in hepatomicrosomal CYP2E1 protein (Western blot) and activity (TBA assay) in DB rats indicated that bioactivation-based potentiation of CCl_4 hepatotoxicity could not be the reason for markedly amplified hepatotoxicity. Hepatic glutathione at 0, 6, 12 and 24 h after CCl_4 administration were unchanged in DB rats compared to non- diabetic (non-DB) rats, obviating the role of glutathione in potentiation. Tissue repair assessed by ^{3}H-T incorporation in hepatonuclear DNA and confirmed by proliferating cell nuclear antigen assay was inhibited in DB rats after CCl_4 administration due to which injury progressed and resulted in death between 24 to 48 h. In contrast, non-DB rats exhibited robust repair response resulting in complete recovery and survival. IL-6 measured in plasma of DB rats compared to non-DB rats significantly declined at 6 h after CCl_4 administration, suggesting a potential role in tissue repair. In conclusion, high fat diet yields a robust model of type 2 diabetes and CCl_4 hepatotoxicity is amplified in this model due to inhibited tissue repair. (Kitty DeGree Endowment & LBRSP)

957 MULTIPLE DRUG RESISTANCE GENE MODULATION BY STREPTOZOTOCIN.

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Xenobiotic transporters enable ionic and water-soluble xenobiotics to enter cells, as well as aid the excretion of these xenobiotics and/or their metabolites out of cells. Multiple drug resistance (mdr) genes encode P-glycoproteins, which are responsible for resistance to some cancer chemotherapeutic drugs and efflux of xenobiotics out of cells. Thus, mdr can protect organs from xenobiotics. In rats, there are two mdr1 genes capable of xenobiotic transport, mdr1a and mdr1b. A third gene product, mdr2, is associated with phospolipid transport and excretion of lipid into bile. Diabetes mellitus is associated with changes in the disposition of chemicals through altered biliary excretion. For example, the biliary excretion of digoxin and ouabain are significantly increased in diabetic rats. The purpose of this study was to determine whether streptozotocin (STZ)-induced diabetes regulates mdr transporters involved in biliary excretion. While mdr1a and 1b gene expression was not readily increased by microsomal enzyme inducers, in contrast to Phase I and II drug-metabolizing genes, there were specific chemicals such as STZ which induced

mdr mRNA levels. Bile flow remained normal at 5 and 10 days, but significantly increased at 20 and 30 days following STZ treatment. In a time-course study, there were modest increases in liver mdr1a mRNA levels. Liver mdr1b mRNA levels steadily increased with time more than 10 days after STZ treatment. Liver mdr2 mRNA levels tended to increase following STZ treatment, reaching statistical significance at days 10 and 30. In summary, STZ increased liver mRNA levels of mdr1a, mdr1b, and mdr2. The increase in mdr1a and 1b mRNAs may ultimately be responsible for the increased biliary excretion of cardiac glycosides, and the increase in mdr2 mRNA might be responsible for increased bile flow in STZ-induced diabetic rats. (Supported by NIH Grant ES-090716 and ES-03192)

958 INDUCTION OF FACIT COLLAGENS XII AND XIV DURING CARBON TETRACHLORIDE-INDUCED HEPATIC FIBROSIS.

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Prolonged exposure to carbon tetrachloride (CCl4) is associated with the development of hepatic fibrosis. The etiology of liver fibrosis includes the excessive accumulation of type I collagen leading to an abnormal thickening of the collagen fibrils. As a consequence, fibrotic liver has a reduced functional capacity and is unable to regenerate effectively. Collagens XII and XIV sit on the surface of collagen I fibrils and play a role in organizing collagen fibrils, assisting in the maintenance of uniform fibril size. Abnormal expression of these proteins can cause fibril thickening. In the present studies we determined if chronic carbon tetrachloride toxicity altered hepatic expression of collagen XII and XIV. Fibrosis was induced by intraperitoneal injection of CCl4 (0.3 ml/kg, twice per week for eight weeks) into female C57BL mice. Liver samples were collected 1, 3, 6, and 9 weeks after CCl4 or mineral oil control and fibrosis evaluated histologically. Hepatic expression of collagens I, XII, and XIV was quantified by immunohistochemistry and relative reverse transcription polymerase chain reaction. Increased collagen I expression in the liver was gradual, but continuous, throughout the study, beginning within one week of CCl4 exposure, reaching maximal levels by 9 weeks (a more than 15 fold increase). In contrast, expression of collagen XII was 2-4 fold greater than the controls within 3 weeks following CCl4 treatment. Unexpectedly, levels of collagen XIV remain unchanged throughout the fibrotic process. These changes in collagen levels corresponded with the increasing fibrosis of the livers over time. Increasing amount of collagen I with a constant amount of collagen XIV led to an overall dilution effect of collagen XIV. Our results are consistent with the idea that modulating the ratios of collagens I, XII and XIV play an important role in the matrix fibril organization during fibrosis.(Supported by NIH grants HL67708 and ES05022).

959 ANALYSIS OF GENDER DIFFERENCES IN EXPRESSION OF IGF-1, CYP1A2 AND CYP3A1 IN HUMAN LIVER.

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Gender differences in hepatic gene expression may explain occasional differential responses to drugs or chemicals. With the advent of new genomic and proteomic analyses, it is now possible to ascertain if there are gender differences in gene expression that can be detected over inter-individual variation. In this study, the effects of gender on the mRNA or protein levels of several genes including insulin-like growth factor (IGF1), CYP1A2 and CYP3A1 were evaluated. Whole cell protein lysates were extracted from the liver of six adult male and six adult females. Western blot analysis of these samples indicated no gender differences in expression of IGF-1 or CYP3A1, however CYP1A2 protein levels were higher in males than females (P<.01). In addition, the CYP1A2 antibody reacted with a cytosolic protein, not evident in the microsomal fraction that was also expressed at higher levels in males than females (P<.05). A homology search of human protein databases using the CYP1A2 peptide used to generate the anti-CYP1A2 antibody tentatively identified this unknown protein as a methyltransferase. These results suggest there are gender differences in the expression of some hepatic proteins.

960 GLIBENCLAMIDE AND TROGLITAZONE INHIBIT THE CUMULATIVE UPTAKE AND BILIARY EXCRETION OF TAUROCHOLATE (TC) IN SANDWICH-CULTURED RAT HEPATOCYTES (SCRH).

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The canalicular bile salt export pump (Bsep; Abcb11) excretes conjugated and unconjugated bile acids into bile. Inhibition of Bsep may cause hepatocellular accumulation of bile salts leading to hepatotoxicity. The SCRH model mimics extracellular matrix geometry, prolongs hepatocyte viability, preserves liver specific protein synthesis, re-establishes canalicular networks, and maintains the functional activity of basolateral and canalicular transport proteins. Freshly isolated hepatocytes from male Wistar rats were cultured for 4 days between two layers of gelled collagen. Culture media consisted of Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 5% fetal bovine serum and insulin (4 mg/L) for the first 24 hrs; subsequently DMEM was supplemented with 1% ITS (insulin, transferring, selenium, linoleic acid and bovine serum albumin) replaced every 24 hrs. SCRH were used to examine alterations in the hepatic uptake and biliary excretion of TC by hepatotoxic drugs. Differential accumulation (mean+/-SEM) of [3H] TC (10mM) in Day 4 SCRH (n = 3 livers in triplicate) incubated in standard Hanks Balance Salt Solution (HBSS pH = 7.4) or Ca2+-free HBSS (disruption of tight junctions), and the biliary excretion index (BEI; percentage of accumulated substrate in canalicular networks) were quantitated. The cumulative uptake of [3H] TC in hepatocytes preincubated 10 min with glibenclamide (10 mM), or troglitazone (10 mM) in standard HBSS decreased from 523+/-124 to 375+/-11 pmol/mg protein with glibenclamide, and 324+/-78 to 159+/-24 pmol/mg protein with troglitazone. In the presence of glibenclamide or troglitazone, the BEI of TC was reduced from 60+/-16 to 40+/-10% by glibenclamide, and 53+/-17 to 25+/-5% by troglitazone. Based on data generated in SCRH, glibenclamide and troglitazone inhibited both basolateral uptake and canalicular excretion of taurocholate. Supported by NIH GM41935.

961 FIBRATES INDUCE PEROXISOMAL AND MITOCHONDRIAL PROLIFERATION IN CYNOMOLGUS MONKEYS WITHOUT CAUSING CELL CYCLE ALTERATIONS OR OXIDATIVE STRESS.

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In mice and rats, peroxisome proliferator-activated receptor (PPAR) α agonists cause a plethora of hepatic effects including liver and hepatocellular hypertrophy, hepatocellular hyperplasia, increased β and ω oxidation of lipids, increased oxidative stress, increased hepatocellular proliferation and ultimately hepatocellular neoplasms. In these species hepatocarcinogenesis likely results from a combination of cell cycle alterations and oxidative stress. Humans and other primates are generally thought to be refractory to peroxisome proliferation (PP). PP has not been adequately evaluated at clinically relevant exposures (CRE) in primates. We treated male cynomolgus monkeys with one of two marketed hypolipidemic fibrates (PPAR α agonists). They received either fenofibrate (0, 250, 1250 or 2500mg/kg/d) or ciprofibrate (0, 3, 30, 150 or 400mg/kg/d) for 15 days. Significant hepatic (up to 2 fold weight increase) and hepatocyte (qualitative) hypertrophy, increased peroxisome numbers (up to 2.7 fold), and mitochondria numbers (up to 2.5 fold) were observed at exposures that were ~4X of CRE. Mitochondrial but not peroxisomal area was increased. In general, acyl-CoA oxidase mRNA and activity increases correlated with PP. Catalase activity and mRNA was unchanged. Cell proliferation (apoptotic/mitotic activity and Ki-67 expression) was not increased. Contemporaneously, transcriptome profiling using human oligonucleotide arrays failed to show significant evidence of cell cycle alterations, increased apoptosis, oxidative stress or DNA damage. In conclusion, numeric PP occurs at ~4X the CRE in non-human primates but is not associated with an increase in peroxisomal area. The weight of evidence reveals a lack of cell cycle alterations, DNA damage, or oxidative stress at either the cytologic or the transcriptomic level.

962 EFFECT OF ILEAL RESECTION OR CHOLESTYRAMINE TREATMENT ON SERUM TRANSAMINASES IN RATS.

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Most hypocholesterolemic compounds, despite acting by different mechanisms, have been associated with mild, unexplained, asymptomatic elevations of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in humans. In this study, using the cholestyramine-treated and ileal resection rat models, we investigated whether interruption of the enterohepatic circulation of bile acids would affect the serum levels of ALT and AST in rats. In addition, we examined the potential mechanisms for these changes in serum ALT and AST following inhibition of bile acid reabsorption. In the first model, Sprague-Dawley IGS rats were treated for 2, 4, 13 and 26 weeks with a standard diet or a standard diet containing 4% cholestyramine. In the second model, rats were subjected to a sham surgery or to an ileal resection, and sacrificed 2, 4 or 13 weeks following surgery. As anticipated, the activity and mRNA levels of cholesterol 7alpha-hydroxylase and HMG-CoA reductase increased following cholestyramine treatment or ileal resection, indicating a

disruption of the enterohepatic circulation of bile acids in both models. In both genders, there were mild, reversible increases in serum ALT and AST following cholestyramine treatment for 2, 4 and 13 weeks, although serum ALT and AST levels returned to control levels after treatment for 26 weeks. In the ileal resection model, there were also mild increases in serum ALT and AST 2 and 4 weeks after surgery. In both models, there was no histologic evidence of liver change. Furthermore, the elevations in serum ALT and AST were not due to increases in hepatic synthesis, since ALT and AST mRNA levels after cholestyramine treatment or ileal resection were similar to those of control rats. In conclusion, our results provide evidence that transient, reversible elevations of serum ALT and AST observed following ileal resection or treatment with cholestyramine or other cholesterol-lowering agents are more consistent with a pharmacologic response and are not related to hepatotoxicity.

963 ALTERED SUBCELLULAR DISTRIBUTION OF HEAT SHOCK PROTEIN 90 (HSP90) IN LIVER OF DIELDRIN-FED RAINBOW TROUT.

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Feeding rainbow trout 0.3 mg dieldrin/kg/day for 9-12 weeks stimulates biliary excretion of intraperitoneal challenge doses of 3 mg [^{14}C]dieldrin/ kg 5-fold and 3 mg [^{14}C]benzo[a]pyrene(BP)/kg 2-fold. This occurs without induction of the hepatic microsomal cytochrome P450 system (Fund. Appl. Toxicol. 30:187) or epoxide hydrolase. However, dieldrin increases [^{3}H]BP binding in rainbow trout hepatic cytosol from 22 to 50 pmol/mg protein. Purification of proteins from hepatic cytosol by size exclusion chromatography yields a fraction with high [^{3}H]BP binding affinity that contains a 90 kD protein. Electrospray ionization mass spectrometry identifies this protein as HSP90 (Toxicologist 66:368). The working hypothesis is that HSP90 functions as a constituent in a protein complex that traffics dieldrin and BP to intracellular sites of metabolism. If feeding dieldrin stimulates assembly and/or intracellular movement of this putative trafficking complex, subcellular distribution of HSP90 may reflect it. Western blots indicate hepatic cytosol from dieldrin-fed fish contains about 65% less HSP90 than cytosol from control fish. Hepatic microsomes from dieldrin-fed fish contain up to 3-fold more HSP90 than controls. This is consistent with the hypothesis that dieldrin stimulates trafficking of a protein complex that contains HSP90 to an intracellular site of metabolism. Supported by PHS grant P30 ES03850.

964 LATE ADMINISTRATION OF COX-2 INHIBITORS MINIMIZE HEPATIC NECROSIS IN CHLOROFORM INDUCED LIVER INJURY.

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Our previous studies have described the protective effects of hepatoprotective agents against liver injury elicited by chloroform even when given 24 hours after the toxicant, at a time when the liver injury is taking place and rapidly developing. However, the mechanisms involved in this protection remain unknown. The cytoprotective mechanism of these hepatoprotectants such as DMSO, may be due to a dramatic shift in the production of prostaglandins that are responsible for controlling the degree of inflammatory response that can affect blood flow in the liver. In this study, NS-398, a specific COX-2 inhibitor, and indomethacin, a COX-1 and COX-2 inhibitor, were administered 24 hr after chloroform dosing to determine their effect on liver injury in Sprague-Dawley rats. The extent of necrosis was evaluated by H&E staining, while injury to hepatocytes was evaluated by measuring plasma levels of alanine transaminase (ALT). Both COX inhibitors, indomethacin and NS-398, prevented an increase in (ALT) at 48 hr after initial toxicant insult and attenuated further liver necrosis. No changes in cellular proliferative activity occurred in all the treatment groups, which indicates that protection from the COX inhibitors did not have an effect on regeneration of cells at 32 hr and 48 hr. These results indicate COX inhibitors provide a significant protective effect on liver cells against CHCl3 injury and may provide further insight into therapeutic interventions against hepatotoxicants. (NIH ES 08414)

965 TOXICOKINETICS OF THIOACETAMIDE EXPLAINS LACK OF DOSE-RESPONSE FOR LIVER INJURY IN *AD LIBITUM* AND DIET-RESTRICTED RATS.

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Previous studies have established that thioacetamide (TA) is bioactivated by CYP2E1 to TA sulfoxide (TASO), which is further bioactivated to sulfdioxide (sulfone), an unstable ultimate reactive metabolite that initiates centrilobular hepatic necrosis. Earlier studies have shown that despite higher hepatotoxic injury of TA by enhanced bioactivation, diet restriction (DR) protected the rats from a lethal dose of TA. Low dose of TA (50 mg/kg) produced a 6-fold higher injury whereas a 12-fold higher dose of TA produced only 2.5-fold higher and delayed liver injury compared to ad libitum (AL) fed rats. The objective of present work was to determine if lack of dose response for injury in AL rats and higher injury in DR rats could be explained by toxicokinetics of TA. Male Sprague-Dawley rats were 35% diet restricted for 21 days and on the 22nd day, both AL and DR rats were administered 50 or 600 mg TA/kg ip in saline. TA and its major metabolite TASO were quantified in plasma at various time points. In the AL rats, with increasing doses (i.e., from 50 to 600 mg/kg), the half-lives of both TA (18 to 270 min) and TASO (38 to 280 min) were increased, indicating that TA bioactivation exhibits mixed kinetics: first order at low dose and zero order at high dose. Increasing TA dose resulted in a marked increase in plasma TA and TASO. The TASO/TA ratio was highest for the 50 mg/kg dose while it was the lowest for the 600 mg/kg dose. This may explain the significant liver injury seen upon administration of a low dose (50 mg/kg), and lack of appreciable increase in bioactivation-based liver injury with the high dose (600 mg/kg) in AL rats. In the DR rats, for both doses (600 and 50 mg TA/kg), there was a decrease in the half-life of TA (270 to 220 min. and 18 to 13 min) and TASO (280 to 240 min. and 38 to 31 min.) compared to AL rats suggesting that there is higher bioactivation resulting from higher CYP2E1 seen in the DR rats. (Supported by ES09870)

966 KAVA INDUCES HEPATOTOXICITY IN MALE B6C3F1 MICE.

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Kava (Piper methysticum), an herbal supplement used as an anxiolytic, has been reported to cause liver injury in humans. No published studies demonstrating hepatotoxicity of Kava in other species have been located. Pharmacological activity of Kava has been associated with six major kavalactones: Dihydromethysticin (DHM), Kawain, Yangonin, Methysticin, 7, 8 Dihydrokawain (7, 8 DHK), and 5, 6 Dehydrokawain (5, 6 DHK). The present study examined the effect of Kava and each of the six-major kavalactones on toxicity (LDH release) in B6C3F1 primary mouse hepatocytes. Hepatocytes were cultured for 24 hours with Kava (0 to 0.6 mg/ml kavalactones) and toxicity measured. A dose related increase in LDH release was seen with Kava. The non water-soluble fraction of Kava (kava resin) was purified from Kava root for examination of toxicity. Hepatocytes dosed with Kava resin (0 to 500 mg/ml) for 24 hours showed an increase in LDH release at 250 and 500 mg/ml (~5.5 fold over control). Toxicity of the 6 kavalactones was also assessed (0 to 125 µg/ml). DHM was the most toxic, producing LDH release at and above 40 µg/ml (3.9 to 7.6 fold). Yangonin and 7, 8 DHK induced toxicity at and above 50 µg/ml (2.1 to 2.6 fold) and 75 µg/ml (2.55 to 3.5 fold), respectively. Methysticin was toxic at 100 and 125 µg/ml (2.8 to 3.3 fold). Kawain was toxic only at 125 µg/ml (2.7 fold). 5, 6 DHK was not toxic at the highest concentration examined. Co-treatment of DHM (10, 25, and 75 µg/ml) with aminobenzotriazole (0.1 and 0.5 mM), a P450 inhibitor, had no effect on DHM toxicity. Similarly, co-treatment of DHM (10, 25, and 75 µg/ml) with buthionine sulfoximine (1.0 and 5.0 mM), which depletes glutathione, had no effect on DHM toxicity. Kava resin (20 mg/kg, i.p.) was also administered to B6C3F1 male mice for 5 days producing slight liver toxicity as measured by elevated AST and focal hepatic necrosis. These studies provide evidence that Kava and selective kavalactone components are hepatotoxic.

967 *o*-HYDROXYPHENYLACETALDEHYDE (*O*-HPA) DETOXIFICATION IS A MAJOR DETERMINANT OF COUMARIN-INDUCED HEPATOTOXICITY.

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o-HPA is a toxic metabolite formed in the absence of glutathione (GSH) following the oxidation of coumarin to coumarin 3, 4-epoxide (CE). Coumarin is toxic in rats, and formation of CE is thought to be the requisite step leading to hepatotoxicity. Metabolic formation of CE is greatest in mice, a species showing no evidence of toxicity. Since CE formation is not directly related to hepatotoxicity, and GSH conjugation of CE is similar in mice and rats, *o*-HPA detoxification is implicated as the major determinant of hepatotoxic potential. The purpose of the present work was to determine whether the detoxification reactions of *o*-HPA contribute to species differences in coumarin-induced hepatotoxicity. In F344 rat liver cytosol, *o*-HPA was oxidized to *o*-hydroxyphenylacetic acid (*o*-HPAA) with a Vmax of 1.2 nmol/min/mg protein and a Km of 7.4 µM. In contrast, the Vmax and Km for *o*-HPAA formation in B6C3F1 mouse liver cytosol were 5.4 nmol/min/mg protein and 2.2 µM, respectively yielding an intrinsic clearance through oxidation that was 15-times higher than the rat. Similarly, the Vmax and Km for oxidation were 5.2

nmol/min/mg protein and 0.5 μM, respectively in pooled human liver cytosol, representing an intrinsic clearance that was 62-times higher than the rat. All species reduced *o*-HPA to *o*-hydroxyphenylethanol (*o*-HPE), and the Km for reduction was 8.7, 65.4 and 10.5 μM in rat, mouse and human cytosol. The similar Km for oxidation and reduction in rats suggests they are equally likely to reduce or oxidize *o*-HPA, whereas mice and humans favor oxidation to non-toxic *o*-HPAA. The fate of ring-opened coumarin metabolites was evaluated in mouse liver microsomes containing mouse, rat or human hepatic cytosol. *o*-HPAA was the only metabolite formed in mouse and human cytosol, whereas the rat formed *o*-HPAA, *o*-HPE and *o*-HPA. Collectively, these data implicate *o*-HPA detoxification as the major determinant of coumarin-induced hepatotoxicity in the rat and suggests humans are unlikely to be sensitive to this toxicity.

968 EFFECTS OF STRUCTURAL MODIFICATIONS ON THE HEPATOTOXICITY OF 3-(3, 5-DICHLOROPHENYL)-2, 4-THIAZOLIDINEDIONE (DCPT) IN FISCHER 344 RATS.

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3-(3, 5-Dichlorophenyl)-2, 4-thiazolidinedione (DCPT) is hepatotoxic in rats. The thiazolidinedione (TZD) ring is also present in the insulin-sensitizing drugs that are used for treatment of type II diabetes. These drugs have been shown to cause liver damage in some patients. To further investigate DCPT-induced hepatotoxicity, 3-phenyl-2, 4-thiazolidinedione (PTZD) and 3-(3, 5-dichlorophenyl)-5-methyl-2, 4-thiazolidinedione (DPMT) were synthesized. Male, Fischer 344 rats were administered DCPT (0.6 mmol/kg), DPMT (0.6 and 1 mmol/kg) or PTZD (0.6 and 1 mmol/kg) ip in corn oil. Control animals received corn oil (4 ml/kg) only. Liver and kidney function and morphology were assessed at 24 hrs. Compared to controls, the other treatment groups showed mild to moderate ketonuria and marked diuresis within 6 hrs. There was no change in liver or kidney weights. Serum alanine aminotransferase (ALT) levels were significantly elevated in the DCPT-, PTZD- and DPMT-treated rats compared to the controls. In contrast, blood urea nitrogen (BUN) levels were not altered by any of the compounds. Also blood glucose, urine protein and glucose levels were unaffected. The histological results showed pockets of liver necrosis with infiltration of neutrophils in the PTZD-treated animals, suggesting a possibility of widespread hepatic infarction. DCPT- and DPMT-treated animals showed no necrosis, but the hepatocytes were clearly damaged near the centrilobular vein and the cytoplasm in the cells was condensed and irregularly stained. Except for minor cellular swelling in some proximal tubules, there was no evidence of kidney damage in any of the treatment groups. In conclusion, DCPT, PTZD and DPMT produce significant liver damage, but only mild kidney damage, in rats. Our results show that DCPT-induced hepatotoxicity is not sensitive to the presence of chlorines in the phenyl ring or a methyl group in the TZD ring. This suggests that hepatotoxicity is inherent in the TZD ring itself; however, this will require further investigation.

969 RESPONSE OF LIVER SLICES TO HEPATOXICANTS ASSESSED USING TRADITIONAL CLINICAL CHEMISTRY MARKERS.

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Precision-cut liver slices have long been of interest to toxicologists as a optimal model for the study of hepatotoxicity mechanisms and screening but have had limited use in such applications, in part because of their relatively short survival time in culture. Recent reports from different laboratories show progress in addressing this shortcoming, prompting reexamination of the potential this model offers. We chose to examine the method developed by Saulnier and Vickers (The Toxicologist, 2002) for this purpose. Following their modified isolation procedure, liver slices were prepared from male Sprague-Dawley rats and cultured in various media under a 70% O_2-containing atmosphere for up to 5 days. Good retention of viability (80% or higher) was obtained in Waymouths-like media based on biochemical and morphological assessments. The response to 6-mercaptopurine (6MP) and geldanamycin (GEL) compounds, which exhibit anticancer activity but different toxicity profiles in rat liver, was studied using traditional clinical chemistry markers in 3-day incubations. 6MP at 0.25 and 5.0 mM produced concentration- and time-dependent reductions in slice contents of AST, ALT and LDH and media levels increased correspondingly. Changes in slice biliary markers were more complex and inconsistent, due in part to the relatively lower levels measurable. Morphological examination of the tissues showed biliary cells to be intact and viable under conditions where hepatocyte viability had substantially degenerated. GEL in the 0.10 to 10 μM range produced analogous effects on ALT and LDH but with higher loss of alkaline phosphatase compared with 6MP. Morphologically, both liver and biliary system viability was markedly lower in concert at enzyme reductions of approximately 50% or greater. These investigations are being extended to determine the value of this improved system for discriminating different types of liver injury. This work was supported by NCI grant R21CA93262.

970 TEMPORAL EXPRESSION PATTERNS OF GENES IN THE LIVERS OF IMMATURE, OVARIECTOMIZED MICE TREATED WITH ETHYNYL ESTRADIOL.

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Changes in global gene expression were examined in the livers of immature ovariectomized C57BL/6 mice treated with ethynyl estradiol (EE), a synthetic estrogen. Mice were gavaged once or 3x24hr with 0.1mg/kg EE or vehicle and livers were harvested at 0, 2, 4, 8, 12, 24 or 72 hrs. A cDNA microarray was used to assess the relative expression levels of 3068 genes using a reference design in which samples from EE treated mice were co-hybridized with samples from time-matched vehicle controls (TMV). To identify genes with significant expression changes, least squares means from a general linear mixed model of EE samples were compared to TMV samples using t-tests. Approximately 4-8% of the genes examined at each time point exhibited significant changes in expression ($p<0.01$). A gene annotation tool that interfaces with our toxicogenomic supportive relational database, dbZach (http://dbzach.fst.msu.edu), was used to associate Gene Ontology functional categories with members of the significant gene set. This identified a large number of second messenger, transcription factor and signal transduction genes (e.g. c-fos, c-jun, signal transducer and activator of transcription 5A, transcription factor 12, transglutaminase 2, and laminin 3B) and various cytochrome P450 enzymes involved in steroid and cholesterol biosynthesis that were significantly altered in response to EE. The promoters of many of these genes were retrieved from the UCSC database, screened for the presence of perfect estrogen response elements (EREs) and were found to contain this motif suggesting direct regulation by the estrogen receptor. The dramatic alteration of diverse signaling cascades illustrates the complexity of estrogen action in the liver. This, combined with the importance of the liver in metabolism and toxicity, suggests that evaluating hepatic responses will be important in future assessments of estrogen action in the intact organism. Supported by ES011271

971 PROTECTION BY NITRIC OXIDE AND CGMP AGAINST ISCHEMIA/REPERFUSION INJURY IN CULTURED RAT HEPATOCYTES.

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BACKGROUND: Nitric oxide (NO) is an important signaling mediator involved in ischemic preconditioning, a phenomenon that confers tolerance of tissue against ischemia/reperfusion. Onset of the mitochondrial permeability transition (MPT) causes apoptotic and necrotic cell death to hepatocytes after ischemia/reperfusion. The **AIM** of this study was to investigate whether NO can block MPT-dependent ischemic hepatocellular killing after reperfusion. **METHODS:** To simulate ischemia, 1-day cultured rat hepatocytes were incubated in anoxic Krebs-Ringer-HEPES (KRH) buffer at pH 6.2 for 4 h. To simulate reperfusion, hepatocytes were then reoxygenated at pH 7.4 for 2 h. In some experiments, hepatocytes were exposed to NO from 200 μM S-nitroso-N-acetylpenicillamine (SNAP), a slow NO donor, from the beginning of reoxygenation. In other experiments, cells were incubated with 100 μM 1H-(1, 2, 4)-oxadiazolo-(4, 3) quinoxalin-1-one (ODQ, a guanylate cyclase inhibitor), 50 μM 8-Br-cGMP (a cGMP analogue), or 5 μM KT5823 (a cGMP-dependent kinase inhibitor) beginning 20 min prior to reoxygenation. Cell viability was determined by propidium iodide fluorometry. **RESULTS:** After reperfusion at pH 7.4, cell viability decreased to 36.3 ± 1.9% after 2 h of reperfusion (n=16). Viability increased to 76.5 ± 3.0% with SNAP but not with the oxidized product of SNAP. Reoxygenation at pH 6.2 led to similar protection. 8-Br-cGMP also blocked cell killing. In contrast, ODQ and KT5823 reversed cytoprotection by SNAP. ODQ and KT5823 alone did not affect cell viability. **CONCLUSION:** These results suggest that NO prevents MPT-dependent cell killing of hepatocytes after ischemia/reperfusion by a guanylate cyclase-and kinase-dependent pathway.

972 ACTIVATION OF SIGNAL TRANSDUCTION PATHWAYS IN HEPATIC PARENCHYMAL CELLS ARE REQUIRED FOR NEUTROPHIL-DEPENDENT KILLING.

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Neutrophils (PMNs) are cells of the immune system which are part of the first line of defense against invading pathogens. Under certain conditions, however, these cells can damage host tissue. For example, they produce hepatic parenchymal cell

(HPC) injury after exposure of rats to bacterial lipopolysaccharide and can magnify injury during chemical hepatotoxicity. *In vitro*, proteases, such as elastase and cathepsin G, released from activated PMNs damage HPCs. These proteases activate signal transduction pathways in other cell types. Therefore, the hypothesis was tested that killing of HPCs by PMNs requires activation of signal transduction pathways in HPCs. To this end, killing of primary rat HPCs by medium isolated from activated PMNs (PMN conditioned medium) was evaluated in the presence of inhibitors of various signaling pathways. The nonspecific serine/threonine kinase inhibitors, H7 and HA-1004, completely prevented killing of hepatocytes by PMN conditioned medium; whereas, selective inhibitors of protein kinase C, A, and G did not. In addition, genistein, a nonspecific tyrosine kinase inhibitor, prevented killing of hepatocytes by PMN conditioned medium. Finally, incubation of HPCs with PMN conditioned medium caused production of reactive oxygen species (ROS) in HPCs, and desferrioxamine, an iron chelator, completely prevented killing of HPCs by PMN conditioned medium. These results suggest that reactive oxygen species and activation of serine/threonine and tyrosine kinases in HPCs are important for their killing by activated PMNs. (Supported by NIH grants ES04139)

973 NITRIC OXIDE-MEDIATED SUPPRESSION OF FLAVIN-CONTAINING MONOOXYGENASE (FMO) ACTIVITIES IN CULTURED PRIMARY RAT HEPATOCYTES BY DESTABILIZING THE MRNA AND S-NITROSYLATION OF FMO1.

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Objectives: The present work is to investigate the effect of nitric oxide (NO)-donors treated exogenously on the metabolic activities of flavin-containing monooxygenase in cultured primary rat hepatocytes. Background: Overproduction of NO by inducible NO synthase (iNOS) is a common phenomenon in inflammation. At this time, the NO has been implicated as the mediator of decreased catalytic activity and expression of drug-metabolizing enzymes such as cytochrome P450s (CYPs) and flavin-containing monooxygenases (FMOs). Previous work in our laboratory suggests that FMO activities in rat liver treated with LPS are decreased by down-regulation of FMO1 mRNA expression *in vivo* (Park et al., 1999, Mol Pharmacol). Methods: Rat hepatocytes were isolated from rat livers perfused with collagenase. The cells were then treated for 4 h with NO-donors like SNAP or SIN-1. The FMO1 mRNA expression was compared after pretreatment of actinomycin-D to examine the effect of NO on the mRNA stability. The metabolic FMO activities were determined by ranitidine N-oxidation and thiobenzamide S-oxidation estimated with HPLC and spectrophotometer, respectively, in the absence or presence of DTT, a sulfhydryl-reducing agent. Results: FMO activities determined in hepatocytes treated with NO-donors were significantly suppressed to 20-40% of those in untreated control hepatocytes. However, the expression of FMO1 mRNA was not decreased or slightly decreased. Interestingly, stability of FMO1 mRNA after pretreated with actinomycin-D was decreased significantly by exposure to NO donors. Furthermore, the inhibition of *ex vivo* and *in vitro* FMO activities estimated with microsomes exposured to NO donors was reversed completely by addition of DTT. Conclusions: These results suggest that FMO activities in hepatocytes can be decreased both by enhanced unstability of FMO1 mRNA leading to decreased FMO1 expression and by reversible S-nitrosylation of the existing FMO1.

974 INVOLVEMENT OF PHOSPHATIDYLINOSITOL 3-KINASE IN HEPATIC STELLATE CELL ACTIVATION AND ANTIOXIDANT RESPONSE ELEMENT-REGULATED GENE INDUCTION.

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Liver fibrogenesis is dependant upon transdifferentiation of hepatic stellate cells (HSC) to a profibrogenic myofibroblastic phenotype. Electrophilic intermediates generated during prooxidant-induced liver injury, including 4-hydroxynonenal (4-HNE), purportedly modulate HSC gene expression and possibly HSC transdifferentiation. The extent to which 4HNE modulates HSC gene expression remains unclear. The objective of this study is to characterize the response of HSC to electrophiles, and the role of 4HNE in profibrogenic HSC activation. To this end, quiescent HSC were treated with 4HNE or tert-butylhydroquinone (tBHQ), both of which are known inducers of antioxidant response element (ARE)-regulated genes. Electrophilic agents elicit ARE-mediated gene induction through release of the cis-acting transcription factor Nrf2 from its cytoplasmic anchor protein (Keap1), allowing Nrf2 to translocate into the nucleus and participate in ARE-regulated gene induction. These studies demonstrate that both 4HNE and tBHQ induce nuclear translocation of Nrf2 and expression of GSTP1, a gene that is solely regulated by the ARE. This observation is further supported by Nrf2-mediated induction of luciferase in cultured HSC. In contrast to ARE induction, tBHQ but not 4HNE promotes myofibroblastic transdifferentiation of HSC. Pretreatment of cultured HSC with a phosphatidylinositol 3-kinase (PI3K) inhibitor blocked tBHQ-mediated ARE-dependant gene induction and HSC activation, but has no effect on HNE-mediated gene induction. These data implicate involvement of the mitogen-activated protein kinase (MAP kinase) pathway in tBHQ-mediated HSC activation and ARE gene regulation; while the trigger by which 4HNE mediates ARE-dependant gene induction is PI3K independent. We hypothesize 4HNE-mediated ARE activation may involve direct interactions between 4HNE with any of the 25 cysteine residues that compose Keap1. (This work is supported by NIH/AA05578-03 and NIH/AA09300.)

975 THE ROLE OF THE ALPHA$_1$ ADRENERGIC RECEPTOR IN THE RESTRAINT-INDUCED PHOSPHORYLATION OF STAT3.

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Stress causes physiological changes in most organ systems in intact organisms. The Janus kinases-signal transducers and activators of transcription (JAK-STAT) signaling pathways are believed to be crucial in these changes but have received limited investigation utilizing *in vivo* stress models. We previously showed that the restraint of mice results in a substantial activation of STAT-3 in liver but the mechanism responsible for this activation is unknown. One critical component of the response to stress involves activation of the sympathetic nervous system and release of the catecholamines epinephrine and norepinephrine. In this work we used agonists and antagonists of the alpha adrenergic receptor to examine the role of the catecholamines in the activation of STAT-3 in liver. C57Bl6J mice were restrained in centrifuge tubes (2.5 cm inner diameter) for 2 or 4 hours and killed immediately afterward by focused microwave irradiation. The phosphorylation state of STAT3 was determined by western blotting and chemiluminescence detection using phospho state-specific antibodies. In confirmation of our previous work restraint produced large increases in p-STAT3. Phentolamine, an alpha-adrenegic antagonist given (10 mg/kg s.c.) 30 minutes before the start of restraint completely blocked the phosphorylation of STAT3. Further, the alpha1-adrenergic agonist phenylephrine when given (10 mg/kg s.c.) to unrestrained mice stimulates phosphorylation of STAT3 by more than 10 fold within 2 hours of the injection. Our data suggest that catecholamines acting through alpha adrenergic receptors play a crucial role in the activation of STAT3 induced by restraint.

976 15-DEOXY-PROSTAGLANDIN J2 ENHANCES ALLYL ALCOHOL-INDUCED TOXICITY IN RAT HEPATOCYTES.

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Allyl alcohol (AA) induces hepatotoxic responses that are potentiated by small doses of bacterial lipopolysaccharide (LPS). Previous research from this laboratory has shown that cyclooxygenase-2 is involved in the augmentation of AA liver damage by LPS *in vivo*. Studies *in vitro* showed a significant enhancement of hepatocyte (HC) killing by prostaglandin D2 (PGD2), but no effect by PGE2. In studies presented here, 15-deoxy-PGJ2 (15d-PGJ2), a non-enzymatic product of PGD2, enhanced AA cytotoxicity *in vitro* to a much greater extent than PGD2. HCs were freshly isolated from rat livers, adhered to tissue culture wells and exposed to PGs for 2 h. HCs were then exposed to various concentrations of AA for up to 90 min. HC killing was assessed from the release of alanine aminotransferase (ALT). The concentration required for half-maximal cytotoxicity by AA (EC50) was reduced by half with 15 μM 15d-PGJ2 (from 50 μM to 25 μM AA). Though 15d-PGJ2 has been identified as a peroxisome proliferator-activated receptor γ (PPARγ) agonist, it did not appear to act through PPARγ to elicit this activity because a synthetic PPARγ agonist neither augmented AA cell killing by itself nor affected the activity of 15d-PGJ2. 15d-PGJ2 has been suggested to act *via* cyclic AMP (cAMP) in macrophages, but in HCs augmentation of cAMP activity with IBMX, or inhibition of protein kinase A with HA-1004, had no effect on enhanced cell killing by 15d-PGJ2. Protein synthesis was markedly decreased by 15d-PGJ2 in HCs, but inhibition of protein synthesis did not appear to cause cell death, as shown by a lack of ALT release with treatment of cells with cyclohexamide alone. 15d-PGJ2 inhibited translocation of the p65 fragment of nuclear factor kappa B (NF kappa B) from the cytosol to the nucleus in a manner additive with AA. In addition, an inhibitor of NF kappa B enhanced AA-induced HC death. Together, these results indicate that 15d-PGJ2 augments HC killing by AA, and the mechanism may be related to the inhibition of NF kappa B activation. (Supported by NIEHS 08789)

977 MODEST INFLAMMATION RENDERS RANITIDINE HEPATOTOXIC: IS THERE A RELATIONSHIP TO DRUG IDIOSYNCRASY?

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Drug idiosyncrasy is an adverse event of unknown etiology that occurs in a small fraction of people taking a drug. Some idiosyncratic drug reactions may occur from episodic decreases in the threshold for drug hepatotoxicity. Previous studies in rats have shown that modest underlying inflammation caused by bacterial lipopolysaccharide (LPS) reduces the threshold for several hepatotoxicants. The H2-receptor antagonist ranitidine (RAN; ZANTAC®) causes idiosyncratic reactions in people, with liver as a frequent target. We tested the hypothesis that RAN could be rendered hepatotoxic in animals undergoing a modest inflammatory response. Male rats were treated with a nontoxic dose of LPS (44 x 10^6 EU/kg, iv) or its vehicle, then two hours later with a nontoxic dose of RAN (30 mg/kg, iv) or its vehicle. Rats were sacrificed 3, 6, 12 or 24 hours after RAN administration. The doses of RAN and LPS used were not hepatotoxic when given alone. By 6 hr, injury to hepatic parenchymal cells and bile ducts was evident only in animals treated with both RAN and LPS, as estimated by increases in serum alanine aminotransferase and γ-glutamyl transferase activities. RAN/LPS cotreatment resulted in midzonal liver lesions characterized by acute necropurulent hepatitis. Famotidine (PEPCID®) is an H2-antagonist for which the propensity for idiosyncratic reactions is far less than RAN. Rats given LPS and famotidine at a dose pharmacologically equipotent to that of RAN did not develop liver injury. The results suggest that a response resembling human RAN idiosyncrasy can be reproduced in animals experiencing a modest inflammatory response. Furthermore, they raise the possibility that concurrent inflammation precipitates idiosyncratic responses to some drugs and may lay the framework for developing a predictive animal model.

978 ENDOTOXIN, NOT OXIDATIVE STRESS, MEDIATES ACUTE ETHANOL-INDUCED HEPATIC TNF-ALPHA PRODUCTION.

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Tumor necrosis factor-alpha (TNF-alpha) production is a critical factor in the pathogenesis of alcoholic liver injury. Both oxidative stress and endotoxin have been implicated in the process of ethanol-induced TNF-alpha production. However, the cause-and-effect relationship between these factors has not been fully defined. The present study was undertaken to determine the mediators of ethanol-induced TNF-alpha production using a mouse model of acute alcohol hepatotoxicity. Ethanol administration *via* gavage at a dose of 6 g/kg to 129/Sv mice induced TNF-alpha production in the liver in association with increases in hepatic lipid peroxidation and plasma endotoxin. Treatment with an antioxidant N-acetyl-L-cysteine significantly inhibited ethanol-induced hepatic lipid peroxidation, but not plasma endotoxin elevation or TNF-alpha production. By contrast, treatment with an endotoxin neutralizing protein significantly suppressed ethanol-induced elevation of plasma endotoxin and inhibited TNF-alpha production, but did not reduce hepatic lipid peroxidation. Furthermore, time course analysis revealed that lipopolysaccharide (LPS) injection induced TNF-alpha production, reaching a peak value at 1.5 h. LPS also caused hepatic lipid peroxidation, but it appeared at 6 h and 12 h after treatment, a delayed time period following TNF-alpha production. This study thus systemically dissected the relationship among oxidative stress, endotoxin release, and TNF-alpha production following acute ethanol administration, and the results demonstrate that ethanol-induced hepatic TNF-alpha production is mediated by endotoxin, but not by oxidative stress. (Supported by AA13601, AA01762, AA 10496 & HL63760)

979 THE ROLE OF THE COAGULATION SYSTEM IN SYNERGISTIC LIVER INJURY CAUSED BY MONOCROTALINE AND BACTERIAL ENDOTOXIN COEXPOSURE.

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Synergistic, acute liver injury develops in rats from the administration of a small, noninjurious dose of bacterial endotoxin (lipopolysaccharide; LPS; 7.4 x 106 EU/kg) given 4 hours after a nontoxic dose of the pyrrolizidine alkaloid, monocrotaline (MCT; 100 mg/kg). Both centrilobular (CL) and midzonal (MZ) liver lesions occur, similar to those seen from larger, toxic doses of MCT or LPS, respectively. A previous study demonstrated that coadministration of the thrombin inhibitor heparin to MCT/LPS-cotreated animals reduced hepatic parenchymal cell (HPC) injury. This suggested that the coagulation system was causally involved. To confirm that the attenuation of MCT/LPS-induced liver injury was due to the inhibition of the coagulation system, another anticoagulant, warfarin, was used. Activation of the coagulation system occurred before the onset of HPC injury. Moreover, extensive fibrin deposition, as quantified by immunohistochemistry, was evident in CL and MZ regions within 12 hours after MCT administration. Warfarin (7.5 mg/kg) given 34 and 10 hours before MCT administration in MCT/LPS-cotreated animals abrogated activation of the coagulation system, attenuated HPC injury (as marked by decreased plasma alanine aminotransferase and aspartate aminotransferase activities) and reduced sinusoidal endothelial cell injury (evident as decreased plasma hyaluronic acid concentration). Warfarin pretreatment decreased the size and frequency of CL and MZ lesions in MCT/LPS-treated rats. Collectively, these results suggest that the coagulation system plays a critical role in synergistic liver injury caused by MCT and LPS coexposure. (*Supported by NIH Grant ES 04139.*)

980 MATRIX METALLOPROTEINASES IN MONOCROTALINE-INDUCED LIVER INJURY.

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Monocrotaline (MCT) is a pyrollizidine alkaloid that causes liver injury in animals. In rats, injury is characterized by sinusoidal endothelial cell (SEC) damage and centrilobular parenchymal cell necrosis. Endothelial dysfunction occurs as early as eight hours, and loss of SECs is seen by twelve hours after administration of MCT. Loss of endothelium is a possible outcome of the action of matrix metalloproteinases (MMPs, collagenases), specifically MMP-2 from SECs and MMP-9 from neutrophils and SECs, on basement membrane collagen. Accordingly, the dynamics of MMPs in MCT-induced sinusoidal endothelial damage was studied. Rats were treated with a single dose of MCT (300 mg/kg, ip), and livers were collected at 8, 12 and 18h. Immunofluorescence analysis of frozen sections of livers from MCT-treated rats revealed a progressive reduction in distribution of basement membrane heparan sulfate proteoglycan and collagen IV. A time-dependent increase in tissue total MMP activity and MMP-9 activity occurred in livers of MCT-treated rats, as measured by fluorescent collagenase activity assay and gelatin-zymography, respectively. Progressive neutrophil accumulation was demonstrated by an increase in myeloperoxidase (MPO) activity in liver after MCT treatment. A sinusoidal endothelial cell line, NP-26, was used for *in vitro* studies. Treatment of NP-26 cells with 0 to 0.8 mM MCT resulted in a dose-dependent release of both MMP-9 and MMP-2 from the cells as early as 4h. The results demonstrate the degradation of basement membrane collagen IV with a concurrent increase in amount and activity of MMP-2 and MMP-9, likely originating from sinusoidal endothelial cells and neutrophils. This suggests a possible role for MMPs in SEC destruction that occurs during MCT hepatotoxicity. (Supported by NIH grant ES04139)

981 S-ADENOSYL-L-METHIONINE ABSORPTION AND TRANSPORT BY CACO-2 CELLS.

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S-adenosyl-L-methionine (SAMe) is a ubiquitous endogenous molecule that is known to be protective against hepatotoxic injury. Although oral SAMe appears to be absorbed across the intestinal mucosa its systemic bioavailability is very low. The reason for the low systemic bioavailability of SAMe is still unknown. Using the Caco-2 cell culture model for enterocyte absorption we determined whether SAMe could be taken up by the cells, i.e. cross the apical membrane, and whether it could be transported transcellularly as well. Caco-2 cells cultured in 6-well culture plates were used for the uptake studies. The amount of uptake of ^{14}C-carboxyl-SAMe into the cells was determined as a function of time. There was significant uptake of radioactivity into the Caco-2 cells, suggesting transport across the apical membrane. Peak levels (350 DPM/10^6 cells) occurred 1 hour after addition with a subsequent apparent decline. For transcellular transport studies, Caco-2 cells cultured on Transwell culture inserts were treated with ^{14}C-carboxyl-SAMe in the apical compartment. Apical to basolateral transport of SAMe was determined by measuring the radioactivity in the basolateral compartment over time. A time-dependent appearance of radioactivity on the basolateral side of the culture inserts was observed. The apparent permeability coefficient (Papp) for transport of SAMe was 0.5 X 10^{-6} cm/s. This Papp is similar to that observed for mannitol transport (0.5 x 10^{-6} cm/s), which is a paracellular transport process. The similarity in Papp for SAMe and

mannitol transport suggests that SAMe may be transported paracellularly. Alternatively, SAMe may be transported by an apical or basolateral efflux pump, limiting its transcellular absorption.

982 RESILIENCY OF F344 RATS TO CHLORDECONE POTENTIATED CCL_4 HEPATOTOXICITY AND LETHALITY IS AGE-DEPENDENT.

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The objective of the present study was to investigate the effects of Chlordecone (CD) + CCl_4 in young adult (3 months) middle aged (14 months) and old (24 months) male F344 rats. After pretreatment with either dietary CD (10 ppm) or normal diet for 15 days, rats were challenged with a single non-toxic dose of CCl_4 (100 µl/kg, ip, 1:4 solution in corn oil) or corn oil (500 µl/kg) on 16th day. Liver injury was assessed by plasma enzymes (ALT and AST) as well as histopathology during a time course of 0 to 96 h. Liver tissue repair was measured by [^{3}H]-thymidine (^{3}H- T) incorporation into hepatic nuclear DNA. Plasma glucose and liver glycogen were measured. As expected, no mortality occurred in any age group exposed to CCl_4 injection alone. Exposure to CD + CCl_4 resulted in 50% mortality of young adult rats at 48 h followed by gradual increase, reaching 100% mortality by 96 h. However, there was no mortality in 14 and 24 month old rats. All rats exposed to CD + CCl_4 had a gradual and equal elevation of plasma transaminases regardless of age up to 36 h. Thereafter, liver injury escalated in young adults. Whereas it declined in middle and old aged rats, indicating that bioactivation-mediated liver injury did not differ between young adult and older rats. Plasma glucose did not change in any age group. Significant depletion of glycogen was observed only in young adult rats exposed to CD + CCl_4. As indicated by [^{3}H-T] assay, in aged rats liver regeneration was robust beginning at 24 h and peaking at 48 h. However, such an increase in tissue repair was not observed in young adults. Because of prompt and robust compensatory tissue repair middle aged and old rats could overcome liver injury, whereas in the young adults liver injury progressed leading to 100% lethality. These findings suggest that both middle aged and old rats are resilient to CD + CCl_4-induced hepatotoxicity by virtue of robust compensatory tissue repair. (Supported by NIH/AG19058)

983 QUANTITATION OF INDIVIDUAL BILE ACIDS IN PLASMA BY LC/MS/MS.

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An increase in circulating bile acid concentrations can be an indicator of hepatobiliary diseases. Knowing how individual bile acid concentrations change with hepatotoxicity may help to understand the mechanisms behind them. This information may provide insight for further investigation, or establish a biomarker for a particular disease state. Here we describe an analytical method to profile 21 individual bile acids, including tauro conjugated, glyco conjugated, and unconjugated, in plasma as a tool in investigative studies. Plasma samples from pre-clinical studies are assayed in triplicate using solid phase extraction followed by liquid chromatography/mass spectrometry (LC/MS) analysis. Individual bile acids are separated by reverse-phase LC and monitored in negative ion mode by electrospray ionization (ESI)-MS/MS. Calibration curves are constructed from a linear regression analysis of the peak areas for samples fortified with known concentrations of analyte. Deuterated bile acids are used as internal standards for each sample, and appropriate blanks and quality controls are included in all analyses. Assay validation was conducted using control rat plasma fortified with bile acids at different concentrations. A majority of the bile acids were determined to deviate by ±30% on average from expected values for standards. The technique described has several improvements over similar methods used to quantitate bile acids. The assay is capable of measuring more individual bile acids than any other method reported in the literature. Another benefit is that sample preparation times are shorter due to the 96-well plate format and no need for sample derivitization. The assay also was more sensitive for some bile acids compared to other current methods. Further development is needed to improve overall accuracy values.

984 LEVAMISOLE ATTENUATES DICLOFENAC-INDUCED ENTEROPATHY IN RATS.

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Background: Therapeutic usage of diclofenac, similar to that of other conventional NSAIDs, is frequently associated with small intestinal inflammation, ulceration and protein-losing enteropathy. Therefore, strategies are needed to minimize this complication. Objective: Based on a report of levamisole protection against the gastropathy of other NSAIDs, we investigated the effects of this agent on diclofenac enteropathy using our established rat model. Experimental Design: Fed adult male Sprague-Dawley rats were treated orally with diclofenac [0 or 50 mg/kg] with or without levamisole [100 mg/kg at - 6 hr and 50 mg at + 1 hr]. At 12 hr, extent of enteropathy was measured by counting ulcer numbers and analyzing serum for total protein and albumin concentrations. At 3 hr, a well characterized antibody was used to assess the patterns of diclofenac-protein adduction in liver and in the intestine. Results: Compared to treatment with diclofenac alone which typically produces ~ 80 intestinal ulcers and >40% leakage of serum albumin by 12 hr, animals that were co-treated with levamisole showed a 96% decrease in ulcer numbers and retention of serum proteins. At 3 hr, a pre-ulceration time point suitable for examining early events in diclofenac enteropathy, substantially less drug-protein adduction was detected in both livers and intestines of animals co-treated with levamisole. Conclusions: Our observations of attenuated enteropathy in association with a diminution in diclofenac-protein adduction support our hypothesis that adduction is a causal event in diclofenac enteropathy. The diminished adduction in the livers of animals also given levamisole suggests that the mechanism for protection by this agent stems from its influence on pre-adduction events, such as hepatic bioactivation of diclofenac to an acyl glucuronide and/or biliary secretion of this reactive metabolite. (NIH DK 56494, NIEHS T32-07254)

985 DNA DAMAGE AS A CONSEQUENCE OF CHLOROFORM-INDUCED CYTOTOXICITY IN MALE F344 RAT AND B6C3F1 MOUSE HEPATOCYTES *IN VITRO.*

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Chloroform is hepatotoxic and hepatocarcinogenic in rodents but is not mutagenic. We have characterized the metabolism-dependent cytolethality of chloroform toward rodent hepatocytes at physiologically relevant exposure concentrations and times. We hypothesize that chloroform cytotoxicity produces indirect DNA damage and replication of hepatocytes, leading to mutational events involved in liver tumor development. Hepatocytes from male F-344 rats and B6C3F1 mice were incubated with chloroform (0 to 3.8 mM) for up to 3 hr. After incubation, some cells were cultured for 24 hr to determine cytotoxicity, as measured by LDH leakage. Some cells from the same incubations were used to measure cytotoxicity-related DNA damage. DNA from lysed cells was separated by centrifugation. The PicoGreen reagent was used to quantify the amount of DNA in the pellets (intact chromatin) and supernatants (fragmented DNA). DNA damage was measured as the percentage of fragmented DNA divided by total DNA. Two-parameter flow cytometry analysis of BrdU incorporation was used to evaluate cell cycle effects. Chloroform treatment produced dose- and time-dependent decreases in cell viability. Chloroform induced dose- and time-dependent DNA damage in rat hepatocytes *in vitro*, which preceded cell death. DNA damage was not evident until 2 hr of exposure. Inhibition of biotransformation by the cytochrome P450 inhibitor 1-phenylimidazole (1 mM) prevented both cytolethality and DNA damage, indicating that metabolism was required. The basal level of DNA damage in mouse cells (5.1%) was higher than that in rat (2.6%, $P<0.001$), which might contribute to species differences in susceptibility to tumor induction by chloroform. The labeling index of cells treated with 2.5 mM chloroform for 3 hr (43.1±6.4%) was significantly greater than the control (15.2±1.6%), indicating the induction of cell growth. These results show that acute exposure to chloroform induced cytotoxicity, DNA damage, and cell growth in isolated rodent hepatocytes, which are likely to be involved in the development of liver cancer.

986 METALLOTHIONEIN-III PROTECTS AGAINST THE HYDROGEN PEROXIDE-INDUCED CYTOTOXIC AND DNA DAMAGE.

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Metallothionein (MT)-III, the brain specific member of MT family, is a low molecular weight, heavy-metal binding protein, in contrast to the ubiquitous MT isoforms, MT-1 and MT-2, that are found in most tissues. Results in genetically altered mice indicate that MT-III may play neuroprotective roles in the brain, but the mechanisms through which this protein functions have not been elucidated. The present study was undertaken to assess whether MT-III is able to directly protect DNA from damage and lipid peroxidation induced by hydroxyl radical and to assess its abilities to scavenge superoxide radicals. DNA damages were detected by the mobility of plasmid DNA in electrophoresis and deoxyribose degradation. Lipid

peroxidation was measured by a thiobarbituric acid reactive substance. DNA damages and lipid peroxidation were induced by ferric ion-nitrilotriacetic acid and hydrogen peroxide, which produces hydroxyl radical. DNA damages and lipid peroxidation were prevented by MT-III in a dose-dependent manner. Preincubation of MT-III with EDTA and N-ethylmaleimide, to alkylate sulfhydryl groups of MT-III, resulted in MT-III that was no longer able to prevent DNA damages. This result indicates that the cysteine thiol groups of MT-III may be involved in its protective effects of DNA from hydroxyl radical attack. MT-III scavenges superoxide radical generated from xanthine/xanthine oxidase reaction system in a dose-dependent manner. MT-III is superior in protection of DNA damages and scavenging superoxide radicals compared to MT-I and MT-II; however, there was no difference in inhibition of lipid peroxidation. These results provide direct support for an antioxidant role for MT-III that can scavenge free oxygen radicals and might be protect cells from oxidative stress.

987 STRUCTURE-ACTIVITY RELATIONSHIPS FOR GENOTOXICITY AMONG A SERIES OF SULFONIC ACID ESTERS.

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The chemical synthesis of pharmaceutical structures often involves the use of reactive starting materials or intermediates. Further, the salt/solvent matrix may generate genotoxic esters of sulfonic acid. Both characteristics may lead to the presence of genotoxic impurities in drug substances, which would have to be limited in the final product at low ppm levels. In silico structure-activity prediction can help to proactively identify structures of potential concern in drug substance syntheses. Such structure-activity prediction systems need to be trained with reliable experimental data, which are often not available. We tested a series of esters of benzyl and toluylsulfonic acid for genotoxicity in bacteria (Salmonella typhimurium TA98 and TA100) and mammalian cells (micronucleus formation in L5179Y mouse lymphoma cells). While ethylmethane sulfonate and methylmethane sulfonate are established mutagens across a variety of mutagenicity systems, little is known about the corresponding esters of benzene and toluene. Most esters tested (methyl-, ethyl-, propyl-, isobutyl-, n-butyl-esters) displayed a mutagenic activity in Salmonella. Not unexpectedly, some indications for an inverse relationship of the mutagenic potency with the side chain length was found. Similar results were obtained with the micronucleus test in mouse lymphoma cells. However, for this assay, a clear dependency of the results from distinct structural features could not be obtained. These results have implications for the use of tosylates and besylates as pharmaceutical salts.

988 CYTOTOXICITY AND GENOTOXICITY OF BENZOQUINONE IN HUMAN BONE MARROW CD34+ PROGENITOR CELLS.

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Benzene induces chromosome aberrations, DNA strand breaks, and a p53 response in the bone marrow of mice. Bone marrow stem cells are progenitors for leukemia and a target cell population for benzene toxicity. Benzene is metabolized to several active metabolites, including the toxic and mutagenic benzoquinone (BQ). We used human CD34+ hematopoietic stem cells (HSC) to assess the effects of BQ exposure relative to DNA damage, DNA repair, and the molecular mechanisms associated with these events. We exposed CD34+ HSC from 10 male and 10 female donors to 1, 5, 10, 15, and 20 μM BQ for 72 h. which allowed for at least one cell division. In both male and females, cell viability and apoptosis were dose-dependent with an approximate 60% viability compared to controls observed at 10 μM BQ and a two-fold increase in apoptotic cells compared to unexposed controls observed at 15 μM BQ. The percentage of micronucleated CD34+ cells observed in treated male and female donors was 4.5 and 7 times higher respectively than unexposed controls. p21 gene expression was elevated approximately 2 to 2.5-fold at 10 and 20 μM of BQ respectively for both males and females, suggesting that, similar to mice, human cells utilize the p53 pathway in response to BQ-induced DNA damage. To gain insight into the mechanism of DNA repair of BQ-induced DNA damage, expression of the DNA repair genes Rad51, APEX, Ku80, XPA, and XPC was evaluated by quantitative RT-PCR from cell populations that remained approximately 50% viable relative to unexposed controls following BQ treatment. There was no significant increase in mRNA levels for Rad51, APEX, Ku80, XPA, and XPC in either male or female donors following a 72-h treatment. Preliminary immunohistochemistry revealed the lack of Rad51 foci in BQ-exposed cells, suggesting that homologous recombination does not occur following a 72-h BQ exposure. These results indicate that BQ exposure induces cytotoxicity, apoptosis, and DNA damage in human HSC. The role and mechanism of DNA repair in BQ-induced genotoxicity require further evaluation.

989 DOES FLUMEQUINE HAVE A GENOTOXIC POTENTIAL TO THE LIVER OF MICE?

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In order to elucidate the tumor initiating potential of flumequine (FL) in the liver, male C3H mice were given dietary administration of 4000 ppm FL throughout the study or for 2 weeks at the initiation stage and then received two intraperitoneal injections of D-galactosamine (Gal) at weeks 2 and 5, with or without 500 ppm phenobarbital (PB) in their drinking water for 13 weeks. In addition, an alkaline single cell gel electrophoresis (comet) assay was performed using adult, infant or partial hepatectomized male ddY mice to evaluate the potential of FL at 500 mg/kg or less to act as a DNA damaging agent. Furthermore, *in vitro* assays were conducted to investigate the potential of FL to inhibit eukaryotic topoisomerase II as well as bacterial gyrase. In the 13-week study, hepatocellular foci were observed in 2 out of 8 and 6 out of 7 animals in the FL/PB+Gal and FL/FL+Gal groups, respectively, none being evident in the other treated mice. The comet assay demonstrated FL to dose-dependently induce DNA damage in the stomach, colon, and urinary bladder of adult mice 3 h but not 24 h after its administration. Similarly, DNA damage was noted in the regenerating liver and the livers of infant mice at the 3 h time point. Inhibitory effects of FL on topoisomerase II were high relative to the influence on bacterial gyrase. The results of our studies thus strongly suggest that FL has initiating potential in the livers of mice and that this is attributable to its induction of DNA strand breaks, possibly through inhibition of topoisomerase II.

990 A COMPARISON OF *IN VITRO* TOXICITIES OF CIGARETTE SMOKE CONDENSATE FROM FOUR COMMERCIALLY AVAILABLE ULTRA LOW-TAR CIGARETTES.

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Eclipse is a cigarette that primarily heats rather than burns tobacco. RJRT has previously reported the results of *in vitro* toxicity studies comparing Eclipse with K1R5F and K1R4F reference cigarettes. In order to characterize the differences between Eclipse and very low yielding ultra low-"tar" (vULT) cigarettes, RJRT conducted a comparative evaluation of the genotoxicity and cytotoxicity of mainstream cigarette smoke condensate (CSC) from Eclipse and three vULT tobacco-burning cigarettes (Now 85 Box, Carlton Soft Pack, and Merit Ultima) as well as the leading market ultralight brandstyle (Marlboro Ultra Lights) under four smoking regimens. These smoking regimens were: 1) FTC - (35 ml puff volume every 60 seconds for a 2 second duration [35/60/2]); 2) 50/30/2, 0% vent block); 3) Massachusetts - 45/30/2, 50% vent blocked); 4) Canadian - 55/30/2, 100% vent blocked). Ames testing indicated that Eclipse CSC was less ($p< 0.05$) mutagenic than the CSC from the four cigarettes under all smoking regimens when compared on a revertants per mg Total Particulate Matter (TPM) basis. When mutagenicity was calculated on a revertants per cigarette basis the mutagenicity of Eclipse CSC was lower ($p< 0.05$) than the mutagenicity of Carlton Soft Pack, Merit Ultima, and Marlboro Ultra Lights regardless of the puffing regimen. On a per cigarette basis, the calculated mutagenicity of Eclipse was higher ($p< 0.05$) than Now 85 Box cigarettes under the FTC and 50/30/2 regimens but lower ($p< 0.05$) under the Massachusetts and Canadian regimens. Eclipse CSC was less ($p< 0.05$) cytotoxic as measured in the Neutral Red test than the CSC from the four test cigarettes regardless of the regimen used. Collectively, these data demonstrate that the *in vitro* toxicity of CSC from Eclipse is significantly reduced relative to the activity of CSC from the vULT cigarettes and the Marlboro Ultra Lights.

991 COLLABORATIVE ASSESSMENT OF THE YEAST GENOTOXICITY SCREEN.

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The Yeast Genotoxicity Screen has been developed to provide a rapid genotoxicity assessment of compounds with interesting pharmacological or chemical properties. The objective of this collaborative study was to establish the reproducibility and reliability of the DNA damage reporter system in an independent laboratory. Yeast is an attractive model system for the detection and evaluation of carcinogens, as being eukaryotic, it is useful for the testing of highly bactericidal compounds, which preclude the use of the regulatory or screening Ames Test. The yeast has been genetically engineered (incorporation of reporter gene *RAD54*) to produce a fluorescent protein in proportion to the activation of their DNA repair systems. The harder its

DNA repair systems work the brighter and stronger the fluorescence. The yeast cells, serial dilution of test article solutions, genotoxic (MMS) and cytotoxic (methanol) controls and diluent (2 % DMSO) were manually mixed in 96-well microplates, then incubated overnight at 25 °C. Cell density and fluorescence measurements provided quantitative measures of cytotoxicity (positive, weak or negative, proportional to proliferation, lowered by toxic analytes) and genotoxicity (positive, weak or negative, proportional to fluorescence, increased by genotoxic analytes). The data were compared to an extensive *in vitro* and *in vivo* literature base. MMS and MNNG were confirmed genotoxins; Ampicillin and Caffeine were confirmed non-genotoxins; Urethane and Nitrosodimethylurea were confirmed Ames false negatives and 8-hydroxyquinoline was confirmed Ames false positive. The remaining compounds, Cisplatin, Phenol, Sulfamethoxazole, Bleomycin sulphate, Benzoyl chloride and Hydroxyurea required re-tests at different dose ranges, due to variable data. The test was performed with rapid end-point determination, with good concordance of results, confirming the lack of inter-laboratory and inter-personnel variation, therefore supporting the use of this screen as a useful and rapid genotoxicity assessment tool.

992 TOXIC AND MUTAGENIC EFFECTS OF BASE ANALOGS.

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Base analogs can be powerful mutagens in both eukaryotic and prokaryotic systems. Due to their similarity to normal DNA bases they may be readily incorporated in DNA; however, due to their ambivalent base-pairing properties, this results in high levels of mispairings and, hence, mutations. Base analogs can also be used as antiviral or chemotherapeutic agents, based on their toxic effects due to interference with normal nucleotide metabolism. Our laboratory has initiated a detailed study of the mutagenic and toxic mechanisms of the purine analogs N6-hydroxylaminopurine (HAP), 2-amino-N6-hydroxylaminopurine (AHAP), and 2-aminopurine (AP), using the model system Escherichia coli in which all three are powerful mutagens. Specifically we have investigated (i) the enzymatic systems that E. coli might use to protect itself against the effects of analogs, and (ii) the pathways by which the analogs are activated through conversion to the nucleotide level. Our experiments have revealed that E. coli possesses a powerful defense system acting against HAP that is dependent on the molybdenum cofactor. Strains defective in the biosynthesis of this cofactor (such as moa, moe or mod mutants) are extremely sensitive to both the toxic and mutagenic effects of HAP. None of the known E. coli molybdoenzymes tested so far is responsible for this protective effect, suggesting the operation of a novel pathway. Another class of HAP-sensitive mutants found are dam strains, which lack DNA adenine methylation. This finding suggests that DNA mismatch repair plays a role in the recognition and protection against base analogs-induced DNA mispairs. The base-analog activation experiments that we have conducted have shown that HAP can be converted to the nucleoside monophosphate (HAPMP) by any of the three E. coli purine phosphoribosyl transferases (gpt, hpt, and apt gene products), while AHAP is converted to AHAPMP by guanine phosphoribosyl transferase (gpt gene product) exclusively. In contrast, AP appears converted to APMP primarily by the serial action of purine nucleoside phosphorylase (deoD gene product) and guanosine kinase (gsk gene product).

993 TOXICITY OF CHRONIC AZATHIOPRINE ADMINISTRATION IN SOMATIC AND GERM CELLS OF C57BL/6 MICE.

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Azathioprine (Aza), a pro-drug of the toxic purine analogue 6-mercaptopurine, is an immunosuppressant used to prevent transplant rejection and for treating autoimmune disease. Patients undergoing chronic Aza therapy can have large increases in *HPRT* lymphocyte mutant frequency (MF), presumably due to drug selection of pre-existing *HPRT* mutant lymphocytes. A similar selection of germ line *HPRT* mutations could result in an increased incidence of the Lesch-Nyhan syndrome, a devastating neurological disease, in subsequent generations. We have begun to develop a mouse model to explore the possibility of increased germ line transmission of *Hprt* mutations. Male C57Bl/6 mice were treated p.o. 3 times /wk with 0, 5, 10, 25, 50 and 100 mg/kg Aza for up to 23 wks. Mice treated with 25-100 mg/kg Aza were all dead by 14 wks of treatment. *Hprt* mutant assays performed on spleen lymphocytes indicated that most treated mice had MFs similar to control mice ($\leqq 10 \times 10^{-6}$), however, a few had clearly elevated Mfs [(e.g.), 233×10^{-6} after 10 weeks of 5mg/kg]. Measurements of testicular weight, and sperm count, viability, morphology, and motility found that 5 and 10 mg/kg Aza produced only low levels of toxicity in germ cells. The data indicate that mice chronically treated with 5 and 10 mg/kg Aza (doses comparable to those given to humans) have elevated *Hprt* MFs similar to humans. Futhermore, the results suggest that mice treated with these doses of Aza retain reasonable fertility, and will be useful for planned breeding experiments to examine the germ line transmission of *Hprt* mutations.

994 MUCOCHLORIC ACID INDUCES PARP ACTIVATION.

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Water chlorination generates halogenated by-products with potential public health risks. While current levels of exposure are low, chronic exposure may lead to significant risk. The halogenated hydroxyfuranones (e.g. mucochloric acid; MCA) have not been studied in detail, but may be an important source of risk. Our goal was to better understand possible mechanisms of MCA-induced genotoxicity. We used a highly sensitive slot-blot assay to characterize aldehydic DNA lesions (ADLs), which include AP sites, and to quantitate 8-oxoguanine (8oxoG; marker for direct base oxidation) by HPLC-ECD *in vivo* after MCA exposure. Five dose groups, each with 6 male F344 rats, received a single gavage dose of 0, 10, 30, 100 or 300 mg/kg MCA. Initial results indicated that there were no significant differences in ADLs and 8oxoG between control and exposed rat liver DNA. We are repeating these analyses and using a new assay where oxidized purines are excised from the DNA backbone by Ogg1, resulting in AP sites detectable by our slot-blot method. We then used a newly developed, sensitive assay which indirectly monitors the status of DNA single strand break (SSB) repair enzymes by circuitously measuring poly(ADP-ribose) polymerase (PARP) activation through depletion of intracellular NAD(P)H. PARP detects, for e.g., direct SSBs arising from sugar damage or spontaneous cleavage at AP sites. We applied this assay to XRCC1-proficient and XRCC1-deficient isogenic Chinese hamster ovary cells exposed to MCA. A water-soluble tetrazolium salt was used to monitor NAD(P)H concentrations in living cells through its reduction to a yellow dye detected spectrophotometrically. After 4h, XRCC1-deficient cells contained significantly less NAD(P)H than proficient cells. To distinguish whether the reduction of NAD(P)H was due to decreased mitochondrial function or due to NAD+ depletion by PARP activation, we co-exposed XRCC1-deficient cells to MCA and a PARP inhibitor. The MCA-induced decrease in intracellular NAD(P)H was almost completely blocked, which strongly suggests that the decrease was primarily due to PARP activation through formation of SSBs.

995 OXIDIZED GUANINE LESIONS IN THE NF-κB REGULATORY ELEMENT ARE SHIELDED FROM REPAIR BY FAPY GLYCOSYLASES WHEN THE TRANSCRIPTION FACTOR IS BOUND.

K. Morrison, B. Martin and K. D. Sugden. *Chemistry, University of Montana, Missoula, MT.* Sponsor: H. Beall.

A number of transcription factor binding sites within inducible promoter regions contain highly oxidizable sequences, namely consecutive runs of guanines. One of these regions, the NF-κB response element displays complex up or down regulation of transcription factor binding depending both upon the nature and position of the oxidized guanine product. It has been reported that the lesions we examined, 7, 8-dihydro-8-oxo-2[1]-deoxyguanosine (8-oxo-dG) and its further oxidized products, guanidinohydantoin/iminoallantoin (GH/IA) and spiroiminodihydantoin (SH) are readily repaired by the bacterial Fapy glycosylase, Fpg (MutM). Therefore we sought to examine the competition kinetics between the DNA binding protein of the NF-κB complex, p50, and bacterial Fpg or its mammalian homolog hOGG1. The terminally oxidized lesions (GH/IA and SH) were generated by the reaction of a high valent chromium compound with synthetic DNA oligonucleotides and purification by HPLC. The oligonucleotides containing a single lesion were reacted with Fpg or hOGG1 in the presence or absence of purified p50. The cleavage efficiency of Fpg was substantially greater than that of hOGG1 for all lesions and the substrate specificity for the two enzymes was also different. Fpg cleaved lesions with the efficiency 8-oxo-dG > GH/IA > SH. Of these lesions hOGG1 also cleaved 8-oxo-dG with greatest efficiency but this was followed by SH while little to no cleavage was observed for GH/IA. The NF-κB transcription factor, p50, efficiently shielded all these lesions from recognition and cleavage by both the bacterial, Fpg, and the human, OGG1, glycosylases *in vitro*. The shielding effect was enhanced with those lesions that show higher binding affinity in a p50 dose-dependent manner. As p50 is translocated to the nucleus under the same oxidative stress conditions under which these lesions can be formed, such shielding from repair may augment any misregulation of gene transcription as consequence of the changes in p50 binding affinity.

996 SYNTHESIS, CHARACTERIZATION, *IN VITRO* AND CALF THYMUS DNA IDENTIFICATION OF N^7-GUANINE ADDUCTS OF 1- AND 2-BROMOPROPANE.

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It has been reported that 2-bromopropane might be a causative agent for reproductive toxicity and have immunotoxic effects. 1-Bromopropane known as an alternative to ozone depleting solvents, which has structural similarity to 2-bromopropane, has been reported to be neurotoxic to rats in long-term inhalation exposure. To elucidate mechanisms of 1- or 2-bromopropane-induced toxicities in the molecular level, formation of N^7-guanine adducts by 1- or 2-bromopropane was investigated *in vitro*. N^7-Guanine adducts of 1- and 2-bromopropane (N^7-propyl guanine and N^7-isopropyl guanine, respectively) were chemically synthesized in three steps in relatively high yields and structurally characterized by analyses of ^{1}H NMR, ^{13}C NMR, UV, HPLC and LC/MS/MS (ESI) to use as reference materials. N^7-Propyl guanine and N^7-isopropyl guanine were detected and identified by UV, HPLC and LC/MS/MS analyses after incubation of 2'-deoxyguanosine with 1- or 2-bromopropane at a physiological condition for 16 hr, followed by thermal hydrolysis. In addition, incubation of calf thymus DNA with 1- or 2-bromopropane at a physiological condition for 16 hr, followed by thermal hydrolysis, produced detectable amount of N^7-propyl guanine or N^7-isopropyl guanine by analyses of HPLC and LC/MS/MS. The present results suggest that 1- and 2-bromopropane may form a DNA adducts at N^7-position of 2'-deoxyguanosine at a physiological condition, which may be responsible for certain toxicities.

997 CONVERSION OF TRIS(8-QUINOLINOLATO-N1, O8) ALUMINUM (ALQ) TO 8-HYDROXYQUINOLINE (8OHQ) AND ACTIVITY IN BACTERIAL REVERSE MUTATION ASSAYS.

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AlQ, an aluminum chelate of 8OHQ, is an important charge transfer molecule in semi-conducting imaging devices. This study was conducted to evaluate AlQ and 8OHQ for the ability to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes, and to determine if AlQ decomposes or is metabolized to 8OHQ under assay conditions. The tester strains used in the mutation assay were S. typhimurium TA98, TA100, TA1535, and TA1537 and E. coli WP2uvrA(pKM101). The assays were conducted in the presence and absence of S9 mix prepared from Aroclor-induced rat liver with appropriate controls and in triplicate. Test article doses were 1 to 1000 µg per plate, and results of the initial assay were confirmed. Stability studies were carried out separately, under conditions designed to closely mimic the mutation assays. Incubation was carried out for 4 hours at 37°C and samples were collected periodically for analysis by HPLC. Analyses by LC/MS were performed to tentatively identify potential metabolites of AlQ and 8OHQ. The results of the bacterial mutagenicity assay indicate that, in the presence of S9 mix, both AlQ and 8OHQ caused positive increases in the mean number of revertants per plate with tester strains TA100 and WP2uvrA(pKM101). No positive increases were observed with any of the remaining tester strain/activation condition combinations. The stability study showed that AlQ degrades readily to 8OHQ under standard mutagenicity test conditions, and the positive test result with AlQ is due to bioactivation of 8OHQ. In the presence of S9, 8OHQ is metabolized to one detectable product with molecular weight indicative of a one-oxygen insertion. 8OHQ N-oxide and 2, 8-quinolinediol were ruled out as possible metabolites; 8OHQ epoxides and other quinolinediols were neither confirmed nor ruled out. Bacterial mutagenicity tests are not predictive of *in vivo* effects of 8OHQ; these assays are similarly expected to be poorly predictive of *in vivo* genotoxic and carcinogenic potential of AlQ.

998 EXAMINATION OF DNA DAMAGE IN ENDOTHELIAL CELLS FOLLOWING TREATMENT WITH 2-BUTOXYETHANOL USING THE SINGLE CELL GEL ELECTROPHORESIS (COMET) ASSAY.

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2-Butoxyethanol has been shown to induce an increase in liver hemangiosarcomas selectively in the mouse following chronic treatment. While the effect for the induction of hemangiosarcomas has not been established, we have postulated that this neoplastic response is indirectly due to the activation of Kupffer cells and hemosiderin deposition subsequent to hemolysis, resulting in oxidative stress in the target endothelial cells. Oxidative stress has been implicated in the induction of DNA damage and modulation of gene expression in chemical carcinogenesis. The present study examined the effect of 2-butoxyethanol, 2-butoxyacetic acid (a major metabolite of 2-butoxyethanol) and iron on DNA damage as measured by the comet assay, in mouse endothelial cells. Treatment with 2-butoxyethanol (0 to 10mM) or 2-butoxyacetic acid (0 to 10mM) failed to induce an increase in DNA damage over control. Iron ($FeSO_4$) (0 to 10µM) and/or H_2O_2 (0 to 5µM) produced an increase in comet measured by olive tail moment and tail DNA. Coincubation of iron and/or H_2O_2 with vitamin E reduced the DNA damage observed with $FeSO_4$ and/or H_2O_2 alone. Incubation of $FeSO_4$ and/or H_2O_2 treated cells with Fpg (an enzyme that cleaves oxidized bases from duplex DNA preferentially) prior to electrophoresis revealed an increase in DNA damage suggesting that the damage was related to an oxidative insult. These data suggest that 2-butoxyethanol and 2-butoxyacetic acid do not directly induce DNA damage in endothelial cells and support our hypothesis that 2-butoxyethanol induces hepatic hemangiosarcomas indirectly, through oxidative stress mechanisms.

999 EXPOSURE OF MICE TO HC RED NO 3 DID NOT INDUCE DNA DAMAGE IN THE COMET AND MICRONUCLEUS ASSAYS.

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This study evaluated the potential for the hair dye HC Red No 3 to induce DNA damage in cells of the liver, urinary bladder, and peripheral blood as well as micronuclei in bone marrow polychromatic erythrocytes (PCE), after oral administration to male B6C3F1 mice. Selection of the liver was based on equivocal data indicating a possible relationship between HC Red No 3 and hepatocellular neoplasms in male mice, while evidence of nephrotoxicity predicates the evaluation of the urinary bladder. Peripheral blood was chosen to evaluate the potential for HC Red No 3 to induce systemic DNA damage. HC Red No 3, the micronucleus assay positive control (cyclophosphamide), and the vehicle control were administered by gavage once daily on four consecutive days (72, 48, 24, and 4 h prior to sacrifice/tissue sampling), while the comet assay positive control (ethylmethane sulfonate) was administered as a single intraperitoneal injection 4 h prior to sacrifice/tissue sampling. HC Red No 3 was administered at 250, 500 and 1000 mg/kg BW per day to 6 animals per dose group. The oral route of administration is the route historically used with this compound. At the time of necropsy, samples of the liver, urinary bladder, and peripheral blood were obtained from each animal for comet analysis, and bone marrow slides for micronucleus analysis were prepared from the femur of each animal. In the comet assay, 100 cells were scored per tissue for the extent of DNA migration. As an indication of the presence of necrosis or apoptosis all dose groups were checked for the frequency of cells with low molecular weight (LMW) DNA. For the MN assay, 2000 PCE were scored per animal. No evidence for apoptosis or necrosis was found using the comet LMW assay. In both assays, HC Red No 3 was negative for genotoxic activity. The values found for the positive and negative controls were within the historical database and confirm the validity of this study. The results of this study support the safety of the direct hair dye HC Red No 3.

1000 *IN VITRO* GENOTOXICITY OF FORMALDEHYDE-RELEASING BIOCIDAL AGENTS IS NOT PREDICTIVE OF *IN VIVO* GENOTOXIC POTENTIAL.

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A series of formaldehyde releasing biocidal agents that produced positive genotoxic responses *in vitro* (chromosomal aberrations and increased mutation frequency in the mouse lymphoma assay) were further evaluated *in vivo* for bone marrow and liver genotoxicity. Bromonitropropane diol, 7-ethyl bicyclooxazolidine, cis-1-(3-chloroallyl)-3, 5, 7-triaza-1-azoniaadamantane chloride, 4-(2-nitrobutyl)morpholine 4, 4'-(2-ethyl-2-nitrotrimethylene)dimorpholine, dimethyl oxazolidine and tris(hydroxymethyl)nitro-methane were evaluated in both the mouse bone marrow micronucleus test (MNT) and the *in vivo*/in vitro rat unscheduled DNA synthesis (UDS) assay. For the MNT evaluation, at least 6 female and/or male CD-1 mice/dose level, at three doses and a vehicle control were treated by a single oral gavage on two consecutive days and sacrificed 24 h after the last dosing. Two thousand polychromatic erythrocytes (PCE) were evaluated from each animal and the frequencies of micronucleated PCEs (MNPCE) were recorded. There were no statistically significant increases in the frequencies of the MNPCE in the test groups relative to vehicle controls. In the UDS studies, at least five male Fischer 344 rats/dose level were given a single oral gavage at two dose levels plus a vehicle control. Livers were perfused at 2-4 and 14-16 h time-points with *in vitro* hepatocyte evaluation of nuclear cell labeling (150 nuclei per animal) after exposure to 3H-thymidine and autoradiography. None of the test materials exhibited statistically significant, compound-related increases in mean net nuclear grains or in the percent of nuclei with five or more net grains relative to vehicle controls. These results clearly demonstrate that *in vitro* genotoxicity associated with formaldehyde is unlikely to have relevance in the whole animal because of the occurrence of efficient detoxification mechanisms.

1001 LACK OF *IN VIVO* GENOTOXICITY OF A DIETARY SOY SUPPLEMENT.

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Numerous epidemiologic studies suggest that diets high in soy products reduce the risk of certain cancers, including breast and colon cancer, which are among the most common cancers in the American population. Some animal studies also suggest that soy fractions can cause deleterious effects under certain circumstances. As part of a study of the effects of diet (including contaminants, secondary plant products, and dietary supplements) on DNA, we examined the effects of two soybean fractions on DNA integrity *in vivo*, using mice and rats. The soy fractions, PCC and Novasoy, are isoflavone-rich ethanol extracts of commercial soybean processing. Outbred CD-1 mice were fed PCC as 1% of a defined, nutritionally complete, semisynthetic diet for up to 5 weeks. Three days before the end of the feeding period, a subset of mice were injected with cytosine arabinoside (ara-c) at a dose previously determined to cause significant DNA damage, to serve as positive controls. At the end of 1, 2, 3, 4 or 5 weeks of eating the defined diet ± PCC, bone marrow from all mice was extracted and examined by flow cytometry. Regardless of the length of time on the defined diet, there were no significant differences between mice given PCC-fed and control-fed mice with respect to flow cytometric measurements. As anticipated, the mice injected with ara-c showed significantly more variation in bone marrow DNA than did uninjected mice In the rat study, outbred CD-1 rats were exposed to PCC and Novasoy while on the same defined, nutritionally complete, semi-synthetic diet. At the end of the feeding period, the rats' bone marrow was extracted and examined for DNA damage. No differences were seen among any of the treatment groups.

1002 HYPOTHERMIA FOLLOWING TREATMENT OF MICE WITH PHENOL IS NOT REVERSED BY THERMOREGULATORY SUPPORT: IMPLICATIONS FOR MICRONUCLEUS (MN) FORMATION.

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Previous investigations revealed an association between high-dose phenol-induced MN in mouse bone marrow and prolonged hypothermia (>8°C decrements for >8 hrs). A study to test the hypothesis that MN in phenol-treated mice could be prevented by maintaining mice at normal body temperature (BT) was inconclusive due to: (a) induction of hyperthermia and MN in controls subjected to the same thermoregulatory support as phenol-treated mice, and (b) the possibility that support measures may not have fully reversed phenol-induced hypothermia. To ascertain whether thermoregulatory support was effective in maintaining phenol-treated mice at normal BT throughout 48-hr observation, frequent BTs were obtained in treated mice using a radio-telemetry device. Male mice (3/dose) were implanted with a small telemetry probe and housed singly in tubs with corncob bedding. BT of mice was sampled every 5 min in standard environmental conditions for 18-20 hrs, then moved to a room providing identical thermoregulatory support as the previous MN study (30°C with warming blankets under half the cage). Mice were dosed with a vehicle or 300 mg/kg phenol (i.p.) and BT monitored every 5-min for an additional 48 hrs. BT measurements indicated that thermoregulatory support altered the normal thermoregulatory pattern of both control and phenol-treated mice. Additionally, thermoregulatory support did not fully prevent hypothermia in phenol-treated mice. In conclusion, these data provide additional evidence for the association between hyper- and hypothermia and MN induction in mice. Attempts to distinguish the potential direct action of phenol on MN formation from the action of hypothermia alone are likely to be confounded by the fact that the maintenance of normal BT in phenol-treated hypothermic mice appears problematic. *Sponsored by the Phenol Panel of the American Chemistry Council, Arlington, VA.*

1003 STRUCTURE-DEPENDENT INCISION OF STEREOISOMERIC BPDE ADDUCTS BY *B. CALDOTENAX* UNIVERSITYvrABC EXCISION NUCLEASE.

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Benzo[*a*]pyrene is metabolized to the highly reactive and mutagenic derivative (+)-benzo[*a*]pyrene-*trans*-7, 8-dihydrodiol-9, 10-epoxide-*anti* (BPDE). BPDE forms adducts mainly on exocyclic amino groups of guanine bases (N2dG) in DNA. N2dG adducts can form in two stereoisomeric configurations, (+)-*trans* and (+)-*cis*, both repaired by nucleotide excision repair (NER). The predominant conformation of the (+)-*trans* adduct has the pyrene system lying in DNA minor groove which causing a minor helix distortion. The predominant conformation of the (+)-*cis* adduct has the pyrene system intercalated in DNA causing a major helix distortion. The (+)-*cis* is excised by human cell extracts 100-fold more efficiently than the (+)-*trans*. *E. coli* UvrABC excision nuclease incises (+)-*cis* ~1.5 fold more efficiently than (+)-*trans*. We examined the role of BPDE-adduct configuration in the recognition and excision of BPDE-adducts by thermal resistant *B. caldotenax* UvrABC excision nuclease. Site-specifically-modified oligonucleotides containing (+)-*trans*, (+)-*cis* and no adduct were ligated into 50 bp DNA fragments and used as substrates for UvrABC. Our results suggest that *B. caldotenax* UvrABC incises *cis*-BPDE- adducts more efficiently than *trans*-BPDE-adducts in the 50 bp DNA fragments (~ 1.6 fold). UvrAB-DNA complex formation was the same with (+)-*trans* and (+)-*cis* substrates suggesting that UvrAB recognizes and binds both with equal affinity and that adduct configuration modifies recognition of UvrAB-DNA complex by UvrC. Discrimination of (+)-*cis* and (+)-*trans* adducts is similar with *E. coli* and *B. caldotenax* UvrABC but much less than with human cell extracts suggesting a major difference between prokaryotic and eukaryotic NER. Stereoisomeric adduct lesions can serve as a good conformational structural tool for exploring the mechanism of DNA damage processing by NER. (Supported by NIH Grant ES06460 and the Commonwealth of Kentucky Research Challenge Trust Fund)

1004 EFFECTS OF EXTRACELLULAR AND INTRACELLULAR FACTORS ON CHROMIUM (III) AND CHROMIUM (VI) GENOTOXICITY.

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Cr (VI) is recognized as a human pulmonary carcinogen. Recently, a debate has been raised as to whether or not Cr (VI) ingestion from drinking water can lead to cancer. An argument against the role of Chromium as an oral carcinogen is that it is reduced to the non-permeable Cr (III) in biological systems. Although it has been shown that both Cr (VI) and Cr (III) have a genotoxic potential, it is still not clear how these two forms of Cr are involved in the mechanism of genotoxicity in an intact cell. In this study, a system screening for deletions was used to compare the ability of Cr (VI) and Cr (III) to induce chromosomal rearrangement in the yeast S. cerevisiae. Induction of intrachromosomal and interchromosomal recombination was measured by growth on selective media. The assay used in this study is sensitive to both oxidation states of Cr. This allows for a comparison of the genotoxic effects of both forms of Cr under identical conditions. At identical concentrations, Cr (VI) induced deletions up six times greater than Cr (III). However, Co-incubation of the test strain with microsomal fractions or N-acetylcysteine had a protective effect against Cr (VI) genotoxicity but had no protective effect against Cr (III). Also, an oxidative DNA damage repair mutant shows a much higher sensitivity to Cr (VI) than to Cr (III). These data imply that oxidative stress is greatly involved in Cr (VI) genotoxicity and not so in Cr(III). An important issue in interpreting the results of this study is the comparison of intracellular concentrations of Cr after exposure to Cr (III) or Cr (VI). This issue is being addressed by analyzing the cells using ICP-MS.

1005 COMPARISON OF DIFFERENT METHODS OF MEASURING CYTOTOXICITY FOR GENOTOXICITY TESTING IN CHL CELLS.

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When substances are tested for their ability to induce structural (clastogenic) or numerical chromosome changes *in vitro* in mammalian cells, the toxicity of a test substance often determines the highest concentration to be assessed. Generally toxicity is measured either by depression in the cell count, or reduction in the mitotic index. The objective of this study was to assess the variability, and to compare the sensitivity, of alternative methods of measuring cytotoxicity. Chinese hamster lung cells (CHL) were exposed for a minimum of 3 hours to Mitomycin C in the absence of a supplemented rat liver homogenate fraction (S9 mix) or cyclophosphamide in the presence of S9 mix. Cytotoxicity was measured from 0 to 72 hours after the end of exposure. Mitochondrial function was assessed using MTT uptake and reduction. DNA synthesis was examined by tritiated thymidine uptake. Cell division was blocked either by cytochalasin B or colcemid and the percentage of either binucleate to mononucleate cells, or mitotic to interphase cells, calculated respectively. The results show considerable variability within and between different methods of measurement. Cell counts and metaphase indices were relatively variable indicators of cytotoxicity. Mitomycin C and cyclophosphamide became more toxic as time passed, as indicated by the MTT assay. It is concluded that the method and time of

measurement of cytotoxicity is likely to influence both the potency of the response and the frequency of biologically irrelevant clastogenic effects. Also, the comparison of *in vitro* micronucleus results between different cell types will be easier if the method of measuring cytotoxicity is the same for both. The MTT assay may be useful, provided that it is used at an appropriate time after the end of exposure.

1006 MINIATURIZED CHROMOSOME ABERRATION TESTING (MINICAT).

J. Lin and R. J. Proudlock. *Genetic Toxicology, CTBR, Senneville, QC, Canada.* Sponsor: L. Dostal.

In vitro genetic toxicology testing is required at an early stage of compound development. For pharmaceuticals, this usually involves a bacterial mutation and a chromosome aberration (or mammalian cell mutation) test before the compound can be administered to man. At this stage the compound is often in short supply and expensive to produce. The dose levels used are dictated by OECD guidelines so that, even for biological molecules such as growth promoters, typically 2 grams of material are used in each assay. A modification of the chromosome aberration test has been developed in this laboratory which reduces the culture volume and, therefore, the mount of test article required for a complete regulatory study to less than 70 mg. Six compounds with various modes of action (including some with a requirement for metabolic action) were evaluated in the standard and miniaturized version of the chromosome aberration test. Benzo(a)pyrene, bleomycin, cyclophosphamide, fluorouracil and mitomycin produced similar levels of chromosome aberrations at comparable doses in both test systems. The intercalating agent, 9-aminoacridine, induced polyploidy but not chromosome damage in both systems. These results show that the MINICAT can be used to meet routine regulatory requirements; it is the recommended *in vitro* mammalian genotoxicity test when test article is in short supply.

1007 PHOTOSTABILITY AND PHOTOGENOTOXICITY OF RETINYL PALMITATE.

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Retinyl palmitate (RP) is currently added to many cosmetic products on the US market. RP is a storage form of retinol (vitamin A) and thermally more stable than retinol, the stability of RP exposed to sunlight and the genotoxicity of RP and its photo-decomposed products have not been described. We determined the photostability and photogenotoxicity of RP and its photoreaction products under UVA, UVB, simulated sunlight (SSL), and visible light. RP photo-decomposed quickly under UVA and UVB irradiation, and at a slower rate in SSL and visible light. The rate of photo-decomposition of RP in a more polar solvent (ethanol) was much faster than that in a less polar organic solvent (hexane). Under similar conditions, retinol was more stable under photoirradiation. RP and its photo-decomposed product mixtures were neither mutagenic in *Salmonella typhimurium* TA98, TA100, and TA102 with and without S9 enzyme activation, nor capable of binding to calf thymus DNA as determined by 32P-postlabeling analysis. Anhydroretinol (AR) was formed as a product in each of the solvents using the difference radiation sources, and exists as a mixture of all-*trans*-AR, 6Z-*cis*-AR, 8Z-*cis*-AR, and 12Z-*cis*-AR, with all-*trans*-AR as a predominant component. AR is considerably stable in hexane, and decomposed photolytically and thermally into multiple products when dissolved in ethanol. It is known that retinoids exert their biological effects, such as cell differentiation and growth, through binding to the RAR and RXR nuclear receptors; however, AR has not been shown to bind to these receptors. As a result, it is possible that photochemical formation of AR from RP may result in altered biological properties when compared to RP exposure without light irradiation.

1008 GENOTOXICANT RESPONSE IN TELOMERASE IMMORTALIZED HUMAN FIBROBLASTS.

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Primary fibroblasts senesce after a limited number of replications. This limitation makes primary fibroblasts unusable for genetic complementation studies requiring selection and cloning. Simian virus 40 immortalization abrogates p53 dependent induction of DNA repair, cell cycle arrest, and apoptosis. Telomerase prevents senescence by maintaining telomere length. We hypothesized that telomerase immortalized cells retain normal p53 activity and provide a good model for DNA repair complementation studies. Therefore, we investigated the use of telomerase immortalized cells for nucleotide excision repair (NER) studies. Primary diploid human fibroblasts (GM00024) from the Coriell Cell Repository were transduced with a telomerase expressing and an empty retrovirus. The empty vector transduced cells senesced after a few doublings. Telomerase transduced cells were cultured continuously for over 6 months indicating immortalization had occurred. Telomerase immortalized GM00024 cells were named tGM24. Responses of tGM24 to ultraviolet light (UV) and benzo[a]pyrene-diol-epoxide (BPDE) exposure were investigated. Cell survival was dose dependent between 0 to 40 J/m^2 UVC. P53 accumulation was UV dose- and time-dependent. At 24 hours post UV, detectable levels were reached at 10 J/m^2 and reached a maximum at 20 J/m^2. At 20 J/m^2, p53 induction occurred between 4 and 8 hours and plateaued between 16 and 40 hours. Caspase-3 activation was detected above 20 J/m^2 48 hours post UV. Apoptosis, as measured by nuclear condensation 48 hours post UV, was dose dependent between 10 and 25 J/m^2. BPDE exposure induced concentration-dependent cell death and p53 accumulation. Our results are consistent with published results using primary diploid fibroblasts. Our findings suggest that the genotoxicant response is unaltered by telomerase immortalization. Telomerase immortalized cells provide an excellent permanent cell culture model for DNA repair complementation studies because they respond to genotoxicants like primary cells. (Supported by the University of Louisville and Kentucky Research Challenge Trust Fund)

1009 MUTAGENICITY AT THE *HPRT* LOCUS IN SPLENIC T-LYMPHOCYTES OF FEMALE B6C3F1 MICE AND F344 RATS AFTER INHALATION EXPOSURE TO *MESO*-1, 2, 3, 4-DIEPOXYBUTANE AND 1, 2-DIHYDROXY-3-BUTENE.

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1, 3-butadiene (BD) is a rodent carcinogen and probable human carcinogen. To evaluate the mutagenic effects following exposure to two important BD metabolites, *meso*-1, 2, 3, 4-diepoxybutane (M-DEB) and 1, 2-dihydroxy-3-butene (DHB), B6C3F1 mice and F344 rats were exposed to 0, or 2 ppm of M-DEB for 3 weeks, or to 0, 6, or 18 ppm of DHB for 4 weeks by inhalation. M-DEB is DNA reactive, while DHB is further metabolized to DNA reactive intermediates. Exposure of mice to 2 ppm of M-DEB yields roughly similar plasma levels of DEB as in mice exposed to 62.5 ppm of BD; exposure of mice to 18 ppm of DHB yields similar plasma levels of DHB as in mice exposed to 200 ppm of BD. *Hprt* mutant frequencies (M*f*s) in splenic T-lymphocytes were determined at multiple time points after exposure using a T-cell cloning assay. M-DEB caused significantly increased *Hprt* M*f*s in both mice and rats. The highest M-DEB-induced *Hprt* M*f*s (M*f*s in exposed animals minus that in control animals) were observed at 4 week post exposure in both mice (2.14 ± 1.06, average $\pm$ SE, $P = 0.046$) and rats (6.99 ± 1.58, $P = 0.016$). The magnitude of increase in *Hprt* M*f*s over background were similar to those observed in an earlier study in mice and rats at the same age exposed to the racemic mixture of DEB under similar exposure conditions. The highest level of induced *Hprt* M*f*s in DHB-exposed mice occurred at 5.5 weeks post exposure (4.08 ± 0.59, average induced M*f*s $\pm$ SE, $P = 0.016$), while *Hprt* M*f*s were not increased in exposed rats. These data indicate that different stereoisomers of DEB have similar mutagenic efficiencies (defined as chemical-induced M*f*s/unit dose); DHB is mutagenic at the *Hprt* locus in mice and the mutagenic potency of DHB is lower than that of DEB. (Supported by the Health Effects Institute)

1010 THE LACK OF MUTAGENICITY FOR THPI (TETRAHYDROPHTHALIMIDE) IN BACTERIAL POINT MUTATION ASSAYS.

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THPI (tetrahydrophthalimide) is the relatively stable degradate of captan, a fungicide that is mutagenic when assayed by *in vitro* bacterial test systems. The US Environmental Protection Agency (EPA) currently classifies captan as a "probable human carcinogen" based on the 1986 cancer guidelines and cites a possible mutagenic component in captan's mode of action (MOA) that promotes tumors in the mouse duodenum. Since captan degrades *in vivo* with a half-life of 0.97 seconds, systemic exposure after captan administration is limited to THPI and subsequent metabolites. In order to show that THPI is not mutagenic, it was tested in six bacterial systems for point mutations. THPI was administered at 0, 0.1, 0.3, 1.0, 3.3 and 10.0 mg/plate to *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA102 and *Escherichia. coli* strain WP2 uvrA. Assays were conducted both with and without activation systems based on the addition of rat liver S-9 preparation. Positive controls varied by test systems and included 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridin, danthron, mitomycin C and 1-ethyl-2-nitro-3-nitrosoguanidine. THPI was uniformly negative in these test systems while the positive controls induced mutations. These data show that THPI is not mutagenic; therefore, it cannot contribute to a "mutagenic component" of captan's MOA. In view of both captan's rapid degradation and the lack of mutagenicity for THPI *in vivo*, a non-mutagenic MOA of captan should be considered.

1011 DETECTION OF SPONTANEOUS AND CHEMICAL-INDUCED DELETION MUTATIONS IN YEAST GENOMIC DNA UNDER NON-SELECTIVE CONDITIONS.

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Mutations are rare genetic events that are difficult to measure directly within a population of wild type sequences. Established methods for detection of mutations rely on selectable loci, as in the gain of function at various his loci in the Ames test or the loss of function in the mammalian hprt assay. Use of selectable loci has been invaluable for understanding mechanisms of DNA repair and mutagenesis in many different species, but this strategy has limitations. For example, mutant frequencies in genes of biological significance cannot be determined because only selectable loci can be used for mutagenesis experiments. In addition, investigators cannot determine when mutations occur because it takes days for selective growth and observation of mutants. We present one solution to this problem and show its application in the yeast DEL assay. In these experiments we use TaqMan to make direct measurements of the yeast chromosomal DNA to detect deletion mutations. The signal/noise ratio of the assay is high, such that mutations can be observed in populations of cells grown under non-selective conditions. Using this assay, chemical-induced deletion mutants can be detected 4 hr after initiation of exposure to mutagens. Dose-response experiments using five direct-acting mutagens showed that the TaqMan assay provided data that were quantitatively similar to those observed in the plating assay, which uses selective conditions. These data therefore support this TaqMan strategy to quantitate deletion mutations in mixed populations grown under non-selective conditions. This TaqMan strategy could also be applied to a broad range of non-selectable loci in any species.

1012 DNA REPAIR AND BREAST CANCER SUSCEPTIBILITY IN PUERTO RICAN WOMEN.

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Cancer is the second leading cause of mortality in the US. Annually more than 300, 000 women are diagnosed with breast cancer (BC) and more than 70, 000 die each year (NCI-SEER). BC represents approximately 31% of all cancers in Puerto Rican women. There is substantial evidence for the involvement of several DNA repair pathways in the carcinogenesis process. The focus of this pilot study was to examine the relationship between DNA repair capacity (DRC), tumor pathology and risk factors of Puerto Rican women with BC. It was hypothesized that a low DRC is a susceptibility factor for BC. A retrospective, case-control study design was used. Peripheral blood lymphocytes were isolated from participants with histopathologically confirmed BC in order to measure DRC using a host cell reactivation assay and a luciferase reporter gene. The age-adjusted DRC of a control group (n=101 women) without cancer was 8.9 ±4.8 (mean ± 1 SD). Participants with BC (n=23) had a mean DRC of 5.7% ±1.6. As DRC decreased, tumor aggressiveness and size and the occurrence of metastasis to axillary lymph nodes increased. Risk factors associated with BC that were statistically significant included: maternal history of BC (odds ratio=OR=11.50), pregnancy (OR= 7.33), familial history of BC or any cancer (OR=5.70 and OR=3.91, and body mass index (OR=3.59). The 36% reduction found in DRC suggests that a low DRC may be a susceptibility factor for BC. Supported by NIH-NCRR grant 2G12RR03050-17 to the PSM RCMI Program.

1013 GENETIC TOXICOLOGY OF 8-2 TELOMER B ALCOHOL.

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8-2 Telomer B Alcohol is a fluorinated chemical intermediate produced and used worldwide to manufacture specialty polymers and surfactants used to a) treat surfaces such as textiles to make them oil and water repellant and b) as a wetting and leveling agent in coatings and cleaning products, respectively. The global telomer producers have initiated a scientific program to develop toxicity data for this chemical. 8-2 Telomer B Alcohol was evaluated for bacterial mutagenicity, in two independent experiments, in S. typhimurium strains TA98, TA100, TA1535, and TA1537 and E coli strain WP2uvrA, at doses of 33.3 to 5, 000 µg/plate with and without metabolic activation (S9). Normal growth was observed in all strains and revertant frequencies for all doses of 8-2 Telomer B Alcohol, with and without S9, approximated vehicle controls. In a micronucleus test, male rats were given a single oral (limit) dose of 2, 000 mg/kg, and the bone marrow cells subsequently examined for the possible induction of micronucleated polychromatic erythrocytes (MN-PCE). As in earlier studies, this limit dose elicited no clinical signs of toxicity. No signs of clinical or bone marrow toxicity, and no statistically significant increases in MN-PCE frequencies were observed. Under the conditions of these tests, 8-2 Telomer B Alcohol did not induce an increase in reverse mutations in S. typhimurium or E. coli, or an increase in MN-PCEs in rat bone marrow. (Studies supported by the TRP Toxicology Program.)

1014 PREVENTION OF ARIPIPRAZOLE–INDUCED HYPOTHERMIA AND HYPOTHERMIA–RELATED MICRONUCLEUS INDUCTION IN MICE.

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Aripiprazole, a novel anti–psychotic agent, induced hypothermia and weak micronuclei induction in mice at 48 hours (but not 24 hours) after dosing. It has been reported that chloromazine– or reserpine–induced hypothermia and induction of micronuclei in mice were prevented by increasing the microenvironmental temperature of the housing conditions. We investigated if the micronucleus induction seen following aripiprazole administration would be observed in aripiprazole–treated hypothermia–rescued mice. Male and female mice were administered a single oral dose of 0, 100 or 200 mg/kg of aripiprazole. Each dose group was housed under different housing conditions; one consisted of housing mice individually in steel cages at 21 — 25°C, and the other consisted of housing mice on chips in a polycarbonate cage placed on a rubber mat heater with cage temperatures of 27 — 31°C. Rectal temperatures were measured. Micronucleated immature erythrocytes stained with acridine orange in the peripheral blood and bone marrow, collected 48 hours post–dosing at necropsy, were counted. Satellite groups of mice housed under the above two conditions were utilized for toxicokinetics. Minimal increases in micronucleated polychromatic erythrocytes were noted at 48 hours in bone marrow of mice rendered markedly hypothermic by treatment with aripiprazole. However, in the groups of mice administered aripiprazole and rescued from hypothermia by heating the microenvironment, no increase in micronuclei was observed. Systemic exposure to aripiprazole was similar between the mice housed under the two different conditions. These data strongly support that the minimal increases in micronuclei observed in bone marrow erythrocytes of mice treated with high toxic doses of aripiprazole were secondary to profound hypothermia.

1015 BIOACTIVATION OF NITROCOMPOUNDS BY PATHOGENIC MICROORGANISMS.

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Mutagenic nitroaromatic compounds are common environmental contaminants. They are the result of atmospheric reactions of polycyclic aromatic hydrocarbons with HNO_3 and NO_2, and of incomplete combustion processes. Their carcinogenic action and mutagenicity depend upon their enzymatic conversion to the corresponding nitroso, hydroxylamine, and amino derivatives by mammalian and bacterial nitroreductases. The objective of the present study was to explore the capacity of cellular extracts from pathogenic microorganisms to activate 1-nitropyrene (1NP) and 2-nitrofluorene (2-NF) to mutagenic derivatives detected in the Ames mutagenicity test. The following microorganisms were tested: *Salmonella typhimurium* knocked out in the main nitroreductase genes (*nfsA* and *nfnB*); *S. typhimurium* double mutant complemented with *nfsA* and *nfnB* from *Escherichia coli*; *Helicobacter pylori* and *Giardia lamblia* clinical isolated strains. Our results show that *S. typhimurium* and *E. coli nfnB* is the main gene involved in the activation of the tested nitrocompounds, although *nfsA* presents some activity. *H. pylori* lysate promoted the activation of 1NP and 2NF increasing the number of mutant colonies of YG7132 *Salmonella* nitroreductase-null tester strain. Finally, *Giardia* lysate activated both nitrocompounds in a minor extent than the observed with bacterial lysates. In summary, the data presented here demonstrate that some of the microorganisms that more frequently infect human populations, possess the enzymatic machinery to activate nitrocompounds that constitute one of the most powerful family of environmental mutagens/carcinogens.

1016 EFFECTS OF CHLOROPHYLLIN ON TRANSPORT OF DIBENZO[*A, L*]PYRENE, 2-AMINO-1-METHYL-6-PHENYLIMIDAZO-[4, 5-*B*]PYRIDINE, AND AFLATOXIN B_1 ACROSS CACO-2 CELL MONOLAYER.

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Chlorophyllin (CHL) is a sodium copper derivative of chlorophyll capable of forming strong non-covalent complexes with several known carcinogens. Anticarcinogenic effects including reduced adduct and tumor formation have been demonstrated for CHL against aflatoxin B_1 (AFB_1), dibenzo[*a, l*]pyrene (DBP) and 2-amino-1-methyl-6-phenylimidazo-[4, 5-*b*]pyridine (PhIP). Alterations in uptake and/or metabolism of planar molecules with at least partial ring structure have been proposed as mechanisms of action for CHL chemoprevention. The Caco-2 cell model of intestinal epithelial transport was used to evaluate the absorption of 1 μM DBP, AFB_1 and PhIP across cell monolayers in the presence of 0, 1, 10, and 100 μM CHL. No significant differences were observed for transport from the basolateral-to-apical (BL→AP) compared to apical-to-basolateral (AP→BL) compartments for DBP and AFB_1, however, PhIP permeability (Pe) from BL→AP compartments (1.26| *times*| $10^{-5} \pm 2.10 \times 10^{-6}$ cm/sec) was significantly higher ($p < 0.001$) than AP→BL ($5.83 \times 10^{-6} \pm 7.56 \times 10^{-7}$ cm/sec) suggesting an active efflux pathway, possibly P-glycoprotein. Transport of DBP from the AP→BL compartment was significantly reduced at all CHL concentrations ($p < 0.05$). AP→BL transport of AFB_1 was significantly reduced by addition of 100 μM CHL ($p < 0.05$) while 1 or 10 μM CHL had no effect. AP→BL transport of PhIP, which has a lower binding affinity for CHL than AFB_1 or DBP, was not significantly altered by addition of CHL. These data suggest that the transport of AFB_1 and DBP can be inhibited by CHL and support a model of direct binding in the GI tract of CHL to these carcinogens as one mechanism of action as a cancer chemopreventive agent. Supported by NIH: ES03850 and ES07316.

1017 DIBENZOYLMETHANE INDUCES PHASE 2 ENZYMES VIA NRF2 ACTIVATION AND INHIBITS BENZO(a)PYRENE INDUCED DNA ADDUCTS IN MICE LUNGS.

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Lung cancer continues to be the leading cause of cancer death in US. Environmental tobacco smoke has been implicated as main agent in lung cancer. Overwhelming the cellular detoxication system, the carcinogens cause DNA damage that sets platform for initiation of cancer. Individual susceptibility to cancer will greatly rely on its detoxification efficacy. Hence, identifying agents that influence the detoxication of carcinogens in lungs can be of significance with respect to lung protection in high-risk population. Dibenzoylmethane (DBM), a minor constituent of licorice and β-ketone analog of curcumin was been identified as an effective blocking agent against carcinogen induced breast cancer however its mechanism is not clear. Further its potency in other organ system has not been studied. Present study reveals the potency of DBM to induce phase 2 enzymes, mechanism of induction and efficacy to inhibit the benzo(a)pyrene(B[a]P) induced DNA adducts in mice lungs. Feeding DBM (500mg/kg/bw) showed induction of phase 2 enzymes and significant inhibition of B[a]P induced DNA adducts however curcumin at a similar dose showed modest effect. To investigate whether DBM mediates phase 2 enzyme induction *via* Nrf2 activation, mouse hepa cells stably transfected with hemeoxygenase ARE luciferase vector p3XstRELuc were exposed to DBM at concentrations 5, 10 and 50 μM. A dose and time dependent increase in mRNA expression of NQO1, GST and GCS was observed. DBM at 50 μM showed a 100-fold increase in luciferase activity. Besides, co-transfection of hepa cells with dominant negative Nrf2 inhibited the reporter activity suggesting the activation of Nrf2 by DBM. EMSA showed increased DNA binding activity of Nrf2 due DBM treatment. In conclusion, DBM inhibits B[a]P induced DNA adducts through phase 2 enzyme induction *via* Nrf2 activation in lungs and thus proves to be promising chemopreventive agent. (This work was supported by the Maryland Cigarette Restitution fund and NIEHS center grant P30 ES03819).

1018 EFFECTS OF PHYTOCHEMICALS ON AFLATOXIN B_1-MEDIATED GENOTOXICITY IN HEPG2 CELLS.

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Aflatoxin B_1 (AFB) is a fungal toxin that commonly contaminates corn, peanuts and other foodstuffs, and is an important risk factor for liver cancer in humans. A variety of phytochemicals found in certain fruits and vegetables show chemoprotective effects towards experimental carcinogenesis in rodents. The objective of this study is to identify whether the human liver-derived cell line, HepG2, can be used to screen phytochemicals for potential chemoprotective activity toward AFB, based on their ability to reduce genotoxic AFB-DNA adduct formation. The five phytochemicals examined, and the concentrations used, were: 1) curcumin (CUR; 5, 10 uM), a component of turmeric; 2) sulforaphane (SFN; 3, 5 uM), 3) phenethyl isothiocyanate (PEITC; 5, 10 uM), and 4) 3, 3-diindolylmethane (DIM; 3, 5 uM), compounds derived from cruciferous vegetables; and 5) iso-xanthohumol (IXN; 5, 10 uM), a hops flavonoid found in beer. After pretreatment of HepG2 cells with the non-cytotoxic concentrations of each phytochemical, we measured changes in AFB-DNA adduct formation using 0.2 uM [3H]-AFB. SFN, PEITC, and IXN significantly reduced AFB-DNA adduct formation in a dose-dependant manner. At the high concentration, SFN, PEITC and IXN reduced AFB-DNA adducts by 57, 69 and 58%, respectively. In contrast, 5 uM DIM significantly increased adduct levels by 121%. Thus, although chemoprotection was observed for some treatments, a modest enhancement of genotoxicity was evident for DIM, which is a known agonist for the Ah receptor and an inducer of CYP1A2. These results demonstrate that common dietary phytochemicals can modulate genotoxicity of AFB in a human liver-derived cell line, and suggest that the cell line may be of use as a screening tool to study phytochemical-induced alterations in gene expression and potential chemoprevention activity. (Supported by grants R01ES05780 and P30ES07033).

1019 EXPOSURE TO SOIL CONTAMINATED WITH AN ENVIRONMENTAL PCB/PCDD/PCDF MIXTURE INHIBITS ULTRAVIOLET RADIATION-INDUCED NON-MELANOMA SKIN CARCINOGENESIS IN THE CRL:SKH1-HRBR HAIRLESS MOUSE.

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Chlorinated aromatic contaminants are active in carcinogenic processes within the skin and may have the potential to modulate ultraviolet radiation (UV)-induced skin carcinogenesis. Exposure to soil bedding containing a complex environmental PCB/PCDD/PCDF mixture during the irradiation phase of photocarcinogenesis was associated with significant ($p < 0.001$) reductions in papilloma incidence and squamous cell carcinoma multiplicity at irradiated skin sites. This protective effect was associated with significantly ($p < 0.0001$) reduced chronic epidermal thickening in UV and contaminant exposed mice compared with mice exposed to UV only and a significant ($p = 0.001$) increase in the amount of methanol-extractable UV-B absorbing material in the skin. This implies that part of the anti-photocarcinogenic effect of the chlorinated aromatic mixture was associated with a sunscreen-like effect. Exposure to the contaminated soil for a 4 week post-irradiation period also resulted in a significantly ($p < 0.015$) reduced tumor multiplicity. This implies that the chlorinated aromatic mixture also inhibited tumor outgrowth during the post-irradiation phase of photocarcinogenesis. Exposure to the chlorinated aromatic mixture was consistently associated with significantly ($p < 0.05$) increased body weight, thus the tumor protective effects observed in these studies cannot be directly explained on the basis of caloric restrictive effects. These results suggest that human chlorinated aromatic exposure may have significant effects on non-melanoma skin cancer development *via* multiple mechanisms.

1020 TWENTY-EIGHT DAY TOXICITY STUDY OF THE CANCER CHEMOPREVENTIVE AGENT 4-BROMOFLAVONE IN DOGS.

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Toxicity of 4-Bromoflavone (4-BF), an experimental cancer chemopreventive agent, was examined after 28 days of daily oral capsule administration to 4 groups of 4 Beagle dogs/sex at 0, 15, 80 and 400 mg/kg/day. Clinical signs included dehydration, diarrhea, decreased activity and abnormally thin dogs among the high dose (400 mg/kg/day) group males. One dog in this group was anorexic, severely dehydrated and exhibited mucoid oculonasal discharge and mucoid greenish feces on day 16. Despite i.v. saline therapy initiated on day 16, the dog lost 30% weight by day 22 and was moribund sacrificed. Females in the high dose group had diarrhea and were dehydrated. Reduced weekly body weight gains were seen on days 11, 18, 25, and 29, resulting in reduced total body weight gain. Reduced food consumption was noted in all 4-BF-treated female groups on day 19. The high and mid dose group males had reduced body weight gain on days 18 and 25 and 25, respectively. Reduced food consumption was noted in these two groups on days 5 - 26. At high dose, hypercholesterolemia and hypocalcemia in males and hypoglycemia in fe-

males were noted. No hematologic changes occurred except reduced MCV in the high dose group males. Treatment-related ophthalmic lesions and changes in electrocardiograms, organ weights, plasma estradiol, testosterone and andrestenediol levels and vaginal cytology were absent. Histopathology revealed that the primary target tissues for 4-BF were liver, kidney, tonsil, epididymis, lymph nodes (mesenteric & mandibular), thymus, ileum, and bone marrow (sternum & rib). Drug-related lesions at high dose were bile duct hyperplasia, hepatic glycogen depletion, oligospermia, immature spermatocytes in epididymis, lymphoid depletion in lymph nodes, bone marrow, tonsils, and gut-associated lymphoid tissue (ileum) and thymic atrophy. (Supported by: NCI Contract No. N01-CN-15141).

1021 TOXICITY EVALUATION OF HALICHONDRIN B ANALOG IN MICE AND BEAGLE DOGS FOLLOWING MULTIPLE INTRAVENOUS EXPOSURES.

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Halichondrin B, a polyeher macrolide, is a tubulin-interactive, antimitotic agent that shows antitumor activity *in vitro*. Multiple (3) i.v. exposures over 9 days to halichondrin B analog produced lethality in male Fischer 344 rats and both sexes of Beagle dogs exposed to 4.43 mg/m2/day and 1.5 mg/ m2/day, respectively, with nonlethal toxicity evident in rats exposed to 2.95 and 1.48 mg/ m2/day. The focus of the current study evaluated the toxicity of halichondrin B analog in both rats and dogs using lower doses than previously studied and the multiple i.v dose exposure regimen. Fisher 344 rats were exposed over 9 days to 3 i.v. injections of 0, 0.077, 0.767 or 1.18 mg/ m2/day of halichondrin B analog. Beagle dogs were similarly exposed *via* 1-hour continuous infusions to 0, 0.08, 0.6 or 0.8 mg/ m2/day. Toxicity endpoints included clinical observations, body weights, clinical pathology, and macroscopic and microscopic pathology. Dog endpoints also included body temperature, non-compartmental serum pharmacokinetic analysis and food consumption. No remarkable clinical findings were found in either low dose rats or dogs. Clinical findings after a 3 or 26-day recovery period for the mid and high groups were limited to reversible changes in blood chemistry parameters, increased body weight (rats), thymic and bone marrow atrophy (rats), lymphoid atrophy (dogs), skeletal muscle degeneration (rats) and non-reversible testicular degeneration in high-dose rats. Analysis of low dose dog serum indicated detectable levels of halichondrin B analog only immediately post-infusion. A mean high dose peak serum concentration of approximately 34 and 39 ng/mL occurred at the end of infusion on Days 1 and 9, respectively with a mean systemic clearance of approximately 6.6 mL/min/kg on both days. The terminal half-life was approximately 6 minutes, indicating effective elimination of halichondrin B analog. Conducted under contract N01-CM-87028, Division of Cancer Treatment of the National Cancer Institute.

1022 ADMINISTRATION OF 1-O-OCTADECYL-2-0-METHYL-SN-GLYCERO-1-D-DEOXY-MYO-INOSITOL (OMDPI) IN A HYDROXYPROPYL-B-CYCLODEXTRIN VEHICLE REDUCES TOXICITY.

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An aspect of cancer therapy involves inducing tumors to undergo apoptosis. OMDPI (1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-1D-deoxy-*myo*-inositol is thought to inhibit the PI-3 kinase, Akt and induce cells to undergo apoptosis. A previous study revealed that jugular catheter i.v. infusion of rats over a 1 hour period to OMDPI in a phosphate buffered saline vehicle resulted in severe localized toxicity at the dosing site after a single exposure to doses ranging from 240 to 480 mg/m^2. The current study evaluated the toxicity of OMDPI when delivered by i.v. infusion in a vehicle of 20% hydroxypropyl β-cyclodextrin (HPCD). Thirty male Fischer 344 rats were implanted with jugular catheters and exposed to daily 1-hour i.v. infusions of either 0, 60, 120, 240, 360 or 480 mg/m^2/day OMDPI in 20% HPCD for 5 days, which was followed by a 10 day recovery period. Toxicity was based on clinical observations, body weights and clinical and macroscopic pathology. No clear dose-related findings occurred over the study period. Findings attributed to possible extravasation due to catheter implantation and the dose administration procedure occurred on Study Day 3 with the death of 1 animal and the euthanasia of 2 other moribund animals in the 120 mg/m^2/day group. Localized swelling near the dosing site was noted in 2 animals in the 120 and 360 mg/m^2/day group and 1 animal in the 480 mg/m^2/day group. No remarkable clinical signs were observed in the 0, 60 or 240 mg/m^2/day dose groups. These findings indicate that localized toxicity following i.v. infusion exposure to OMDPI is greatly reduced when OMDPI is delivered in a HPCD vehicle. Conducted under contract N01-CM-87028, Division of Cancer Treatment of the National Cancer Institute.

1023 INHIBITION OF HUMAN BREAST TUMOR CELL GROWTH BY CONTROLLING MITOCHONDRIAL BIOGENESIS.

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Earlier studies have indicated that Ca2+ ions and mitochondrial function are involved in the regulation of a certain segment of mitosis beyond metaphase. Mitochondrial proteins such as manganese superoxide dismutase, fumarase, succinate dehydrogenase, and the peripheral benzodiazepine receptor are considered to be tumor suppressors. In mammalian cells, gene products from two physically separated genomes: one contained within an organelle and the other within the nucleus; are involved in the biogenesis of mitochondria. Although mitochondrial biogenesis is controlled by nuclear transcription activators and co-activators, the role that mitochondrial biogenesis plays in controlling nuclear gene expression and cell growth is not clear. In this study we present the data in support of our hypothesis that that the growth of breast tumor cells can be controlled through the modulation of mitochondrial biogenesis. Specific inhibitors of mitochondrial biogenesis-chloramphenicol, rhodamine 6 G, and ethidium bromide were used to study the influence of mitochondria on cell growth, cell proliferation, cell cycle progression, and cell cycle genes. Our results show that: 1) Inhibitors of mitochondrial biogenesis significantly reduced breast tumor cell growth as determined by cell counts and the sulforhodamine B assay. 2) DNA synthesis was significantly reduced (50-60%) as measured by BrdU incorporation and further verified by flow cytometry. 3) ATP assays revealed that the inhibition of S-phase was not due to a lack of ATP. 4) Super GEArray analysis of cell cycle genes revealed that the modulation of mitochondrial biogenesis significantly inhibited the expression of cell cycle genes involved in the progression from G1 to S phase. The biological significance of our findings from this study provide the basis for the development of a novel breast cancer therapy, which targets the biogenesis of mitochondria through the use of chemical or peptide inhibitors.

1024 CALORIC RESTRICTION REDUCES BODY FAT, LEAN BODY MASS, AND PALPABLE MASSES WHILE INCREASING THE 24-MONTH SURVIVAL RATE IN SPRAGUE-DAWLEY RATS.

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Caloric Restriction (CR) has been shown to increase the life span of laboratory animals. The goals of this study were to determine the effects of CR on mortality, body weights, clinical pathology, incidence and time of onset of palpable masses, and total body composition in rats. Sixty rats/sex were fed either *ad libitum* or fed a CR diet (70 percent of age-matched *ad libitum* fed rats) and monitored for 24 months. At the end of the 24 months and prior to the scheduled necropsy, total body composition was evaluated using dual energy x-ray absorptiometry (DEXA) on 5 animals/sex/group. The survival rate at 24 months was increased from 47% to 65% for males and from 23% to 78% for females as a result of CR. Average maximum body weights were reduced in both male and female CR rats by approximately 35% compared to *ad libitum* fed rats. Serum chemistry and hematology parameters were analyzed at 12, 25, 51, 77 and 104 weeks and were unaffected by CR. The percentage of animals with palpable masses was slightly reduced in CR vs *ad libitum* animals (27% vs 33%, and 65% vs 78% for males and females, respectively). However, no age-related difference was noted in the mean number of days to the discovery of the first mass (ranged from 401 to 463 days for both groups and sexes). Correlating to the lower percent mortality in the CR group were reductions in total body fat of 57% and 92% and reductions in lean body mass of 36% and 31% for males and females, respectively. There were no discernable differences in bone mineral content or density detected for males or females. As has been noted in numerous studies, rats with CR diets have increased percent survival over 24 months. This study indicated a slight decrease in palpable masses in the CR group, but no changes in bone mineral content, bone mineral density, or clinical pathology parameters. However, the increased survival correlated with a lower body weight in CR animals, which is directly reflected by a decrease in body fat and lean body mass.

1025 COMPARISON OF ONSET AND PROGRESSION OF FOUR COMMON LESIONS/ TUMORS IN *AD LIBITUM* AND RESTRICTED FEEDING REGIMENS FOR CHRONIC STUDIES USING THE SPRAGUE-DAWLEY RAT.

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Previously published research has shown that a reduction in caloric intake of 25 to 40% (of maximal caloric intake) supplemented with essential nutrients, retards the onset of aging and disease in rodents. Following these and similar findings, studies

are now being performed using restricted feeding as opposed to ad libitum feeding in an attempt to increase animal survival by improving the overall health status of the animals. Data collected at CTBR and other research facilities has shown that animals on these studies had improved survivability and had a lower body weight at the end of the dosing period (104 weeks). Analysis of the tumor data showed a reduced incidence of spontaneous and endocrine tumors for these animals when compared to ad libitum feeding. Data obtained at these laboratories for four common lesions/tumors from control animals from carcinogenicity studies using Sprague-Dawley rats from Charles River Canada were compared between restricted and ad libitum feeding regimens. The incidence of pituitary adenoma pars distalis; mammary gland fibroadenoma, mammary gland adenocarcinoma and chronic progressive nephropathy was determined from individual histopathological data. From these data the percentage incidences (number of masses/ number of rats/group) were determined for each study over the course of the 104-week dosing period. Analysis of the data showed a generalized earlier onset of the lesions and in general an increased incidence of the lesions for all four specific lesions/tumors in animals on ad libitum feeding when compared to those on a restricted diet. In conclusion, restricted feeding increased survival in the following ways each being inter dependant; 1) reduced body weight gain; 2) delayed onset of lesions and 3) reduced the overall incidence of the lesions contributing to an overall healthier animal and thus increasing the terminal data population.

1026 EFFECTS OF DIETARY GLYCINE ON THE GROWTH OF PRENEOPLASTIC HEPATIC LESIONS IN MALE F344 RATS.

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The Kupffer cell has the capacity to produce mitogenic factors upon activation, however its contribution to the growth of preneoplastic lesions in the liver is not clear. Phenobarbital (PB), classified as a non-genotoxic carcinogen, enhances the growth of diethylnitrosamine (DEN)-initiated preneoplastic lesions, and has also been shown to activate Kupffer cells. It has been demonstrated that glycine treatment inactivates Kupffer cells. The purpose of this study was to examine the role of Kupffer cell activation as it relates to the tumor promotional effects of PB. Following the initiation of preneoplastic lesions in male F344 rats treated with DEN, groups of 8 initiated-rats were given the following treatments: 0 and 500 mg PB/L (drinking water) for 7 and 30 days. Four rats from each treatment group received 5% GLY (diet) three days prior to additional treatments and this diet continued throughout the study. With respect to cell proliferation, PB caused significant increases in non-focal hepatocyte DNA synthesis, an effect that was not affected by GLY co-treatment. GLY alone had no effect on basal levels of hepatocyte non-focal DNA synthesis. PB also caused an increase in GSTP positive focal lesion DNA synthesis, an effect that was decreased by GLY treatment. PB also caused an increase in the volume of GSTP positive foci, however this effect was reduced by 50% with GLY treatment. GLY alone caused an approximate 40% decrease in the volume of GSTP positive foci, while no effect was seen with respect to the number of GSTP positive foci. These data demonstrate that Kupffer cell inhibition by glycine attenuates the growth of DEN-initiated GSTP positive foci. Kupffer cell inhibition also decreases the promotional properties of PB, indicating a possible growth regulatory role for the Kupffer cell. The results of this study suggest that the Kupffer cell plays an important role in modulating the selective growth of preneoplastic lesions in the liver.

1027 CHEMOTHERAPEUTIC EFFECTS OF PERILLYL ALCOHOL (POH) ON LUNG CANCER CELL LINES.

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POH is being tested in clinical trials as an anti-cancer agent, though the mechanism of action has not been definitively established. We treated 2 lung cancer cell lines, H322 and H838, with POH to determine the anti-tumor properties of this agent. A sulforhodamine B cell proliferation assay was used to determine the effects of POH after 1 and 5 days of treatment with 0.25 mM, 0.5 mM, 0.75 mM, 1.0 mM, and 1.5 mM POH. After 1 day of treatment little difference could be seen between the control and highest concentrations of POH. However, after 5 days, H322 cells showed a dose-dependent decrease in cell proliferation ranging from 15%-83%. H838 cells were less responsive and showed a maximal decrease in proliferation of 70% at 1.50 mM POH. A clonogenic assay demonstrated that while there was no significant effect of POH after 1 day of exposure, a dose-dependent decrease in colony formation, ranging from 15%-97% in H322 cells and 20%-100% in H838 cells, was seen after 5 days of treatment. Using a colormetric enzymatic assay, we observed that POH activated caspase-3 activity 3-6 fold. Time-lapse video microscopy revealed that apoptotic cells were evident within 24 to 48 hr of treatment with 1.5 mM POH, with apoptosis occurring earlier in the H322 than the H838 cells by approximately 20-24 hr. At the doses employed the cells appeared to enter apoptosis in an asynchronous manner, reflecting the use of clinically relevant but not maximally toxic doses of POH. The appearance of apoptotic cells coincided with the increase in caspase-3 activity and cleavage of poly (ADP-ribose) polymerase. Nuclear staining with DAPI confirmed the classical characteristics of apoptosis in POH treated cells. RNA microarray analysis is in progress to determine alterations in expression of signaling molecules. POH may thus be an effective anti-cancer drug that stimulates apoptosis in lung tumor cells. (MX and HSF contributed equally to the work. Supported by the Vaughn-Jordon Foundation, Inc.)

1028 INHIBITION AND REVERSAL OF CELLULAR TRANSFORMATION BY THE HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A.

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The histone deacetylase inhibitor trichostatin A (TSA) inhibited the ability of nickel, chromate and benzo[a]pyrene to transform mouse embryo fibroblast (PW) cells to anchorage-independent growth. TSA to a lesser extent also inhibited the ability of these agents to transform the human osteoblastic (HOS) cells to anchorage-independent growth. Exposure of a variety of nickel or chromate-transformed clones to TSA at 5 and 25 ng/ml followed by a recovery period of 2 to 3 weeks substantially reverted the transformed phenotype from anchorage-independent growth to anchorage-dependent growth. However, TSA was not able to revert other cells as readily as the nickel or chromate-transformed cells. We have observed changes in p21 gene expression and various hypoxia inducible factor related genes such as Cap 43 and VEGF in normal and Nickel transformed cells. We have also analyzed using the gene chip the pattern of gene expression changes found in normal cells, or cells transformed by Ni or those transformed cells reverted by Trichostatin A. Further work in this area should help delineate the specific genes that are silenced by transformation and reactivated by TSA exposure during the course of its reversal of cellular transformation. Supported by grants ES 05512, ES 10344, ES 0260 and CA 16087 from NIH.

1029 CAFFEIC ACID PHENETHYL ESTER (CAPE) INHIBITS ARSENITE-INDUCED HUMAN CELL TRANSFORMATION AND INDUCES G2/M PHASE ARREST OF ARSENITE-TRANSFORMED HUMAN CELLS.

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Inorganic arsenic is a common environmental and occupational pollutant and a human carcinogen, but the mechanisms of its carcinogenicity have not been well defined. Increasing evidence suggests that inflammatory factors play a pivotal role in chemical carcinogenesis. The present study was designed to investigate if CAPE, an anti-inflammatory and immunomodulatory agent, has an effect on arsenic carcinogenicity. Arsenite-induced transformation of human osteogenic sarcoma (HOS) cells, which are immortal but not tumorigenic, was determined by anchorage independent growth assay. An 8-week arsenite (Na2AsO2, 0.3 uM) exposure caused HOS cell transformation, whereas simultaneous treatment with CAPE (0.5, 1.0 or 2.5 uM) completely suppressed it. To examine the effect of CAPE on the growth of HOS cells, arsenite-transformed HOS (As-HOS) and non-transformed parent HOS (N-HOS) cells were treated with CAPE (0.5, 1.0 or 2.5 uM) for 24 h. The results showed that CAPE suppressed As-HOS cell growth dose-dependently, with no effect on growth of N-HOS. Cell cycle analysis revealed that CAPE induced As-HOS cell cycle arrest at the G2/M interphase, with the percentage of cells in G2/M increasing from 14.8% in control As-HOS to 41.8% in CAPE-treated As-HOS (2.5 uM). Western blot analysis indicated that CAPE treatment significantly inhibited the expression of cyclin B1 and cdc2 (CDK1) in As-HOS cells. It is concluded that CAPE is capable of inhibiting arsenite-induced human cell transformation and suppressing growth of arsenite-transformed cells. These results suggest that inflammatory factors may play an important role in arsenic carcinogenesis, and anti-inflammatory CAPE could be a promising preventive and therapeutic agent, particularly for human cancers resulting from arsenite exposure. [Supprted in part by ES00260 & ES10344]

1030 INDUCTION OF DIFFERENTIATION BY KAEMPHEROL IN GJIC-SUFFICIENT BUT NOT IN GJIC-DEFICIENT COLON CANCER CELLS.

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The consumption of flavonoids has been linked to the prevention and therapy of some human cancers. The underlying mechanisms by which the flavonoid could contribute to cancer prevention and therapy are not known. Inhibition of GJIC by oncogenes has been implicated in the removal of the suppression of growth and differentiation by neighboring cells on the initiated cells in the tumor promoting/progression steps of cancer, while upregulation of gap junctional intercellular communication (GJIC) has been correlated with anti-tumorigenicity. We determined the effects of kaempherol on the differentiation of a KNC (well-differentiated colon carcinoma; GJIC+) and a HCT116 (colon carcinoma cell line; GJIC-), as measured by changes in the activity of alkaline phosphatase (ALPase) and morphological shape of the cells. Compared to the highly malignant and GJIC—HCT116 cell line, the ALPase activity of the GJIC+-KNC cells was approximately 3x higher. Kaempherol further increased GJIC (1.3x) and ALPase activity (2.5x) and changed the morphology of the KNC cell to a more normal epithelial cell appearance but had no effect on these same parameters in the HCT116 cell line. As measured by Western blot analysis, the phospho-ERKp42 was more highly expressed than phospho-ERKp44 in the KNC cell, and decreased by 20% with kaempherol. STAT3 was also decreased by 3% in KNC with kaempherol. In conclusion, kaempherol induced further increase in GJIC of the GJIC sufficient colon cancer cell that correlated with a decrease in both ERK and STAT3 and an increase in cell differentiation, but had no affect on the GJIC-deficient cell line. Funded by the NIEHS Superfund grant #P42 ES04911-07.

1031 INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY CHLOROHYDROXYFURANONES IN BALB/C 3T3 CELLS.

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One of the main groups of mutagenic by-products in chlorinated drinking water is the chlorohydroxyfuranones (CHFs). Of the CHFs, MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), MCA (3, 4-dichloro-5-hydroxy-2(5H)-furanone), CMCF (3-chloro-4-(chloromethyl)-5-hydroxy-2(5H)-furanone), and MCF (3-chloro-4-methyl-5-hydroxy-2(5H)-furanone) have been shown to be genotoxic *in vitro* in bacteria and in mammalian cells. MX is genotoxic *in vivo* and a multisite carcinogen in Wistar rats. The cellular mechanisms by which MX induces tumors in rats are not known. A two-stage cell transformation assay *in vitro* suggests that MX has the potential to promote tumor development. Inhibition of gap junctional intercellular communication (GJIC) is supposed to be one mechanism in tumor-promotion. In the present study, the effects of three CHFs (MX, MCA, and CMCF) on GJIC in Balb/c 3T3 cells were measured using the scrape loading dye technique. The cells were exposed to MX (0.005, 0.05, 5, and 50 μM), MCA (0.05, 0.5, 5, and 50 μM), and CMCF (50, 75, 100, and 125 μM) for 30 minutes. The known tumor promoter, TPA (12-O-tetradecanoylphorbol-13-acetate) was used as a positive control. All three CHFs inhibited GJIC in a dose-dependent fashion in the order of potency MX⟩MCA⟩CMCF. The maximum inhibition was 50% of the control level. MX was nearly as potent (nM concentrations) as TPA, while MCA was 10 times weaker and CMCF 10 000 times weaker than MX. The results indicate that MX is a potent inhibitor of GJIC *in vitro*, while the other CHFs have a lower inhibition activity.

1032 A COX-2 INHIBITOR INHIBITS POST-INITIATION PHASE OF N-NITROSOBIS(2-OXOPROPYL)AMINE-INDUCED PANCREATIC CARCINOGENESIS IN HAMSTERS.

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The modification effects of nimesulide, a cyclooxygenase (COX)-2 inhibitor, administration during the post-initiation phase of pancreatic carcinogenesis were investigated in hamsters treated with N-nitrosobis(2-oxopropyl) amine (BOP). Male Syrian hamsters were given four weekly s.c. injections of BOP at a dose of 10 mg/kg and thereafter they were administered 0, 100 or 400 ppm nimesulide in the diet for 36 weeks. Additional groups of hamsters were fed 400 ppm nimesulide without prior BOP-initiation or non-treated. At week 40 of the experiment, all surviving animals were sacrificed, and development of neoplastic and preneoplstic lesions was assessed histopathologically. The incidence of pancreatic adenocarcinomas was significantly ($p<0.05$) decreased in the BOP/400 ppm nimesulide group as compared to the BOP alone group. The multiplicity of total lesions of pancreatic adenocarcinomas plus atypical hyperplasias was also significantly ($p<0.05$) lowered. Immunohistochemically, COX-2 was expressed in pancreatic adenocarcinoma cells whereas the severity of expression was not remarkably affected by the nimesulide treatment. The incidence and multiplicity of neoplastic lesions in other organs did not significantly differ among the BOP-treated groups. No neoplastic lesions were detected in animals receiving nimesulide alone. Our results clearly indicate that nimesulide protects against BOP-induced pancreatic tumors in hamsters.

1033 COLON CANCER AND VITAMIN E SUPPLEMENTATION IN YOUNG AND OLD RATS.

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Previous studies have reported conflicting effects of vitamin E on cancer development. In this study, we examined the effects of d-alpha tocopheryl succinate (TS) in female Sprague-Dawley rats of either 20 (OLD) or 2 mo (YNG) of age. Treatment groups from each age group were fed commercial rodent chow or the same feed supplemented with either 1 (YNG) or 2 (OLD) g/kg TS/kg diet. These animals were injected ip with the carcinogen, azoxymethane (AOM)(10 mg/kg) on days 8 and 15 of the study. After 42 days exposure the animals were sacrificed and serum and a section of colon were collected for analysis of d-alpha-tocopherol (aT) and gamma-tocopherol (gT) content. The remainder of the colons were removed and examined for the presence of aberrant crypt foci (ACF), a preneoplastic marker for colon cancer. Dietary TS supplementation significantly increased aT concentrations in the serum and colons of all rats compared to control diets. The OLD group fed the TS diets had significantly increased colonic aT and gT levels as compared to the YNG group fed similar diets. There were consistently fewer total ACF in the colons of groups treated with TS regardless of age compared to their controls. This reduction was significant in larger ACF (>3 aberrant crypt/foci) of the OLD group. It appears that the larger ACF, which are more likely to develop into tumors, are reduced by TS treatment in the OLD group and this appears to correspond with increased aT or gT levels in the colon. This data supports previous studies by others that show dietary vitamin E supplementation may be protective against the development of colon cancer. Surprisingly, our study shows that older rats retain significant levels of gT in the colons compared to young rats, even in the presence of high aT supplementation. We speculate that the retention of gT in the colons of older rats may play a chemopreventive role.

1034 EFFECT OF LOW DOSE MATERNAL DIETARY VITAMIN E ON EMBRYONIC OXIDATIVE DNA DAMAGE IN P53 KNOCKOUT MICE.

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Reactive oxygen species (ROS) have been implicated in cancer initiation and promotion, and the embryo has limited protective antioxidative mechanisms, leaving it at potentially greater risk. We have shown that *in utero* exposure of pregnant p53 knockout mice, which exhibit spontaneous tumorigenesis, to a high level (10% [w/w]) of the antioxidant vitamin E (VE) alters conceptal DNA oxidation and enhances postnatal tumor development in the offspring. Since we previously found that low dose (0.1% [w/w]) dietary vitamin E *in utero* reduces embryonic DNA oxidation and embryopathies in outbred CD-1 mice, here we detemined the effect of this VE dose on embryonic DNA oxidation on p53 knockout mice during organogenesis (gestational day [GD] 13) to assess its potential for cancer prevention. Heterozygous (+/-) p53-deficient females were placed on either a normal or 0.1% [w/w] VE (dl-α-tocopherol-acetate)-supplemented diet for 4 weeks and mated with +/- p53-deficient males. Vitamin E supplementation continued until GD 13, when the embryos were extracted, p53-genotyped, and analyzed for DNA oxidation, reflected by 8-hydroxy-2'-deoxyguanosine formation. Unlike in CD-1 mice, 0.1% VE exposure did not alter embryonic DNA oxidation levels during organogenesis for any p53 genotype. However, since carcinogenic initiation/promotion also may occur later in gestation, it remains to be determined whether fetal DNA oxidation in the last trimester may be reduced by 0.1% VE, in which case that dose would be reasonable for attempting to inhibit tumorigenesis in p53 knockout mice. (Support: National Cancer Institute of Canada; Canadian Institutes of Health Research)

1035 SELENOMETHIONINE AND VITAMIN E IN SMOKERS: A PHASE I STUDY OF A POTENTIAL CHEMOPREVENTIVE REGIMEN.

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We have completed a Phase I study of l-selenemethionine (SeMet) and vitamin E in chronic smokers, characterizing safety and examining biomarkers for compliance and effects. Thirty-two healthy subjects with a minimum of ten pack-years of smoking were enrolled. Following a four week single-blind placebo period, each subject was examined and blood and bronchoalveolar lavage samples were obtained. Subjects then were randomized to four arms of this double-blind study: placebo, low dose SeMet (200 μg Se/day), high dose SeMet (400 μg Se/day), or high dose SeMet plus 400 I.U. vitamin E/day. All subjects also received 250 mg vitamin C/day. After 16 weeks of treatment subjects had repeat bronchoscopies and blood and lavage specimens were obtained. Markers of compliance included plasma vitamins C and E and selenium, whereas biomarkers of effect included glutathione peroxidase, catalase, and superoxide dismutase activities and PGE2 levels in bronchoalveolar lavage fluid, and DNA damage in blood mononuclear cells and in alveolar macrophages. DNA damage was determined using the single cell electrophoresis (SCE) assay. Selenium levels in alveolar macrophages also were determined for some subjects. The treatment was well tolerated, with no serious adverse effects noted. Compliance was supported by significant increases in plasma vitamin E for subjects in the fourth arm, however neither vitamin C nor selenium levels in plasma were altered by the treatments. Among the effect biomarkers, no statistically significant changes could be associated with SeMet. All groups showed some improvement in SCE scoring in alveolar macrophages, but this was not SeMet dependent. Interestingly, examination of eight high dose SeMet subjects demonstrated a significant increase in alveolar macrophage Se content. This effect in a presumed Se-replete cohort suggests that tissue-specific levels of Se may be increased by supplementation, independent of effects on Se levels in plasma. Supported by NCI-NO1-CN-65019.

1036 ARSENIC AS A DEVELOPMENTAL TOXICANT: LOW DOSE EXPOSURE *IN UTERO* INDUCES ABERRANT GENE EXPRESSION IN THE EMBRYONIC LUNG.

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The human health effects of low dose arsenic exposure in the drinking water remain controversial. The adult lung is a target organ for arsenic toxicity, however, the molecular effects of maternal arsenic exposure on the developing lung are poorly understood. We hypothesize that *in utero* exposure to inorganic arsenic causes altered gene expression in the lung, leading to molecular and functional changes. We exposed pregnant Sprague-Dawley rats to 500 ppb arsenic in the drinking water, in the form of sodium arsenite or sodium arsenate, from conception to embryonic day eighteen. Subtractive hybridizations of embryonic lung cDNA from control and treated day 18 embryos yielded numerous differentially expressed cDNA clones. We have sequenced 352 differentially expressed cDNA clones from these subtractive hybridizations and have spotted them on a custom cDNA microarray. Using a rat alveolar type II cell line exposed to arsenic as a model for lung gene expression effects, we have carried out a dose and time-response study of subacute, low dose arsenic exposure (10, 50, 100, and 500 ppb for 1, 7, and 14 days). Using these samples for cDNA microarray hybridizations, we have profiled gene expression changes induced by arsenic exposure. Using both subtractive hybridization and cDNA microarray, we have found differential expression of genes involved in branching morphogenesis and in formation of the extracellular matrix and cytoskeleton, thus implicating arsenic as a toxicant effecting structure and function in the developing lung. (Supported in part by the NIEHS Superfund Basic Research Program Grant P42 ES04940).

1037 ABERRANT GENE EXPRESSION IN LIVER AND LIVER TUMORS INDUCED BY TRANSPLACENTAL EXPOSURE TO INORGANIC ARSENIC.

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Arsenic is a known human carcinogen that targets the liver. We have found exposure of pregnant mice to inorganic arsenic in the drinking water induces hepatocellular carcinoma (HCC) in the offspring. In addition, adult mice chronically exposed to inorganic arsenic in the drinking water (37.5 ppm, 48 weeks) show hepatic overexpression of the estrogen receptor-alpha (ER) and cyclin D1 genes, both of which are implicated in hepatocarcinogenesis. Thus, we examined ER and cyclin D1 expression in liver and liver tumors in adult mice that resulted from transplacental exposure to inorganic arsenite (0 or 85 ppm from gestation day 8 to 18). HCC or surrounding normal liver samples were taken at necropsy from control or arsenic-exposed mice which were typically over 1-year old. Immunohistochemistry revealed the increased expression of ER and cyclin D1 protein in HCC and normal liver of arsenic-exposed mice. Microarray and real time RT-PCR analysis confirmed the increased expression of ER (2.5- to 3.5-fold over control) and cyclin D1 (1.7- to 6.8-fold) at the RNA level in HCC and normal liver after arsenic exposure. Methylation-specific PCR of DNA from HCC and normal liver showed arsenic had induced extensive hypomethylation in the promoter region of the ER gene. To define the human significance of these data, RT-PCR analysis was applied to liver biopsy samples from a population in Guizhou, China, heavily exposed to environmental arsenic that develop HCC. ER and cyclin D1 expression was 2 to 5 times higher with arsenic exposure than in normal human livers. These data show that transplacental arsenic carcinogenesis is linked with enhanced expression of ER and cyclin D1. The overexpression of the ER gene may be due to promoter region hypomethylation. Both ER and cyclin D1 can play important roles in liver tumor formation, and their overexpression may be an important element in the hepatocarcinogenic effects of inorganic arsenic.

1038 TOXICOGENOMIC ANALYSIS OF ABERRANT GENE EXPRESSION IN LIVER AND LIVER TUMORS INDUCED BY TRANSPLACENTAL EXPOSURE TO INORGANIC ARSENIC IN MICE.

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Arsenic is a known human carcinogen. We have reported that brief exposure of pregnant mice to arsenite in the drinking water induces hepatocellular carcinoma (HCC) in the offspring. In this prior work, adult male mice, which were the offspring of dams exposed to sodium arsenite (85 ppm as arsenic) in the drinking water from gestation day 8 to 18, showed a 61% rate of HCC while control animals showed a spontaneous rate of 12%. Liver tumor or surrounding pathologically normal liver tissue samples were taken at necropsy and quick frozen for later use. In the present study, total RNA was extracted from these liver samples, and subjected to microarray and quantitative RT-PCR analysis. Among 600 genes, arsenic-induced HCC showed a higher rate of aberrant gene expression (>2 fold increase or decrease, 31% of all genes tested) than spontaneous tumors (20% of all genes tested). Overexpression of estrogen receptor-α, cyclin D1, c-*myc* and suppression of p27 was associated with arsenic exposure. Expression of α-fetoprotein and downregulation of syndecan-1, markers for aggressiveness of HCC, were seen only in arsenic-induced tumors. The non-tumorous hepatic tissue of animals transplacentally exposed to arsenic also showed various significant alterations in the expression of genes associated with cell proliferation, oxidative stress, signal transduction, DNA damage/repair, and hepatic biotransformation. Real time RT-PCR largely confirmed these findings (with a 75% accordance among 32 selected genes from microarray). These aberrantly expressed genes could be important in arsenic carcinogenesis. This genomic analysis revealed aberrant gene expression patterns associated with transplacental arsenic carcinogenesis, indicating that arsenic can alter gene expression long after a brief exposure during gestation. This study points towards the importance of protecting pregnant women in areas where arsenic exposure is common.

1039 TOXICOKINETIC AND GENOMIC ANALYSIS OF CHRONIC ARSENIC EXPOSURE IN MULTIDRUG-RESISTENCE DOUBLE KNOCKOUT MICE.

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Multidrug-resistance gene double-knock out mdr1a/1b(-/-) mice, which are deficient in drug-transport type I P-glycoprotein, are more sensitive than wild-type (WT) mice to acute arsenic toxicity. This study assessed the toxic manifestations of chronic oral arsenic in mdr1a/1b(-/-) mice, including altered gene expression, and investigated altered toxicokinetics as a potential basis of enhanced arsenic toxicity. Thus, mdr1a/1b(-/-) and WT mice were exposed to sodium arsenite (0 to 80 ppm as arsenic) in the drinking water for 10 weeks at which time hepatic arsenic accumulation, redox status, lipid peroxidation (LPO) and aberrant gene expression (using cDNA microarray) were assessed. During the exposure period, all mice survived, but body weight in the highest dose (80 ppm) group was less than control in both mdr1a/1b(-/-) and WT mice. Arsenic induced pathological changes, increases in LPO and enhanced glutathione S-transferase (GST) activity in the liver, to a greater extent in mdr1a/1b(-/-) than in WT mice. Enhanced expression of hepatic

cyclin D1, a potential hepatic oncogene, was observed in arsenic-exposed mdr1a/1b (-/-) mice when compared to WT mice. The expressions of certain genes, such as those encoding GST, heat shock proteins and metabolic enzymes, were modestly altered in arsenic-exposed mice. At the highest level of exposure, the hepatic arsenic content was higher in mdr1a/1b (-/-) mice than in WT mice, suggesting that enhanced accumulation due to transport deficiency may account for the enhanced toxic manifestations observed in these double knockout mice. In summary, this study shows that chronic arsenic toxicity, including liver pathology and oxidative stress, is enhanced in mdr1a/1b(-/-) mice, possibly due to enhanced accumulation of hepatic arsenic as result of transport system deficiency.

1040 CHROMOSOME ALTERATIONS AND HYPERPROLIFERATION ASSOCIATED WITH CHRONIC ARSENIC EXPOSURE.

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Epidemiological studies link arsenic exposure to cancers of the skin, lung, liver and other sites in human, although the underlying carcinogenic mechanism remains incompletely understood. Our previous studies have shown that chronic (> 18 weeks) low level (125 to 500 nM) exposure to sodium arsenite induces malignant transformation in the TRL 1215 rat liver epithelial cell line, which is normally diploid, and non-tumorigenic. Malignant transformation was confirmed in these chronic arsenic exposed (CAsE) cells by tumor formation upon inoculation in Nude mice. Chromosomal aberrations have been linked to arsenic exposure in both epidemiological and laboratory studies. The aim of present study was to better characterize the cytogenetic effects of the arsenic during arsenite-induced transformation in CAsE cells by assessing *in vitro* changes in mitotic index (MI, related to cell proliferation), Sister-chromatid exchange (SCE, correlated with chromosome damage), and polyploidy (numerical chromosomal aberration related to tumor development). Our results showed that MI was increased a maximum of about 4-fold in a concentration dependent manner in CAsE cells compared to control cells after continuous exposure to 125, 250 or 500 nM sodium arsenite for 24 weeks. The average generation time (AGT), which is inversely related to growth rate, was reduced in CAsE cells compared to control cells after 24 weeks of arsenite exposure, but not after arsenic exposure for only 6 weeks, a time point prior to malignant transformation. Polyploid, which was typically tetraploid in nature, was increased in CAsE cells compared to control, but not changed in pretransformed cells. The frequency of the SCE was significantly increased in CAsE cells. Taken together, these results indicate chronic arsenic exposure resulting in malignant transformation induces chromosome aberrations and enhanced proliferation, factors that might be an important in arsenic carcinogenesis.

1041 MALIGNANT TRANSFORMATION AND DNA HYPOMETHYLATION IN HUMAN PROSTATE EPITHELIAL CELLS CHRONICALLY EXPOSED TO INORGANIC ARSENITE.

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Arsenic is an established human carcinogen that some epidemiological evidence suggests may target human prostate. We set out to malignantly transform the human prostate epithelial cell line RWPE-1 by chronic inorganic arsenite exposure to determine if this cell type was directly susceptible to the transforming effects of arsenic. Cells were exposed continuously to 5 mM sodium arsenite and assessed for signs of transformation, such as increase matrix metalloproteinase (MMP) secretion. After 29 weeks of exposure, the chronic arsenic-exposed human prostate epithelial cells (CAsE-PE) exhibited a 2.2-fold increase in secreted MMP-9 activity compared to passage-matched RWPE-1 cells maintained in medium only. As definitive proof of malignant transformation, the cells were inoculated into nude mice to assess tumorigenicity. Five of five mice inoculated with CAsE-PE cells developed tumors within 10 weeks of inoculation while none of the mice inoculated with passage-matched RWPE-1 cells developed tumors. CAsE-PE cells produced undifferentiated epithelial tumors that were highly invasive into the surrounding capsular muscle and adjacent normal renal parenchyme and stained positive for prostate-specific antigen, confirming their origin. We next compared the genomic DNA methylation status of CAsE-PE and passage-matched RWPE-1 cells. Our results revealed significant DNA hypomethylation in the arsenite-transformed cells, which is consistent with previous studies using rodent cell models. We are currently comparing gene expression patterns in these two lines to determine the functional consequences of this hypomethylation. The present study represents the first report of arsenite-induced malignant transformation of human epithelial cells and provides a human-based model in which to examine the mechanisms underlying arsenic carcinogenesis.

1042 ALTERED GENE EXPRESSION ASSOCIATED WITH ARSENITE-INDUCED MALIGNANT TRANSFORMATION OF HUMAN PROSTATE EPITHELIAL CELLS.

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Arsenic has long been known to cause cancer in humans and epidemiological studies have shown an association between long-term environmental arsenic exposure and cancer in a number of tissues, including potentially the prostate. However, the mechanisms underlying arsenic carcinogenesis remain unclear. We have recently developed an *in vitro* model of arsenic-induced prostate cancer by malignantly transforming the immortalized, non-tumorigenic human prostate epithelial cell line RWPE-1 by continuous exposure to 5 μM sodium arsenite for 29 weeks. Malignant transformation was established by the ability of the chronic arsenite-exposed prostate epithelial cells (designated CAsE-PE) to produce tumors after inoculation into nude mice. To understand the molecular mechanisms underlying arsenic carcinogenesis, we performed gene expression comparisons between CAsE-PE and passage-matched control RWPE-1 cells. Initially, we used northern blot analyses to examine expression of several oncogenes and tumor suppressor genes. Our studies revealed increases in c-*myc*, K-*ras* and *p53* mRNA in the CAsE-PE cells (1.5-fold, 2.6-fold and 2-fold, respectively, of RWPE-1 levels). As an expansion of the above studies, cDNA array experiments have been initiated in order to perform more extensive gene expression comparisons between CAsE-PE and RWPE-1 cells. The initial array studies have provided preliminary data suggesting altered expression of oncogenes as well as genes linked to apoptosis, cell cycle regulation and signal transduction in CAsE-PE cells. While these results suggest that arsenic may alter oncogene and tumor suppressor gene expression in the course of transformation, further study is clearly needed to determine the role of the observed changes in the transformation process. Further genomic analysis should provide additional clues as to the molecular changes associated with arsenic-induced malignant transformation.

1043 SPECIFIC CHANGES IN CADHERIN EXPRESSION AND LOCALIZATION ARE ASSOCIATED WITH CADMIUM-INDUCED MALIGNANT TRANSFORMATION OF HUMAN PROSTATE EPITHELIAL CELLS.

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Cadmium is a carcinogenic metal implicated as a human prostatic carcinogen based on epidemiological studies, rodent studies and *in vitro* experiments. However, since the evidence is still considered equivocal, identifying the molecular changes specifically associated with cadmium-induced malignant transformation of human prostate epithelial cells would help clarify the role of this metal in human prostate cancer. In order to identify cadmium-specific changes, we compared the gene expression pattern of cadmium-transformed human prostate epithelial cells to those of the parental cell line as well as Ki-*ras*- and MNU-transformants derived from the same cell line. cDNA array experiments revealed a specific increase in P-cadherin in the cadmium transformed cells that was later confirmed by western blot analyses. Surprisingly, the increase in P-cadherin resulted from overexpression of several truncated species of P-cadherin that exhibited perinuclear, rather than membrane, localization. Pan-cadherin westerns showed that these truncated molecules were missing their C-terminal regions, which explains the altered localization since the C-terminus contains the transmembrane domain. The pan-cadherin westerns also revealed specific loss of N-cadherin in the cadmium transformed cells, even though N-cadherin did not show up in the array analysis. In addition, preliminary data show that, while there are no differences in E-cadherin levels between the various cell lines, the cadmium-transformed cells showed specific alterations in E-cadherin localization. While the mechanistic implications of these changes are unclear, it is known that alterations in cadherin expression and/or localization can have profound effects on tumor cell migration and metastasis. Additionally, these alterations in cadherin expression represent potential specific biomarkers for cadmium-induced prostate cancers in men.

1044 MICROARRAY ANALYSIS OF BONE CELL GENE EXPRESSION EARLY AFTER CADMIUM GAVAGE IN MICE.

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We developed an *in vivo* model for Cd-induced bone loss in which mice excrete bone mineral in feces starting 8 h after Cd gavage. In both mice and dogs, this bone response starts at 2-5 μg Cd/l blood, below current USOSHA standards for industry. Female mice of 3 strains [CF1; metallothionein-wildtype (MTN); MT1, 2-deficient (MT1, 2KO)] were placed on a low Ca diet for 2 wk. Each mouse was gavaged with 200 μg Cd or vehicle. Fecal Ca was monitored for 8 d to document the bone response. For CF1 mice, bones were taken from 4 groups: +/- Cd, 2h after Cd; +/- Cd, 4h after Cd. The MTN and MT1, 2KO strains had two groups each: +/- Cd, 4 h after Cd. PolyA+ RNA was isolated from marrow-free shafts of femura and tibiae, and each +/- Cd pair was submitted to Incyte Genomics for microarray analysis. To validate microarray results, the same CF1 4h RNA preparations were subjected to Northern analysis; probes were prepared from 18 clones of genes shown by microarray to be key to the bone cell Cd response, standard housekeeping genes, and other genes with no Cd response *via* microarray. Fecal Ca results showed that bone calcium excreted after Cd differed for the three mouse strains: CF1 0.24+0.08 mg; MTN 0.92+0.22 mg; MT1, 2KO 1.7+0.4 mg. Microarray results showed that, among the ~8500 arrayed genes, three categories were significantly induced by Cd: cell protector genes (e.g., MT1, MT2, transferrin receptor); cell signaling genes (e.g., p38 MAP kinase); and genes involved in osteoclast-mediated bone resorption (e.g., vacuolar proton pump, integrin alpha V, src-like adaptor protein). Cd also induced two genes encoding unknown proteins, one by 18-fold. No genes were clearly down-regulated by cadmium. In particular, genes for bone formation, stress response, growth factors, and signaling molecules other than p38MAPK did not change in expression in bone cells early after cadmium gavage. Results support the hypothesis that Cd increases bone demineralization *via* a p38 MAPK pathway that results in stimulation of osteoclast-mediated bone resorption.

1045 METAL RESPONSE ELEMENT BINDING TRANSCRIPTION FACTOR-1 (MTF1) IS ESSENTIAL FOR ACQUIRED RESISTANCE TO CADMIUM TOXICITY.

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Metallothioneins (MTs) are low-molecular-weight cysteine-rich heavy metal-binding proteins, which function to protect cells against many forms of oxidative stress including cadmium (Cd) toxicity. The four Mt genes are regulated through metal-response-element (MRE) DNA motifs, to which MRE-binding transcription factor-1 (MTF1) binds for up-regulation. The cell culture line dko7 lacks MTF1. Using these cells, MTF1 was re-introduced using a retrovirus to produce mdko7 cells. Surprisingly, mdko7 cells resist Zn, but not Cd, toxicity. When dko7 and mdko7 cells (107 cells) were cultured in 5 mM Cd, resistant mdko7 clones were observed at a frequency of 1 in 105 cells. On the other hand, resistant dko7 clones were not observed. Cd-resistant clones could be cultured in the absence of Cd for >60 population doublings, and they remain Cd-resistant. Slot-blot analysis (to detect Mt gene amplification) showed that resistance was not a result of increased Mt copy number; nevertheless, all resistant mdko7 clones had high constitutive levels of MT mRNA accumulation. These data suggest that MTF1 is essential for acquired resistance to Cd. Further, resistance to Cd may depend on activation of Mt gene expression, and this resistance, which is dependent on MTF1, is likely the result of constitutive MTF1 activation. (Supported, in part, by NIH grants P30 ES06096 and R01 ES10416)

1046 ABERRANT ONCOGENE EXPRESSION ASSOCIATED WITH CADMIUM-INDUCED MALIGNANT TRANSFORMATION IN RAT LIVER CELLS.

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Cadmium (Cd) is a recognized human carcinogen that potentially targets the liver, although its mechanism of action is incompletely defined. This study attempted to induce malignant transformation of normally nontumorigenic rat liver epithelial cell line TRL 1215 by chronic, low level *in vitro* Cd exposure and to define associated aberrant gene expression. Cells were cultured in 1.0 μM CdCl2 for up to 28 weeks and compared to passage-matched control cells. These chronic Cd exposed (CCE) cells were hyperproliferative with a growth rate about 3-fold higher than control cells. CCE cells showed frequent foci of cell mounding, even when subconfluent, which were absent in passage-matched control cells. CCE cells produced aggressive tumors upon inoculation into nude mice confirming malignant transformation. cDNA gene microarray showed an increase in the steady state expression of the oncogenes c-*myc* and c-*jun* in CCE cells, compared to control. To confirm the overexpression of c-*myc* and c-*jun*, northern blot analyses were performed. By this analysis, transcript levels of c-*myc* and c-*jun* were increased by over 10-fold and 2-fold, respectively, in CCE cells compared to control cells. Western blot analysis showed that the steady state level of c-Myc protein in CCE cells was increased over 10-fold compared to the control, demonstrating enhanced expression at the translational level. Further study revealed a significant increase in expression of the transcription factors AP-1 and NF-κB. Taken together, this work suggests that chronic low level Cd leading to malignant transformation triggers oncogene overexpression possibly by altering transcription factor expression. Such changes in cellular gene expression likely culminate in the loss of growth control and neoplastic transformation.

1047 INCREASED EXPRESSION OF THE ECT2 ONCOGENE IN NICKEL COMPOUND INDUCED, TRANSFORMED 10T1/2 MOUSE EMBRYO FIBROBLAST CELL LINES.

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Nickel is an economically important element, particularly for its presence in stainless steel. In the past, workers in specific refining operations, who were exposed to mixtures of soluble and insoluble nickel compounds, had increased incidence of nasal and lung tumors. The exact mechanism of nickel carcinogenesis is still largely unknown. In order to study the molecular mechanism of nickel carcinogenesis, we used C3H/10T1/2 (10T1/2) mouse embryo fibroblast cells and nickel oxide/nickel monosulfide induced, transformed 10T1/2 cells as a model system. To characterize the molecular events associated with nickel-induced carcinogenesis, mRNA from these cell lines was isolated and analyzed by RAP-PCR mRNA differential display. Gene fragments whose expression patterns differed between the non-transformed and transformed cell lines were isolated. One of these isolated fragments, R2-5, was a 293bp fragment that is over-expressed in nickel-induced transformed cell lines. R2-5 was subcloned, sequenced and subjected to nBLAST homology analysis. R2-5 was found to be 100% homologous to a portion of the mouse Ect2 proto-oncogene. Ect2 is known to be a GTP-exchange factor for the Rho family of GTPases. Increased expression of Ect2 keeps Rho constitutively active and results in an increased cytoskeletal remodeling and cytokinesis, which could eventually leading to morphological transformation (Miki, et al.). Southern analysis shows the presence of gene amplification of the Ect2 gene in the transformed cell lines. Multiple copies of the Ect2 gene could be responsible for its over-expression. Western analysis of Ect2 showed higher steady-state levels of Ect2 protein in the transformed cell lines. Higher steady-state levels of Ect2 may contribute to the induction of the nickel compound induced morphological transformation.

1048 ANALYSIS OF CHROMATIN STRUCTURE IN NICKEL SILENCED G12 CHINESE HAMSTER CELLS.

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We have previously reported that the gpt transgene in the G12 Chinese hamster cells could be silenced by water-insoluble nickel compounds nickel sulfide (NiS) or nickel subsulfide (Ni3S2), and showed that the transgene was silenced by de novo DNA methylation and chromatin condensation. To further understand the nature of the silencing induced by nickel, we used Chromatin immunoprecipitation (ChIP) assays to elucidate the chromatin structure in nickel-induced silenced G12 clones. We also analyzed the effects of a DNA methyltransferase inhibitor 5-azacytidine (5-AzaC) and a histone deacetylase inhibitor trichostatin A (TSA) on histone H3 and H4 acetylation and gpt gene expression in N37 cells. We observed that both histone H3 and, H4 were hypoacetylated, and methyl DNA-binding protein MeCP2 was targeted to the gpt gene locus, resulting in a localized inactive chromatin configuration in nickel silenced cell clones. The histone H3K9 was also found methylated in 3 of 4 nickel silenced cell clones, whereas the histone H3K9 was deacetylated in all 4 cell clones, indicating that the H3K9 methylation was involved in nickel induced gene silencing. The acetylation of the gpt gene could be increased by a combination of 5-AzaC and TSA treatment, but not by either 5-AzaC or TSA alone. The gpt transcript was studied by either northern blot or by semiquantitative RT-PCR, however only a slightly increase in gpt mRNA could be detected by RT-PCR. These data show that gene silencing induced by nickel in the gpt transgenic cell line involved a loss of histone acetylation and an activation of histone methylation. Both H4 and H3 histone acetylation was lost in the silenced clones and these clones exhibited an increase in the methylation of lysine 9 in histone H3. This work was supported by grant numbers ES05512, ES00260, ES10344 from the NIH/ NIEHS and CA16087 from the NIH/NCI.

1049 GENOTOXICITIES OF NICKEL SAMPLES IN C3H/10T1/2 MOUSE EMBRYO FIBROBLASTS: PREDICTIONS OF CARCINOGENIC POTENTIALS OF NICKEL CARCINOGENESIS.

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Long-term inhalation exposures of nickel refinery workers to mixtures of specific insoluble and soluble nickel compounds in the past were associated with excess lung and nasal cancer. However, animal carcinogenicity data by relevant routes of exposure are not available for all nickel compounds, particles of elemental nickel, or nickel-containing alloys. *In vitro* studies can directly evaluate genotoxicities of nickel-containing substances relative to those nickel compounds that have been well-characterized in animal studies. Previously, we showed that two preparations of nickel subsulfide, one prepared by INCO and another one used by the National Toxicology Program in the 1990-1996 animal cancer bioassay, both induced a high, dose-dependent yield of morphological transformation in 10T1/2 cells. Nickel carbonate hydroxide ($NiCO_3$), nickelic hydroxide ($Ni(OH)_3$), small particles of nickel subsulfide (Ni_3S_2, particle size 1.35 μm), and spherical particles of 316 stainless steel powder, (SS< 3.0 μm) were subsequently studied. The effectiveness of these samples to be phagocytosed by 10T1/2 cells (based on % uptake of samples at 1.0 μg/ml and linear slope) was: Ni_3S_2 > $Ni(OH)_3$ > $NiCO_3$ > SS (<3.0 μm). The order of cytotoxic potency for this set of samples was: Ni_3S_2 > $Ni(OH)_3$ > $NiCO_3$ >>> SS (<3.0 μm). Based on percent chromosomal aberrations at 1.0 μg/ml and linear slope estimates, the effectiveness of these samples to induce chromosomal aberrations in 10T1/2 cells was: Ni_3S_2 = $NiCO_3$ > $Ni(OH)_3$ ≥ SS (<3.0 μm). Based on foci per 20 dishes at 1.0 μg/ml and linear slope estimates, the effectiveness of these samples to induce morphological transformation in 10T1/2 cells was: Ni_3S_2 > $NiCO_3$ > $Ni(OH)_3$≥ SS (<3.0μm). We predict the carcinogenic potential of these samples is Ni_3S_2 > $NiCO_3$ ≥ $Ni(OH)_3$ >> SS (<3.0μmm), and this would be the order to prioritize them for further *in vivo* testing.

1050 DIFFERENT MECHANISMS IN SUPPRESSING MTH EXPRESSION BY NICKEL CHLORIDE AND COBALT CHLORIDE.

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In previous studies, carcinogenic nickel compounds were found to silence senescence gene and transgenic gene expression by epigenetic mechanisms. It is still unclear whether nickel suppresses other gene expression by the same mechanisms. Here, we studied nickel effect on the expression of MTH gene, one of the nickel-down-regulated genes selected from our GeneChip data in mouse cell. MTH (mutT homologue) encodes 8-oxodGTPase, which metabolizes 8-oxodGTP to 8-oxodGMP and thus prevents the incorporation of 8-oxodGTP into newly synthesized DNA and later subsequent mutation. Mouse cell clones, PW, HIF (+/+) and HIF (-/-), were treated with nickel chloride for 24 h. The expression of MTH gene was quantified by northern blotting method. Our data shown that nickel treatment suppresses MTH expression. Cobalt, which has similar biochemical characters as nickel, also abrogates MTH expression in a dose-dependent manner. Interestingly, the treatment of cobalt in HIF (-/-) cell does not suppress MTH gene expression, while no obvious difference on MTH gene expression was found between nickel-treated HIF (+/+) and HIF (-/-) cell. It suggests that cobalt suppress MTH expression through a HIF-dependent pathway, while nickel decrease MTH expression through other mechanisms. Currently, HIF-upstream signal transduction pathway is investigated in cobalt-treated cells. Chromatin immuno-precipitation assay will be used to test the possible change in acetylation level of histone 3 and 4 after nickel and cobalt treatment.

1051 REACTION OF URANYL ACETATE WITH ASCORBATE PRODUCES DNA STRAND BREAKS *IN VITRO.*

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Exposure to uranium in the mining industry has been linked to lung cancer through uranium's radioactive decay product, radon. Previous studies suggesting increased risks of pancreatic, stomach, colon and prostate cancers and birth defects in people exposed to uranium mine tailings may signal a non-radiochemical mechanism of uranium genotoxicity. We propose that hexavalent uranium (U(VI)) may have a similar chemistry to that of hexavalent chromium (Cr(VI)), which is a known human carcinogen that directly damages DNA. Chromium(VI)-induced DNA damage requires metabolism of the metal by biological reducing agents, for example ascorbate (vitamin C). The goal of our research is to measure the chemical genotoxicity of University(VI) in the presence of ascorbate. Results from experiments with plasmid DNA *in vitro* showed that reactions of University(VI) with ascorbate produced DNA single strand breaks in a time- and concentration-dependant manner. Uranyl acetate was also more cytotoxic in the Chinese hamster ovary EM9 line, which is sensitive to DNA strand breaks, than in the parental CHO AA8 line. However, the presence of catalase did not decrease DNA strand breaks *in vitro*, suggesting that a Fenton-type mechanism of free radical generation was not a significant pathway. An alternative hypothesis may be that a uranyl ascorbate complex catalyzes the hydrolysis of the DNA phosphate backbone by a mechanism similar to that known for the lanthanide metals. 1H NMR spectroscopy supported this interpretation and showed the formation of a stable uranium-ascorbate complex. These are the first experiments to demonstrate direct DNA damage induced by uranium and ascorbate. Uranium mining has a strong presence on the Navajo reservation in the Four Corners region of the United States. Understanding the metabolism of uranium and its reactivity with DNA may help to elucidate mechanisms of lung and other cancers in Native American populations exposed to uranium. Supported by NIH grant # CA96302.

1052 LEAD CHROMATE GENOTOXICITY IS MEDIATED BY PARTICLE DISSOLUTION AND NOT PARTICLE INTERNALIZATION.

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Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen that is commonly encountered in occupational settings and in the environment. Water solubility has proven to be a key factor in the carcinogenicity of Cr(VI), with the water-insoluble or 'particulate' compounds the more potent carcinogens. Previous studies in rodent models indicate that extracellular particle dissolution is responsible for the genotoxicity of particulate Cr(VI) compounds in rodent cells, however, it is uncertain what roles occurs in human lung cells. We investigated the roles of particle uptake and Cr ion uptake in WTHBF-6 cells, a human lung cell line, after exposure to particulate lead chromate (PC). We found that PC was clastogenic in a concentration-dependent manner with 0.1, 0.5, and 1 ug/cm^2 PC damaging 15, 34, 42 % of metaphases respectively and a 5 ug/cm^2 concentration causing complete cell cycle arrest. We further found using ICPMS that these doses produced concentration-dependent intracellular Cr levels of 0.7, 1.6, 3.7, 7.8 and 12 uM. We investigated particle internalization using electron microscopy and found that in as little as 1 hr, PC particles were internalized in tight vacuoles after cells were exposed to the four highest concentrations. In addition, there was an apparent relative increase with concentration, however, there was no particle uptake at the lowest dose (0.1 ug/cm^2) even after 24 h. Further, we found no lysosomal association with the vacuoles containing particles suggesting that intracellular dissolution may not have occurred. These data indicate that PC clastogenesis at low doses is mediated by the dissolution of the particles and not their internalization. Further efforts are aimed at determining if dissolution is intracellular, extracellular or some combination of the two. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.).

1053 CHROMIUM IS THE PROXIMATE GENOTOXIC SPECIES IN LEAD CHROMATE-INDUCED GENOTOXICITY IN HUMAN BRONCHIAL CELLS.

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Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen with potentially widespread exposure. Solubility is a key factor in the carcinogenicity of Cr(VI), with the water-insoluble or 'particulate' compounds the more potent carcinogens. Currently data from our laboratory indicates that particle dissolution and not particle uptake is responsible for the genotoxicity of lead chromate (PC) particles in human lung cells, however, it is uncertain whether chromium (Cr), lead (Pb) or some combination of the two is the proximate clastogen. Accordingly, we compared the cytotoxicity and clastogenicity of PC (a particulate Cr(VI) compound) with sodium chromate (a soluble Cr(VI) compound) and Pb-glutamate (a soluble lead (Pb) complex) in WTHBF-6 cells, a human lung cell line. We found that 2 uM Pb was the maximal intracellular level of Pb after exposure to clastogenic concentrations of PC. However, clastogenesis was not observed after exposure to Pb-glutamate even when intracellular Pb concentrations reached 40 uM, indicating that intracellular Pb levels did not reach clastogenic levels in WTHBF-6 cells after PC treatment. By contrast, sodium chromate was clastogenic damaging 26 and 44 percent of metaphase cells at intracellular Cr doses of 0.6 and 1.9 uM respectively, which was comparable to the clastogenesis observed after PC treatment. PC damaged 10, 27 and 37 percent of metaphases at intracellular Cr doses of 1.3, 1.7 and 4.5 uM respectively. These data indicate that with respect to PC-induced genotoxicity, Cr and not Pb is the proximate genotoxic species in human lung cells. Further experiments are aimed at investigating the differences between particulate and soluble Cr(VI). This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.).

1054 THE PHASE OF LEAD CHROMATE INDUCED CELL CYCLE ARREST IS CONCENTRATION DEPENDENT.

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Hexavalent chromium (Cr(VI)) is widely accepted as a human lung carcinogen that is common in the environment as a consequence of industrial pollution. A key factor in the carcinogenicity of Cr(VI) is water solubility. Water-insoluble of 'particulate' Cr(VI) compounds are more potent carcinogens than soluble compounds. However, the mechanisms that explain this difference in potency are poorly understood. One possible explanation is that the cation in the particulate compounds such as lead (Pb) may behave as a cell growth stimulant. Indeed we have found that Pb is a potent mitogen for WTHBF-6 cells (a human bronchial cell line). We found that an intracellular dose of 4 uM Pb can induce a 40% stimulation of growth. We then investigated the cell cycle effects of lead chromate (PC) itself in WTHBF-6 cells. We found a progressive change in the cell cycle arrest induced by lead chromate. At a relatively low concentration (0.5 ug/cm^2) PC induced G1 arrest increasing the number of cells in G1 by 15%. The arrest changed to a G2/M arrest when concentrations of 1.0 and 5.0 ug/cm^2 increased the number of cells in G2/M by 24 and 124% respectively. At the highest dose (10 ug/cm^2), the arrest became an S-phase arrest with an 86% increase in the cells in S-phase. We also found a concentration dependent increase in the total amount of chromosome damage at these doses suggesting that the arrest may be mediated by the amount of genetic damage that occurred. Future investigations are aimed at investigating the cell cycle effects at lower concentrations and understanding the time-course of damage formation and repair.

1055 DNA DAMAGE INDUCED BY CHROMIUM PICOLINATE.

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The dietary supplement chromium picolinate (CrPic) has previously been shown to cause chromosomal aberrations and mutations at the hprt locus, and to induce apoptosis in Chinese hamster ovary (CHO) cells. The molecular mechanism underlying this toxicity is unknown. The focus of the current work is to characterize the types of DNA lesions found in cells after exposure to CrPic. Chromium-DNA adducts were measured by ICP mass spectrometry and UV/visible spectroscopy. CrPic produced less Cr-DNA adducts in CHO cells than did chromic chloride and potassium dichromate. Results from single cell gel electrophoresis (the comet assay) suggested that DNA crosslinks were present. Comparison of the effects of MMS and proteinase K on percent DNA in tail and DNA tail length for cells exposed to CrPic, cis-platin and formaldehyde suggests that DNA-DNA crosslinks were more prevalent than DNA-protein crosslinks in CrPic treated cells. The observation of crosslinks in the comet assay was consistent with extents of cell survival in a series of CHO cells deficient in DNA repair. CrPic was most toxic in the CHO UV135 line and least toxic in the parental AA8 line with the following order observed: UV135 < UV41 < UV5 = EM9 < UV20 < AA8. The free ligand, picolinic acid, produced the opposite trend, with cell survival being greater in the repair deficient lines: AA8 < EM9 = UV20 < UV135 < UV5 < UV41. Equivalent doses of chromic chloride were non-cytotoxic in all lines tested. Understanding the mechanism behind CrPic-induced DNA damage will be necessary before genotoxicity results can be extrapolated to humans ingesting dietary supplements. Supported by NIH grant # CA75298.

1056 COMPARATIVE GENOTOXICITY OF TWO PARTICULATE HEXAVALENT CHROMIUM COMPOUNDS IN HUMAN BRONCHIAL CELLS.

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Particulate hexavalent chromium (Cr(VI)) compounds are well-established human lung carcinogens. However, there carcinogenic mechanisms are poorly understood as most investigators have used related soluble Cr(VI) compounds. Currently, work in our laboratory has shown that barium chromate (BC) is also cytotoxic and clastogenic. To better understand how it related to existing data on other particulate Cr(VI) compounds, we compared its cytotoxicity and clastogenicity with lead chromate (PC), which has been used as a prototypical particulate Cr(VI) compound in WTHBF-6 cells, a near-normal human lung cell line. We found that BC is more potently cytotoxic inducing 67, 12, 3, 0, and 0 % relative survival at concentrations of 0.1, 0.5, 1, 5 and 10 ug/cm^2 respectively, while PC induced only 90, 71, 43 and 15 % survival at these same concentrations respectively. We found that BC was also more potently clastogenic damaging 22 percent of metaphases cells at 0.1 ug/cm^2 and causing complete cell cycle arrest at 0.5, 1 and 5 ug/cm^2. By contrast, 0.1, 0.5, and 1.0 ug/cm^2 PC damaged 10, 27 and 37 percent of metaphase cells respectively and complete cell cycle arrest was not observed until a concentration of 5 ug/cm^2. We investigated both particle internalization and Cr ion uptake and found that BC and PC were similar with respect to particle uptake, but more Cr ions were observed in the cells treated with BC than PC. Exposure to 0.1, 0.5 and 1.0 ug/cm^2 BC produced intracellular Cr ion levels of 2.89, 5.87 and 6.44 uM respectively compared to 0.72, 1.55, and 3.69 uM respectively after PC exposure. This suggests that the difference in genotoxicity may be mediated by the difference in intracellular Cr levels. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.).

1057 BARIUM CHROMATE IS CYTOTOXIC AND CLASTOGENIC TO HUMAN BRONCHIAL CELLS.

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Particulate hexavalent chromium compounds are well-established human lung carcinogens. However, there carcinogenic mechanisms are poorly understood as most investigators have used related soluble Cr(VI) compounds. Moreover, those researchers investigating particulate chromium compounds have focused almost exclusively on lead chromate as a prototypical compound as it is the most insoluble and has been directly shown to be carcinogenic in animal models and cellular transformation assays. Almost no data exist on the carcinogenicity of other particulate Cr(VI) compounds. To begin to address this data gap, we determined the cytotoxicity and clastogenicity of barium chromate in WTHBF-6 cells, a near-normal human lung cell line. We found that the barium chromate induced concentration dependent cytotoxicity with concentrations of 0.01, 0.05, 0.1, 0.5, 1 and 5 ug/cm^2 inducing 88, 74, 67, 12, 3 and 0 percent survival respectively. Barium chromate was also clastogenic in a concentration dependent manner damaging 5, 9 and 22 percent of metaphases and inducing 5, 10 and 28 total aberrations per 100 metaphases at concentrations of 0.01, 0.05, and 0.1 ug/cm^2 respectively. The spectrum of chromosome damage induced included chromatid and isochromatid lesions and chromatid exchanges, which is consistent with mechanisms of neoplastic transformation such as deletion of tumor suppressor genes and activation of oncogenes. Overall the data clearly indicates that barium chromate is cytotoxic and genotoxic to human lung cells. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.).

1058 INHIBITION OF PHASE I AND PHASE II METABOLIC ENZYMES *IN VITRO* BY NICKEL CHLORIDE.

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Nickel compounds have been identified as human carcinogens/cocarcinogens using both *in vivo* studies and epidemiological evaluations. The mechanisms involved in the toxicity of nickel have not been fully elucidated. *In vitro* studies were designed to identify potential mechanisms of toxicity by analyzing transcriptional changes caused by soluble nickel. It was observed that nickel chloride can alter the transcription of several phase I and phase II metabolic enzymes, which can play a fundamental role in the detoxification and elimination of xenobiotics. For example nickel downregulated the basal and benzo(a)pyrene inducible expression of both cytochrome P450 1B1 (Cyp 1B1) and quinone reductase (NQO1). Both of these genes were involved in the biotransformation of xenobiotics. Quinone reductase was also involved in the metabolism of endogenous compounds and possibly Cyp 1b1, as well. By suppressing basal level expression of Phase I and Phase II genes, nickel can cause alterations in the metabolism of endogenous compounds. Chemicals that can bind to the aryl hydrocarbon receptor or produce oxidative stress can induce metabolic enzymes. It has been suggested that this upregulation is important to aid in the elimination of harmful compounds from the body. Nickel could block the upregulation of some detoxification enzymes and lead to a decrease in the elimination of toxic compounds. Metabolic enzymes are also important in the biotransformation of chemotherapeutic agents and insights into their regulation could prove useful in cancer treatment. In conclusion, concentrations of nickel chloride that caused low toxicity induced changes in Phase I and Phase II enzymes. This could have toxic effects by affecting the metabolism of harmful compounds in the body. These effects are relatively specific for nickel since other metals like cadmium and chromium failed to produce similar changes.

1059 CYTOTOXICITY AND CLASTOGENICITY OF ARSENIC, CADMIUM AND CHROMIUM IN HUMAN BRONCHIAL CELLS.

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Metals are a significant public health concern. Several are established human lung carcinogens that pose a significant risk for both occupational and environmental exposure. Five metals are listed in the "Top 20 Hazardous Substances" by the Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR), and five metals are listed as "carcinogenic to humans" by the International Agency of Cancer Research (IACR). However, there is limited data concerning the effects of metals in human lung cell models. This is largely because many available human lung cell culture models are already fully transformed. Recently, we developed WTHBF-6 cells, which are a new model system of human bronchial cells that have an extended cellular life-span, but grow in culture and respond to metals similarly to primary human bronchial cells. In this model, we investigated the cytotoxicity and clastogenicity of three metals that are human lung carcinogens chromium, cadmium and arsenic. We found that chromium was the most cytotoxic of the three inducing 63, 8 and 0 percent relative survival at 1, 5 and 10 uM respectively. Arsenic was intermediate inducing 75, 33 and 8 percent survival at these concentrations, while cadmium was the least toxic with greater than 89 percent survival at each concentration. Similarly, chromium was the most clastogenic damaging 26 percent of metaphases at 1 uM and complete cell cycle arrest at 5 and 10 uM. Arsenic was again intermediate damaging 12, 28 and 36 percent of metaphases respectively at these doses, while cadmium was the least clastogenic damaging 3, 2 and 14 percent of metaphases respectively. Thus these data indicate that each of the three metals is cytotoxic and clastogenic to human lung cells with chromium more potent than arsenic; and arsenic more potent than cadmium on the basis of administered concentration. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.).

1060 THE MECHANISM OF RETINOL INDUCED IRRITATION AND ITS APPLICATION TO DEVELOPMENT OF ANTI-IRRITANTS.

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Retinoids, such as retinol and retinoic acid, have been increasingly used for topical and systemic treatment, but they induce mild erythema on the skin. It is not clear if the redness induced by retinoid is caused by the sensitization or irritation. The mechanism of dermatitis induced by retinoid is also unclear. We first clarified that retinol has no sensitization potential using IMDS, an alternative to sensitization test. we analyzed the change of mRNA expression of inflammation related cytokines, such as KC (mouse analogue of human IL-8), JE (mouse analogue of human MCP-1), TNF-a, IFN-r, IL-12p40, IL-6, IL-10, Eotaxin in mouse epidermal cell, and also examined the inflammation related cytokines in the human foreskin cell lines to understand the inflammation mechanism induced by the retinol or retinoic acid. Retinol upregulates the mRNAs of IL-8, MCP-1, TNF-a, IFN-r and IL-12p40 in mouse ear skin when measured using the RT-PCR method. Significant increases in level of IL-8 and MCP-1 protein concentration induced by retinol and retinoic acid are observed in culture supernatants of human epidermal keratinocytes, melanocytes and dermal fibroblasts. We selected several substances that have inhibitory effects against the retinol induced cytokine increase (IL-8 and MCP-1) based on the *in vitro* test using the human foreskin cell lines. These substances were used for *in vivo* test, the skin irritation test in rabbits and the human patch test, to see if those have the inhibitory effects against the retinol induced redness. Afterward, we analyzed the correlation between *in vitro* and *in vivo* data. Most of the substances which lowed the level of cytokine(MCP-1, IL-8) produced by retinol *in vitro*, showed good inhibitory effects against the retinol-induced irritation in the rabbit and human patch test. The present study showed that retinoids induced the skin irritation by the inflammation related cytokines, such as IL-8 and MCP-1. We concluded these cytokines can be applied for the good markers to screen the anti-irritants against the retinol induced irritation.

1061 PREDICTION OF THE LOCAL IRRITATION POTENTIAL OF VARIOUS PHARMACEUTICAL VEHICLES AND BUFFERS USING A NEONATAL RAT MYOCYTE ASSAY *IN VITRO*: COMPARISON WITH RESULTS OBTAINED IN PIGS *IN VIVO*.

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During development of parenteral pharmaceuticals a wide range of vehicles containing the drug may need to be assessed for local irritation, and it is therefore preferable to find an alternative method of screening than use of live animals. In order to select a suitable vehicle for subcutaneous administration of a pharmaceutical, a number of routinely used vehicles and buffers have been tested in a neonatal rat myocyte assay *in vitro*, and the results compared with some of the vehicles and buffers administered subcutaneously to pigs. Pigs were chosen as the animal model because of similarities of their skin with that of humans, particularly the thickness of the subcutaneous tissues. A single cell suspension of myocytes was prepared from the skeletal muscle of 1-day old rat pups, and the cells were allowed to form a confluent layer. They were then incubated for 10 min with the following vehicles and buffers: physiological saline at pH 4.5 & 7.0, isotonic mannitol, lactose and sucrose at pH 4.5 & 7.0, and 10, 25 & 40mM acetate, citrate and succinate buffers at pH 4.3 or 4.5. 0.5% glacial acetic acid was used as a positive control. Cytotoxicity was assessed by the loss of creatine kinase activity (CK) from the cells. Saline, mannitol, lactose and sucrose showed no significant cytotoxicity, irrespective of pH. Acetate and succinate buffers caused 2 to 4 times more loss of CK activity than saline, regardless of molarity. 10 & 25mM citrate buffers caused only a slight loss of CK activity, but 40mM citrate caused a 10-fold loss of CK activity. Acetate and citrate buffers (10, 25 & 40mM) and saline have been injected subcutaneously (1 ml/day) into pigs for 28 days. There were no external signs of local irritation. The severity of pathological changes showed a similar relationship to that in the assay *in vitro*, suggesting that the myocyte model has good predictive value for local irritation.

1062 AN *IN VITRO* TECHNOLOGY FOR FAST IDENTIFICATION OF LESS ALLERGENIC PROTEIN VARIANTS.

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Rationale. The use of proteins in new industrial and pharmaceutical applications is hampered by their allergenicity. Less allergenic protein variants can be obtained by protein engineering (PE) but no methods are available for fast identification of these variants in a protein library. Methods. 1) Epitope Mapping Tool(EMaT): A programme assessing experimentally defined B-cell epitope patterns on the 3D-structure of a target protein. 2) Epitope Mutation Tool(EMuT): An algorithm for identifying mutations with maximal impact on the EMaT-derived epitope profile of the target. 3) Automated screening (AS): The antibody binding capacity of the micropurified variants is assessed in an ELISA-based assay. 4) ImmunoFit: The differences in antibody binding between variants and target are quantified, and analysed statistically (Significance = >2x the standard deviation). 5) MINT study: Mice were immunised intranasally with 0.1, 1 and 10 microgram of protein, and the IgG1 and IgE levels were determined by ELISA after days 15 and 45. Results. 1: EMaT was evaluated on published B-cell epitopes. Overall, 90% of these epitopes were scored for > 50%. Linear as well as conformational epitopes for IgE and IgG antibody from human, mice, rat and rabbit were identified by this in silico method. 2: An example: EMaT revaled on Bacillus subtilisin Protease two important epitope areas. These areas were subjected to restricted site-directed mutagenesis, as predicted by EMuT. More than 90% of the epitope variants revealed a > 2x change ($p < 0.05$) in antibody-binding of rabbit anti-target IgG antibody. The 3 top-scoring variants revealed a 100x lower binding capacity. BiaCore confirmed these variants as having a > 1000x reduction in affinity for the antibody. One variant did not bind the antibody at all. Evaluation of these variants in mice revealed a > 10x reduction in IgE levels. Conclusion. This technology platform allows fast identification of less allergenic protein variants in a protein epitope library. Overall, animal experimentation is reduced with 90%.

1063 ALTBIB: ALTERNATIVES TO ANIMAL TESTING DATABASE.

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The Alternatives to Animal Testing Database is a bibliographic database whose purpose is to assit toxicologists and scientific researchers in accessing information on methods and procedures that support the development, testing, application, and validation of alternatives to the use of live vertebrates in biomedical research and toxicology testing. The database includes citations from published articles, books, book chapters, and technical reports. Content of the database are organized under subject categories, and features citations which deal with methods, tests, assays, or procedures which may prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled by searching TOXLINE and MEDLINE at the National Library of Medicine (NLM). The database is updated periodically, and is available at the Specialized Information Services web site. It is searchable by using the TOXNET search engine which features a relevancy-ranked full text search capability. The content of this database is also available in a downloadable format in 23 separate issues.

1064 EXPLORATORY *IN VITRO* EYE IRRITATION STUDY OF MARKETED ALKALINE DRY LAUNDRY DETERGENTS BY BCOP ASSAY AND PH/RESERVE ALKALINITY (RA) PARAMETERS.

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An exploratory *in vitro* eye irritation study of 11 currently marketed alkaline dry laundry (ADL) detergents was conducted to investigate the correlation between an *in vitro* biological endpoint and pH/reserve alkalinity (RA) ranges of ADL detergents. Marketed products can be considered "safe benchmarks", since they are produced by industry leaders and are assumed to have acceptable pH/RA characteristics. Based on performance in previous eye irritation studies with surfactants and the potential to measure depth of injury, the bovine corneal opacity and permeability (BCOP) assay was selected as an *in vitro* endpoint to evaluate biological effects. Based on preliminary studies, a 10% (w/v) aqueous suspension of each detergent was applied to the corneas for a 30-minute exposure. The degree of epithelial damage is reflected in the increase in fluorescein permeability value. Permeability values (OD_{490}) for the 11 ADL detergents ranged from 0.267 to 0.856 reflecting a moderate range of epithelial damage. The range of opacity scores was more variable (0.3 to 21.5) and showed little consistent change with the permeability values. Anionic/nonionic surfactant formulations often produce little *in vitro* opacity. The pH of each dosing suspension (10% w/v) was measured and ranged from 11.0 to 12.0. The RA was determined for a 0.2% (v/v) aqueous suspension of the supernatant from each dosing suspension (i.e., 20% dilution of supernatant from 10% dosing suspension) titrated to a target pH of 9.5. Titration values ranged from 1.7 to 5.2 ml of 1N HCl. Neither pH nor RA values correlated with the epithelial damage as measured by permeability changes. These data suggest characteristics of the formulation other than pH or RA are responsible for the epithelial damage produced in the BCOP. This protocol, using a 10% (w/v) aqueous suspension with a 30-minute exposure, the permeability endpoint (with histological confirmation) and benchmark formulations, shows promise for evaluating ADL detergents.

1065 APPROACHES TO MINIMISING DOG USE IN PHARMACEUTICAL SAFETY ASSESSMENT: AN INDUSTRY/ ANIMAL WELFARE INITIATIVE.

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The primary non-rodent species used in toxicology is the dog. It is widely agreed that, for ethical and economic reasons, dog use should be reduced to the minimum consistent with maintaining the scientific quality of toxicology and ensuring human safety. Dog use in toxicology has been discussed widely, both from a scientific and ethical viewpoint, and there seems to be real potential for achieving significant reductions in the number of dogs used in safety testing. An Industry/Animal Welfare Initiative was started in 2000 to evaluate and, where possible, put into practice, scientifically valid approaches to minimise dog use in regulatory toxicology. The Steering Group categorised potential approaches into three areas: industrial co-operation/data sharing, best practice in study design, and assessing the need for particular studies. Progress has been made in evaluating and/or implementing approaches in the first two areas. One way to reduce dog use would be to establish a database of effects of vehicles and other non-active ingredients used in drug formulations. Access to such a database would minimise the need for dog studies when it is intended to use a previously tested material or use it under different conditions. The possibility of establishing such a database is currently being explored. A best practice guide in aspects of study design, including appropriate group sizes, use of control animals, single sex studies and design of MTD studies, is currently being prepared and its status will be presented.

1066 EVALUATING THE IRRITANCY POTENTIAL OF SODIUM PERCARBONATE: A CASE STUDY USING THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

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Sodium Percarbonate ($2Na_2CO_3{\cdot}3H_2O_2$) is a component in cleaning products but the neat powder has the potential to be highly irritating to the ocular tissue of rabbits (EPA Category I). This injury results from the chemical's reactivity and dosing method which may trap the powder against the eye. In the BCOP assay, experience has now shown that oxidizing/reactive materials often require a longer post-exposure time to fully manifest cytopathic changes. When testing reactive chemistries, the post-exposure incubation times are increased from 2 hours to 4 and 24 hours. Exposure times of 10, 20, 30 and 60 minutes were used in this study. Sodium percarbonate and percarbonate-based formulations were evaluated as 50% suspensions in water. Abattoir-derived corneas were received, mounted, exposed to test materials, and opacity, permeability and histological endpoints measured as previously reported (Harbell and Curren (1998) *In Vitro* and Mol. Biol. 11:337-341). Opacity and permeability scores increased with increasing exposure times and concentration of percarbonate. After the 10-minute exposure to percarbonate alone, the 4-hour post-exposure corneas showed focal epithelial layer changes which progressed to a loss of epithelium after 24 hours. Stromal damage included collagen matrix vacuolization and loss of basophilic components in the keratocyte cytoplasm. Exposures of 20 minutes or greater led to rapid destruction of both the epithelial and stromal cells and marked collagen matrix swelling. Tissue lesions declined rapidly with decreasing percarbonate concentration. Thus, the marked ocular damage induced by neat percarbonate in the rabbit could also be reproduced in the modified BCOP. These data suggest that the modified BCOP assay can be effectively used to evaluate the safety of percarbonate-based formulations and lead to appropriate labeling decisions.

1067 COMPARATIVE ASSESSMENT OF TWO EYE AREA COSMETIC FORMULATIONS THROUGH EVALUATION OF ALTERNATIVE EYE IRRITATION METHODS RELATIVE TO ENDPOINTS MEASURED IN A HUMAN CLINICAL SUB-ACUTE STUDY DESIGN.

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Evaluating ocular irritation of eye-area cosmetics is essential to their safety assessment. Although eye-area formulations are designed to be mild, subjective/objective ocular responses can limit their acceptability. This study evaluated two similar formulations by comparing alternative assays, selected for their ability to distinguish milder effects, to human objective and subjective endpoints assessed by a sub-acute human eye irritation design. Ocular irritation alternative assays included Hen's Egg Test-Choriollantoic Membrane (HETCAM) assay, Cytosensor Microphysiometer Bioassay (CMB), and EpiOcular® (EPO) tissue construct assay. HETCAM evaluates inflammatory responses in a complete tissue model. CMB uses L929 cells to evaluate cytotoxicity through metabolic rate measurements. EPO uses differentiated human epidermal keratinocytes, having stratified into a squamous epithelium similar to corneal tissue. Human ocular irritation was assessed through subjective and objective measures at post-application intervals. Ocular exams included evaluation of the area/density of fluorescein staining of all tissues utilizing Kanengiser's 13-point scale. Each formulation was assessed simultaneously by randomized, periorbital application to paired contralateral eye areas in an exaggerated use design. Clinical results indicated formulation A elicited a slightly greater magnitude and frequency of subjective and objective findings compared to formulation B. Results of alternative assays predicted both formulations were mild but were not entirely consistent in terms of rank ordering relative to the human response. This study demonstrates the need to consider a battery of alternative assays when screening formulations for distinguishing mild ocular effects and the potential of this clinical protocol in predicting differences in eye irritation potential of mild formulations.

1068 EVALUATING OXIDIZING/REACTIVE CLEANING PRODUCTS IN THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

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Recent trends in household cleaners highlight enhanced cleaning with "natural" materials such as "active oxygen" and make claims such as "harnessing the natural cleaning power of..". The formulations often utilize well-known oxidizing or reactive chemistries in new, innovative ways. However, the formulations have the potential to influence the interaction of the reactive components with tissue. Thus, the irritancy potential of each formulation must be evaluated. To evaluate these formulations, the BCOP assay was selected for this study because of its range of response and capacity to exhibit depth of injury. Abattoir-derived corneas were received, mounted, exposed to test materials, and opacity and permeability endpoints measured as previously reported (Harbell and Curren (1998) *In Vitro* and Mol. Biol. 11:337-341). The BCOP assay was modified to include exposure periods of 3 to 10 minutes and extended post-exposure incubations periods of 4 and 20 hours (with controls). The assay endpoints were opacity, permeability to fluorescein and

histology. The data showed that the opacity and permeability endpoints often underestimated the degree of damage relative to the lesions observed in the corneas. Furthermore, cytopathic changes initially detected after 4 hours were more clearly manifested after 20 hours. After 4 hours, the wing and basal cells in the epithelium often showed increased cytoplasmic vacuolization and occasional nuclear pyknosis while the keratocytes of the stroma showed nuclear pyknosis and some loss of basophilic components in the cytoplasm (eosinophilia). At 20 hours, the cells in the damaged epithelial layers were lost from the corneas. In the stroma, pyknotic keratocytes were lost entirely and the full depth of injury was determined by keratocyte eosinophilia. These data suggest the importance of an extended post-exposure incubation and histological evaluation of the corneas. Understanding how these materials act in the assay enables responsible product labeling.

1069 AN IMPROVED PRIMARY SERTOLI CELL-GONOCYTE CO-CULTURE SYSTEM FROM NEONATE RAT: *IN VITRO* MODEL FOR THE ASSESSMENT OF MALE REPRODUCTIVE TOXICITY.

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Testicular cell growth and differentiation is essential for embryonic, prepubertal, pubertal, and adult testis function. Impairment of proliferation and differentiation of Sertoli cell and/or germ cells during the fetal or/and neonatal period can produce profound negative effects on the reproductive capacity of the adult. In this study, we established an improved *in vitro* culture system that recreates critical *in vivo* characteristics of seminiferous tubules and which will allow for improved evaluation of testicular developmental toxicants. We compared the effect of different ECM concentrations, added as an overlay, on the morphology and the expression of c-kit protein. A sequential enzymatic digestion of testicular tubules from 5-day-old rats with collagenase, DNAse I and hyaluronidase was used to obtain a cell suspension containing primarily Sertoli cells and spermatogonia. After plating, different concentrations of ECM were added directly to the culture medium. Morphological changes were monitored and photographed. For western blot analysis, cells were harvested at 72h after addition of ECM. We found that an overlay of ECM dramatically enhanced the attachment of Sertoli cells, aided in the establishment of the Sertoli cell-gonocyte communication and furthermore, increased the survival of the spermatogonia as demonstrated by c-kit protein expression levels. We also compared the MAPK, SAKP/JNK and AKT signaling and ubiquitinatin pathways in response to different concentrations of ECM. An overlay of ECM at an optimal concentration of 150 μg/ml facilitated the formation of a 3-dimensional architectural structure, and mimicked the *in vivo* testicular-like structure of the neonate testis. The expression of c-kit protein further confirmed the functional integrity of this co-culture system. This refined system will prove useful for studying the molecular mechanisms associated with developmental and reproductive environmental toxicants on Sertoli cells and/or spermatogonia. Supported by NIH ES07033, ES09601-02, USEPA R826886-01

1070 EVALUATING THE OCULAR IRRITATION POTENTIAL OF 54 TEST ARTICLES USING THE EPIOCULAR HUMAN TISSUE CONSTRUCT MODEL (OCL-200).

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Colgate-Palmolive is sponsoring a research program to validate the use of the EpiOcular model in evaluating the eye irritation potential of surfactants. Previously, in a study that demonstrated the reliability of the EpiOcular model, four laboratories using a formal and detailed study protocol tested 19 test materials. In the current study, two laboratories (Institute for *In Vitro* Sciences and MatTek Corp.) have tested 54 test articles using the same study protocol. EpiOcular is a commercially available three-dimensional *in vitro* model of the human corneal epithelium composed of normal human-derived epidermal keratinocytes. Test articles included a shampoo formulation and 30 different surfactants (10-cationic; 11-anionic; 7-nonionic; 1-amphoteric; 1-zwitterionic) which were liquids, powders or creams. Multiple concentrations of 11 of the surfactants were tested to evaluate the ability of the model to predict dose-related differences in the ocular irritation potential of test articles. Testing was conducted in compliance with FDA GLPs. The laboratories were blinded to the identities of the test articles. Test results were compared to previously published animal eye irritation studies. 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. The model also correctly predicted increasing irritation potential of surfactants with increased concentrations. These data provide additional evidence that the EpiOcular model meets the validation criteria, as defined by the Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No. 97-3981), for assessing the ocular irritation potential of certain classes of surfactant and surfactant-based formulations.

1071 ICCVAM/NICEATM EXPERT PANEL RECOMMENDATIONS FOR THE STANDARDIZATION AND VALIDATION OF *IN VITRO* ESTROGEN RECEPTOR (ER) AND ANDROGEN RECEPTOR (AR) TRANSCRIPTIONAL ACTIVATION (TA) ASSAYS.

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A variety of natural and anthropogenic substances appear to interact with the endocrine system. As a result, the USEPA was required by legislation to develop a screening and testing program to identify substances with endocrine disruptor (ED) activity. Within the Tier 1 battery of screening test methods, *in vitro* ER and AR TA assays are proposed to identify estrogenic or androgenic agonists or antagonists. *In vitro* results would be considered together with other Tier 1 assay results in a weight-of-evidence evaluation of the need for testing in the more definitive Tier 2 *in vivo* assays. A comprehensive literature review indicated no adequately validated *in vitro* ER or AR TA assays. After considering the available data, an ICCVAM/NICEATM-sponsored Expert Panel developed recommendations for future development, standardization, and validation efforts. For ER TA assays, a mammalian cell line transfected with a human ER expression vector and a luciferase reporter containing multiple vitellogenin estrogen response elements is recommended. Pre-validation studies should be performed to determine whether transiently or stably transfected cell lines are most useful. For AR TA assays, validation of a mammalian cell line containing an endogenous human AR and transduced with an adenovirus containing a luciferase reporter plasmid is recommended. Recommendations were also developed for minimum procedural standards and substances for validation studies, which together should facilitate standardization and validation of protocols for ER and AR TA assays. Supported by NIEHS Contract N01-ES-85424.

1072 ICCVAM PROPOSED SUBSTANCES FOR THE VALIDATION OF *IN VITRO* ESTROGEN RECEPTOR (ER) AND ANDROGEN RECEPTOR (AR) BINDING AND TRANSCRIPTIONAL ACTIVATION (TA) ASSAYS.

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The US EPAs proposed Endocrine Disruptor Screening Program (EDSP) includes a Tier 1 screening battery composed of *in vitro* and *in vivo* test methods designed to identify substances capable of interacting with the endocrine system. Prior to implementation of the EDSP, the component test methods must be adequately validated. An Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM expert panel evaluated the validation status of *in vitro* ER and AR binding and TA (agonist/antagonist) assays that might be included in the Tier 1 battery. The panel determined that none of the *in vitro* assays had been adequately validated. To facilitate the necessary validation studies, a common list of 78 proposed substances was compiled that addressed the panels recommendations. Substances were selected to ensure that reliability and accuracy of the *in vitro* assays would be adequately characterized across a broad range of chemical classes and responses. Selection criteria included quantity and quality of available data, potency, chemical class, selection for *in vivo* validation studies, and commercial availability. A minimum of 25% of the substances are known or expected to be negative in each of the different assay types. The use of a common substance list for validation will facilitate assessment of comparative assay performance, establishment of minimum performance criteria, and selection of acceptable *in vitro* test methods. Generation of both *in vivo* and *in vitro* data on many of these chemicals during future validation studies will also aid the future development of more predictive *in vitro* endocrine disruptor assays. Supported by NIEHS Contract N01-ES-85424. The views expressed above do not necessarily represent the official positions of any federal agency.

1073 A NON-ANIMAL ALTERNATIVE CARCINOGENICITY ASSAY USING FERTILIZED AVIAN EGGS: THE *IN OVO* CARCINOGENICITY ASSAY (IOCA).

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Carcinogenicity testing of substances is currently assessed *via* costly bioassays utilizing rodents in two year studies. We have developed an alternative assay which is performed on avian embryos as a screening test for carcinogenicity. The *in ovo* carcinogenicity assay (IOCA), is conducted in fertilized turkey, chicken, or quail eggs,

and addresses the three R's (reduction, refinement and replacement) of alternatives to animal testing. *In ovo* administration of known carcinogens on day 0 to 4 of embryonic development for up to 26 days resulted in the induction of pre-neoplastic lesions in the avian liver. Liver effects included changes in liver weight, enlarged nuclei, foci of altered hepatocytes (FAH), hepatocellular reassignment from a trabecular to tubular pattern, as well as damage and breakage of mitochondrial DNA. Diethylnitrosamine, N-nitrosomorpholine, dimethylnitrosamine, and aflatoxin B_1 were all positive carcinogens in this assay. The sensitivity of this assay was similar to rodent tests; A single dose of 1.0 – 5.0 mg of DEN per 100g egg weight induced dose-dependent liver and mtDNA changes whereas 0.5 mg and below did not. Thioacetamide and all dosing vehicles were negative. The IOCA has numerous advantages over the rodent carcinogenicity bioassay, including: 1) it is a rapid test, requiring a maximum of 24 days to perform; 2) testing does not require use of any mammals; 3) testing is performed on eggs, which are kept in incubators, requiring less space and equipment than animal facilities; 4) dose amounts are small, which reduces exposure of humans to potential carcinogens; and 5) cost of analysis is greatly reduced when compared to traditional carcinogenicity testing.

1074 REGULATION OF ANGIOGENESIS FACTORS BY ULTRAVIOLET RADIATION (UVR) OR H2O2 IN THE EPIDERM *IN VITRO* HUMAN SKIN EQUIVALENT.

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Vascular endothelial growth factors (VEGFs) released by epidermis modulate blood vessel growth (angiogenesis), a critical step in skin tumor progression and metastasis. Placenta growth factor (PlGF), an angiogenic member of the VEGF family, is also expressed in epidermis. To gain insights into the relative importance of VEGFs and PlGF in stimulating angiogenesis in epidermis, their expression was evaluated in the EpiDerm *in vitro* human skin equivalent after treatment with UVR or H_2O_2. To evaluate the role of EGR receptor (EGFr) signaling in expression of VEGF and PlGF, the EGFr ligand TGFαand the EGFr tyrosine kinase inhibitor AG1478 were used. At various times after treatment, VEGF and PlGF expression was evaluated by RT-PCR and ELISA. Message for VEGF-A, -B, -C, -D and PlGF was detected in EpiDerm, but only VEGF-A, -D and PlGF were induced by UVA or H_2O_2. These were upregulated at 1 hr and persisted for at least 8 hrs. By ELISA, VEGF-D protein was not detected and only low amounts of PlGF (~25 pg/ml) were observed. In Contrast, abundant amounts of VEGF were detected by non-specific VEGF ELISA after UVA or H_2O_2 treatment (>850 pg/ml). EGFr signaling *via* TGFα strongly induced VEGF-A and PlGF message as well as protein (2646 pg/ml and 84.5 pg/ml for VEGF and PlGF protein, respectively). AG1478 blocked TGFα effects. Induction of VEGF-A message and protein secretion by UVA or H_2O_2 was appreciably blocked by AG1478, while VEGF-D and PlGF induction were less sensitive to AG1478. Surprisingly, in contrast to UVA or H_2O_2, irradiation of EpiDerm with complete solar UVR (UVA + B) did not lead to increased expression of VEGF-A above the basal level. These results indicate that VEGF-A is the most abundant angiogenic growth factor *in vitro* human epidermis, secreted at levels on the order of >30-fold higher than PlGF. The results also indicate that exposure of skin to oxidants, or sunscreens which block UVB but not UVA, may lead to increased expression of VEGF *via* EGFr signaling and thereby promote angiogenesis in human skin.

1075 A SYNTHETIC NON-ANIMAL MODEL OF SKIN PENETRATION.

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Efficacy of topical drugs depends on penetration of active into or through skin. Prior to animal and clinical studies to test topical drug efficacy, *in vitro* penetration is often evaluated to select candidate formulations that demonstrate enhanced penetration or permeation profiles, and thus, potentially enhanced topical or systemic efficacies. Although using excised human skin for penetration studies may show the best correlation to intact human skin, difficulty of procurement and lack of reproducibility among specimens limit this option. Instead, excised animal skin is often utilized. Animal skin is cost-effective and easily obtained; however, it often does not predict drug penetration in humans. Alternatively, investigators have used human-derived or synthetic skin models for penetration studies with varying results. The goal of these preliminary studies was to examine penetration of drug using a synthetic non-animal system, Corrositex® (CTX) biobarrier membranes, and to determine if penetration through this *in vitro* system correlated with *in vivo* efficacy following topical administration. Corrositex® biobarrier membranes (InVitro International, Irvine, CA) are substitute skin-like membranes approved for corrosivity testing; compounds penetrate through a skin-like membrane and participate in a colorimetric reaction in the lower chamber. CTX membranes were adapted for penetration experiments by placing various drug formulations onto the donor side and measuring drug in the receiving chamber. Drug formulations were tested for their ability to promote drug penetration through CTX membranes and for their ability to enhance efficacy against infection in an animal model. Data show that penetration through CTX membranes correlated with efficacy *in vivo*; specifically, formulations that caused greater drug penetration *in vitro* were more efficacious in reducing parameters of infection. These preliminary data point to a non-animal alternative to skin for *in vitro* penetration as well as an alternative use for CTX membranes.

1076 INSULATION AND REPAIR OF YOUR PROFESSIONAL CAREER: INTRODUCTION.

M. L. Biehl. *Pfizer Global Res. & Dev., New London, CT.*

For most SOT members, their job is primarily a vehicle to challenge themselves intellectually and scientifically in a subject matter for which they have both expertise and interest. When stability in those endeavors is threatened or even eliminated, the resulting impact on careers and personal life (e.g. financial and emotional) can be potentially devastating. In today's turbulent economy, many individuals have suddenly found their employment status and stability in jeopardy. These situations have not been localized to one SOT professional segment, but have occurred to all groups. SOT members cannot absolutely protect their job situation from this type of upheaval. However, one can learn to recognize the "warning signs", understand how to prepare for the future possibility, and how to react if it occurs so as to minimize the damage, both professionally and personally. This seminar/discussion brings together 4 experts in subjects germane to this career scenario. Subjects to be covered include: 1) current skills and training in demand by employers, 2) job search sources and "no-strength" interviewing strategies, 3) financial planning and reactions, 4) handling the emotional stress associated with job uncertainties, and 5) "personal reflections" from an SOT member who has experienced sudden job change. Following short individual presentations, the remainder of the session time will be devoted to a panel discussion. Questions (both oral and written) will be solicited from the audience.

1077 CAREER PLANNING FOR TOXICOLOGISTS. STRATEGIES FOR PREPARATION AND RESPONSE TO CHANGING CIRCUMSTANCES.

T. Leyden. *Career Marketing Associates, Greenwood Village, CO.* Sponsor: M. Biehl.

Toxicology careers have been increasingly less stable in the last few years as a result of various economic pressures across the industrial, academic and government sectors. This presentation will discuss strategies which toxicologists may employ to ensure preparedness should a staff reduction, merger, career discontent or related event occur. A number of career options and the means to explore them will be presented. Advertisements, networking and professional recruiting services will be mentioned as effective means for investigating career choices and keeping apprised of current trends in toxicology employment and compensation. A suggested plan of action to make a career change will be presented. This will include a brief review of resume and cover letter format, interview preparation and follow through.

1078 FINANCIAL STRATEGIES FOR THE UNEXPECTED CIRCUMSTANCES.

J. D. Strunk. *Salomon Smith Barney, Mystic, CT.* Sponsor: M. Biehl.

Many people struggle with the anxiety of dealing with personal financial circumstances. You can keep one step ahead of your finances by anticipating problems and taking advantage of opportunities. You can save your money and invest wisely. You can plan to ensure your long-term financial security. Financial planning can help you establish firm control over any situation. This presentation will discuss a practical means for taking stock of your present financial status. You will learn how to formulate effective strategies for building and managing your investments and finances and how to react if you are suddenly in financial difficulty.

1079 HANDLING STRESS ASSOCIATED WITH JOB UNCERTAINTIES.

R. G. Weigel. *University Counseling Center, University of Utah, Salt Lake City, UT.* Sponsor: M. Biehl.

Job termination for any reason more often than not leads to physical, cognitive, and/or emotional problems. These problems may be debilitating, but are natural reactions of normal people thrust into abnormal situations. They reflect our built-in

biological mechanism for fight or flight when threatened. Fight seldom is viable choice of behaviors. Flight from the threat may be either physical or psychological: either avoids dealing with facing up to the situation. Such avoidance, based on shame, guilt, or worries, is effective short-term to lessen anxiety, but is self-defeating over the longer haul because it inhibits the individual from seeking a new position. The longer one is out of work the more anxiety increases, and leads to further avoidance of necessary job-seeking behaviors. Thus, it is important not to succumb to such feelings, but to cope by activating oneself to mount and maintain a full-bore job search. A central component of the search is self-examination of strengths, weaknesses, needs, goals, and generalizable skills. With this increased understanding of self, the individual is better prepared to research potential employers, to consider alternative career paths, to network, and to seek possible positions. As one progresses to making contact with employers, another threat to well-being often emerges: the high risk-reward ratio of such contacts may be so stressful as to drive the job-seeker again into self-defeating avoidance of the anxiety of further rejections, and a re-escalation of debilitating physical symptoms or cognitive and emotional problems. "So, what's new?", you say. "It's predictable." Yes. However, an intellectual understanding of this process may be very difficult to bring to bear when you are the victim experiencing the shock, and sometimes the sheer terror, accompanying termination. Realistic strategies for breaking this self-destructive cycle are discussed, as are ways to prepare early and throughout one's career so as to have in place the skill sets, industry knowledge, personal hardiness, professional contacts, and search plans necessary to effectively bounce back if faced with termination.

1080 CAREER DURING CHAOS: AN INSIDERS PERSPECTIVE.

J. A. Popp. *Purdue Pharmacology L.P., Ardsley, NY.*

Change in the employment world is inevitable with the pace of change rapidly increasing. While change can be a very unsettling experience, it is important for every toxicologist to be prepared even though one's current employment appears to be stable. To be prepared the following points should be considered: 1) Every toxicologist should focus on career development all the time. It is important to have up to date skills and be able to demonstrate the ability to contribute to your current employer or to a potential new employer. 2) Rumors of pending workplace changes should be avoided since it is couterproductive. Most rumors are incorrect. You are unlikely to be able to discern the false rumors from the occassional fact. 3) Reactions of colleagues may be unpredictable. Leadership may not be demonstrated during crisis by those who demonstrate leadership during "normal" times. 4) Change is not bad. In summary, chaos in the workplace is here and it will continue. To be successful, one must be prepared, be able to demonstrate flexibility and view change as an opportunity.

1081 BEYOND GENOMICS: IMAGE ANALYSIS AND COMPUTATIONAL BIOLOGY.

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The reductionist approaches used historically in toxicology to understand the nature of cellular functions, and the processes that collectively constitute the molecular basis of the toxic response, can be overly simplistic. Instead, altered cellular structure and function must be studied in a holistic manner that embraces the immense complexity of biological networks. High-throughput genomic technologies are now allowing scientists to pave the way to a deeper understanding of biological systems and their disruption by chemical and physical agents. This symposium brings together toxicologists, engineers, and mathematicians to evaluate the usefulness of genomic and computational approaches to characterize complex biological networks in health and disease, to dissect complex toxicological responses into cogent, contextually relevant processes and to develop mathematical principals governing biological systems.

1082 EPISTEMOLOGY OF CLUSTERING.

E. R. Dougherty. *Electrical Engineering, Texas A&M University, College Station, TX.* Sponsor: K. Ramos.

Many algorithms are now available to cluster sample data points based on nearness or similarity of measure. Often the implication is that points in different clusters arise from different classes, while those within the same cluster come from the same class. Stochastically, the underlying classes represent different random processes and the inference is that clusters represent a partition of the sample points according to the process they belong to. In the present work, the validity of this assumption was tested using a model-based clustering toolbox that evaluates cluster accuracy. This method modeled each random process as its mean value plus independence noise, and generated sample points for subsequent clustering. The clustering error was then calculated, with error defined as the number of points clustered incorrectly according to the generating random processes. Various clustering algorithms were evaluated based on process variance and the key issue of the rate at which algorithmic performance improved with increasing numbers of experimental applications was demonstrated.

1083 MODELING GENETIC REGULATORY NETWORKS WITH PROBABILISTIC BOOLEAN NETWORKS: FROM INFERENCE TO INTERVENTION.

I. Shmulevich. *MD Anderson Cancer Center, Houston, TX.* Sponsor: K. Ramos.

Probabilistic Boolean Networks (PBN) constitute a class of new models of gene regulatory networks. This model class incorporates rule based dependencies between genes, allows the systematic study of global network dynamics, is able to cope with uncertainty, and permits the quantification of the relative influence and sensitivity of genes in their interactions with other genes. The dynamics of these networks, and in particular long run behavior, can be studied using Markov chain theory. We consider the general question of the potential effect of individual genes on the global dynamical network behavior, both from the view of gene perturbation as well as intervention for eliciting desired network behavior. This has great potential for the identification and discovery of potential targets for therapeutic intervention in diseases such as cancer.

1084 APPLICATION OF CLUSTERING METHODOLOGIES TO THE ANALYSIS OF ALTERED CELLULAR PHENOTYPES INDUCED BY OXIDATIVE STRESS.

K. S. Ramos[1], C. D. Johnson[1], M. H. Falahatpisheh[1], T. Thomas[1], P. Beremand[1], M. Tadesse[1], K. P. Lu[1], Y. Balagurunathan[1], C. A. Afshari[2] and . R. Dougherty[1]. *[1]Center for Environmental and Rural Health, Texas A&M University, College Station, TX and [2]Microarray Center, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

The study of complex patterns of gene expression as cells transition from normal to disease phenotypes has been facilitated by the application of Microarray technologies. The present studies were conducted to compare the transcriptome profiles of oxidatively stressed vascular and renal cells to identify critical genes that mediate acquisition of disease phenotypes. Cultured murine vascular smooth muscle cells and nephroblastema were challenged with three micromolar benzo(a)pyrene (BaP) for various times to evaluate the early adaptive global gene expression response associated with altered cellular phenotypes. Vascular and renal cytochrome P450s metabolized BaP to reactive intermediates that triggered a complex cellular response that culminated in activation/repression of overlapping signal transduction cascades involving oxidative stress and DNA damage. The largest group of responsive genes in vascular cells included lymphocyte antigen-6 complex, Histocompatibility class 1 component B and Q region, lysyl oxidase, secreted phosphoprotein, and several interferon inducible proteins; genomic changes neutralized by N-acetylcysteine (0.5 mM), a soluble antioxidant and precursor of cellular glutathione. In nephroblastemal cells, the largest group of responsive genes included glial derived neurotrophic factor, frizzled receptor, IGF receptor, synecan, Sry, oncostatin M, pinin, GATA-3, Sox-18, nrf2 and fibulin. Application of various clustering algorithms (k-means, fuzzy C means, self-organizing maps, and hierarchical) identified critical genes involved in the cellular response to oxidative stress. These data suggest that deregulation of gene expression by BaP involves disruption of redox homeostasis with several common gene targets involved in the early adaptive response to hydrocarbon injury.

1085 BAYESIAN CLASSIFICATION TOOLS IN TOXICOLOGY.

C. A. Bradfield. *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI.*

One of the major objectives of toxicology is to understand the adverse health effects of chemicals in humans. This understanding could be aided by the ability of group chemicals that generally act the same, or are believed to produce their toxic endpoints through similar mechanisms. In an attempt to identify these toxicologically relevant gene expression patterns, we initiated a survey of chemically induced changes in liver gene expression through the use of custom cDNA microarrays. Five broad chemical classes were selected for study. Microarray analysis was performed on various chemicals within these groups and the results analyzed using a Bayesian

analysis. Using a forward parameter selection scheme, a 'minimal' set of 5 genes was identified that allowed 100% accuracy in classifying the treatments and an 'optimal' set of 12 genes which still provided 100% accuracy but also gave more robust predictions. These results provide significant evidence that the classification of chemicals according to their gene expression profiles is possible and opens the door to a potentially new era of toxicological testing.

1086 TOXICOGENOMICS AND THE QUEST FOR PREDICTIVE TOXICOLOGY.

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One of the major goals in toxicology is the ability to predict an adverse affect on human health following exposure to an environmental stress. The power and promise of genomics is to provide new tools to actually accomplish this goal. By building a robust knowledge of gene expression changes that occur in various tissues following exposures to toxicants, one can then begin to discern early changes that are informative of ensuing pathological alterations. Animals have been exposed to a variety of toxic and nontoxic agents and the gene expression patterns have been analyzed using cDNA microarray analyses. The resulting information has been subjected to a variety of analysis approaches which identify significantly differentially expressed genes within the data set, reduce the complexity of the data and allow for identification of highly informative genes that permit discrimination among classes of agents or among toxicological endpoints. These approaches include hierarchical clustering, analysis of variation (ANOVA), genetic algorithm/K-nearest neighbor (GA/KNN), linear discriminant analysis (LDA), principal component analysis (PCA), and a simplified fuzzy adaptive resonance theory map (SFAM) neural network architecture.

1087 CHILDREN'S HEALTH RISK: WHAT'S SO SPECIAL ABOUT THE DEVELOPING IMMUNE SYSTEM?

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In recent years, there has been increasing regulatory pressure to protect the health of children, with the basic tenet being that children differ significantly from adults in their biological and/or physiological responses to chemical exposures. In a regulatory context, this has been translated to mean a requirement for an additional 10-fold safety factor for environmental contaminants, specialized tests, or both. Much of the initial focus has been on the developing endocrine and nervous systems but increasingly the developing immune system has been identified as a potential target organ for chemically mediated toxicity. More recently, the question has been raised regarding whether the current state of the science supports the creation of developmental immunotoxicology (DIT) test guidelines. What is needed is a risk-based evaluation of the biology associated with the proposed differential sensitivity between children and adults and the impact of that assessment on additional regulatory measures to protect children in risk assessment analyses. Additionally, an understanding of whether the developing immune system shows greater susceptibility, either qualitatively or quantitatively, to chemical perturbation is critical.

1088 ASSESSING THE HAZARD TO CHILDREN OF LOW LEVEL ENVIRONMENTAL EXPOSURES.

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Since about 1995, there has been extraordinary interest in the possible hazard to children posed by exposure to environmental toxicants and pharmaceuticals. Passage of the Food Quality Protection Act (FQPA) in 1996 represents one of the first major national attempts to protect children from chemical exposures by requiring that pesticide residue levels be 10-fold less than would normally have been selected based on the available toxicity data. A Presidential Executive Order on the Protection of Children from Environmental Health Risks and Safety Risks and the USEPA's Office of Children's Health Protection were also established in 1997 to prioritize and coordinate research efforts on health and safety risks to children. Over the past five years, due to improved levels of detection of chemicals, it has been shown that many chemicals which are found in commercial products (carpets, wallpaper, etc.)are present in indoor air and the blood of children. In 2000, the USEPA announced the Voluntary Children's Chemical Evaluation Program (VCCEP)which is intended to provide data to enable the public to understand the potential health risks to children associated with certain chemical exposures. Recent examples of concerns over children's exposures to environmental toxicants include CCA-treated wood, pesticides in carpet from outdoor application and pthalates in toys. Two new journals have been announced which will focus on research in these areas and a dozen centers for the study of Children's Health have been formed at major universities. This presentation will review some of what is known about the enhanced susceptibility of children to low level exposure to environmental chemicals (or lack thereof), current methods for estimating exposure to children, new or proposed models for estimating the toxicological hazard, and some of the current research efforts.

1089 DIFFERENTIAL SENSITIVITY OF CHILDREN AND ADULTS TO CHEMICAL TOXICITY - BIOLOGY, RISK, AND REGULATION.

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Many are questioning the extent to which methods used by regulatory agencies to assess risks from environmental toxicants are adequate to protect children. Arguments for and against an additional 10x safety factor to account for the potentially greater susceptibility of children can be made on both scientific and precautionary grounds. Insults that occur during development *in utero* or during childhood can have tragic consequences in terms of birth defects and greater likelihood of disease throughout both childhood and adulthood, placing great demands on social and emotional resources. The proportion of birth defects, developmental immunotoxicity, and other problems attributable to environmental exposures to chemical toxicants is not known, but even if that proportion is small, it would constitute a public health problem by virtue of the numbers of people affected. Children are at greater risk of chemical toxicity if their exposures are high enough to produce adverse effects whether or not they are more or less sensitive than adults, however. The question then is one of whether current regulatory approaches to limiting chemical exposures are sufficient to protect children from exposures that are toxic; or, are chemical exposures misregulated due to inadequate attention to children's sometimes greater sensitivity to particular chemicals, putting them at greater risk? The uncertainty factors that regulatory agencies use when identifying criteria for limiting chemical exposures are designed to account for differences in susceptibility within and among species and to compensate for limited data availability, when necessary. Where available, quantitative analysis of the extent of toxicodynamic and toxicokinetic variability among humans indicates that relying on a default value of 10 to compensate for variability among humans, including that due to age, and on a default value of 10 to compensate for a limited toxicity database, when necessary, are adequate to protect most of the people - including children - most of the time.

1090 THE DEVELOPING HUMAN IMMUNE SYSTEM: A CLINICAL PERSPECTIVE.

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Sponsor: L. Burns Naas.

Defining critical windows in the development of the immune system is needed to identify periods of specific vulnerability in the continuum of development, which may differ significantly from the mature system. Other fundamental factors must then be integrated that may further predispose the developing immune system to risk. These factors, which may vary significantly between species, include biologic parameters of the fetus, infant, child, and adolescent, such as differences in metabolism, physiology and body surface area ratios. Prenatal events can affect immune responses at birth and, depending on gestational timing, can result in widely varied outcomes. Because birth occurs at various stages of fetal maturity, the significance of parturition in terms of immune susceptibility varies from species to species. At the time of human birth, various compartments of the immune system differ in their numbers and functionality, which can contribute to a sensitivity to chemical immunosuppression. After the early neonatal period, there is continued acquisition of immune competence concomitant with increased antigen exposure throughout years one and two of childhood. However, it is important to consider the fact that attainment of immunocompetence does not necessarily mean closure of 'windows'. For comprehensive progress towards strategies aimed at the needs of the young, the following questions must be considered. Are the parameters that we extrapolate from animal models physiologically and clinically relevant to the developing human? Are the data that we extrapolate from mature individuals relevant during variable stages of immunologic immaturity? Continuing difficulties will be presented because there is inadequate data on exposure, toxic effects, pharmacokinetics and pharmacodynamics during fetal and neonatal life, infancy, childhood and adolescence. Furthermore, assays may be unvalidated for varied states of immaturity. A major collaborative effort is needed in order to facilitate design and development of better strategies to determine the potential impact of chemicals on the developing immune system.

1091 EVOLUTION OF THE SCIENCE OF DEVELOPMENTAL IMMUNOTOXICITY.

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The value of incorporating immunological data for the toxicological assessment of drugs, chemicals, biologicals and medical devices for human risk assessment has been increasingly accepted. Since the 1970s, experimental animal studies, and to a lesser extent human studies, have been published describing immunological effects in neonates exposed to toxic agents during the prenatal or early postnatal period . Of particular concern was that immunotoxicity often appeared more severe and/or persistent when the exposure occurred perinatally when compared to exposure in adult animals. These concerns were addressed in a 1993 report from the National Research Council (NRC) entitled Pesticides in the Diets of Infants and Children in which the immune, as well as the reproductive and nervous systems, were identified as potential targets for pesticide exposure. Efforts are presently being undertaken to identify appropriate methods and approaches to identify and study developmental immunotoxicants. In addition to immunosuppression, there is increasing evidence that transplacental priming of the immune system occurs in response to certain environmental agents which possess allergic or inflammatory properties. This subsequently results in T-helper 1 (Th1) or, more often, T-helper 2 (Th2) skewing of the immune system. Consistent with this general postulate is experimental and clinical evidence that individuals exposed early in life to these environmental agents are predispose to increased prevalence and/or severity of immune diseases

1092 SUSCEPTIBILITY OF THE DEVELOPING IMMUNE SYSTEM TO IMMUNOSUPPRESSIVE AGENTS: DIFFERENTIAL RISK ACROSS LIFE STAGES.

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Recent findings from *in utero* vs. adult exposures to toxicants will be used to demonstrate the specific challenges associated with developmental immunotoxicity evaluation. Differential risk to immunotoxicants can result from life-stage-specific differences in metabolism, differential susceptibility of the developing immune system target, potential latency effects, and heterogeneous susceptibility of populations (i.e. toxicogenomics). Likewise, the challenge in selecting an appropriate age of assessment capable of revealing these comparative risks will be discussed. Data from early exposures to low-levels of the heavy metal, Pb, will be presented to support the concept of critical developmental windows of differential immunotoxic risk. Additionally, the Pb-induced immunotoxicity results are consistent with an emerging paradigm of gestationally-induced immunotoxicity. Disrupted T helper balance with the hallmark of suppressed T helper 1 (Th1) function appears to represent a common immunotoxic outcome of early chemical insults. Because Th1 function develops later in gestation than the default T helper 2 (Th2) function, the potential for impairing Th1 function with *in utero* exposure to toxicants appears to be significant. It should be noted that this type of immune alteration (elevated Th2 function with suppressed Th1 activity) would contribute to an increased risk of childhood asthma and allergic disease.

1093 TEMPORAL SPECIFIC EXPRESSION OF TOXICANT-METABOLIZING ENZYMES: IMPLICATIONS FOR LIFE-STAGE-DEPENDENT TOXICITY.

R. N. Hines. *Pediatrics & Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee, WI.*

Substantial changes in toxicokinetics and toxicodynamics occur during human development that contribute to differential susceptibility. Although certainly not the only component, the temporal-specific expression of toxicant metabolizing enzymes contribute significantly to these changes. A better knowledge of these processes and their underlying mechanisms will be required if we wish to understand and predict the dynamic dose-response relationships that occur during ontogeny and develop strategies that would prevent developmental toxicity. The objective of this symposium is to present recent advances in our understanding of how developmental, genetic, and environmental factors interact to define the risk from toxicant exposure. The symposium will cover research results on longitudinal pediatric phenotyping and present a dramatic example of the importance of phenotyping in identifying potential risk. Studies also will be presented on the characterization of both phase I and phase II developmental expression patterns in the human and animal models, explore molecular mechanisms underlying the regulation of such expression patterns, and begin to explore how genetic factors might contribute to both interindividual differences in expression as a function of age.

1094 PEDIATRIC PHARMACOGENETICS: DEVELOPMENTAL "PHENOTYPES" AND THEIR POTENTIAL CONSEQUENCES.

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Individual cytochrome P450 (CYP) isoforms have characteristic developmental profiles, and phenotypic measures of those activities change throughout maturation. Thus, in children, gene-environment interactions that contribute to the pharmacogenetic determinants of drug disposition and response are influenced by an additional variable, development. The goals of our research program are to define the ontogeny of drug biotransformation pathways *in vivo*, and to identify the pharmacogenetic basis of variation in the developmental patterns observed, a process we refer to as "developmental pharmacogenetics." To achieve these goals, we employ a longitudinal phenotyping strategy using probe compounds that are considered safe by health care professionals and perceived as safe by parents, such as dextromethorphan (DM) and acetaminophen (APAP). Phenotyping studies are coordinated with well baby visits to primary care providers. At two weeks postnatal age, CYP2D6 activity (as estimated by the ratio of DM to its *O*-demethylated metabolite, dextrorphan) is consistent with *CYP2D6* genotype (n=96). However, the data also suggest that DM *N*-demethylation activity, attributed to CYP3A, becomes quantitatively more important to overall DM biotransformation as infants mature and become toddlers. For example, the fraction of total DM and metabolites recovered as 3-hydroxymorphinan increases from 0.156 ± 0.154 at two weeks to 0.531 ± 0.157 at 12 months of age while the fraction recovered as dextrorphan decreases from 0.799 ± 0.164 to 0.450 ± 0.148 over the same period. Developmental changes in CYP activity clearly have the potential to affect dosage requirements at different ages but may have toxicologic significance as well. Following APAP dosing to two week old infants, thioether metabolites represent 10.4 ± 0.4% of recovered drug and metabolites, similar to adults. Further investigation is required to determine if developmental differences in bioactivation and detoxification processes result in periods of susceptibility that are unique to children at a specific developmental stage.

1095 MOLECULAR MECHANISMS REGULATING *FMO* TEMPORAL-SPECIFIC EXPRESSION.

R. N. Hines, S. B. Koukouritaki, K. Hopp and Z. Luo. *Pediatrics & Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee, WI.*

The flavin-containing monooxygenase genes (*FMO1-5*) encode enzymes important for the metabolism of numerous environmental toxicants. Studies to date have demonstrated temporal-, tissue-, & species-specific *FMO* expression patterns that impact susceptibility. However, a complete picture of these patterns and underlying regulatory mechanisms remain unknown. To characterize human hepatic *FMO1 & 3* developmental expression, microsomal protein levels were quantified in 240 liver samples representing ages from 8 wks gestation to 18 postnatal yrs. FMO1 expression was highest at 8-15 wks gestation (7.8 ± 5.3 pmol/mg protein) then slowly declined with complete suppression within 3 postnatal d through a mechanism tightly coupled to birth, but not gestational age. Low FMO3 levels also were detected at 8-15 wks gestation, but at no other prenatal period. Most individuals failed to express FMO3 during the first 21 postnatal days. FMO3 was detected by 10 mo & was expressed at 20% of adult values between 10 mo & 11 yr. Thus, birth is necessary, but not sufficient for the onset of FMO3 expression. A gender-independent increase in FMO3 expression was observed between 11 and 18 yrs (maximum of 26.9 ± 8.6 pmol/mg protein), although adult expression levels were not attained by the latter age. Data from transient expression studies suggest that fetal hepatic FMO1 expression is largely regulated by HNF1α, although as yet unidentified upstream-binding factors also are involved. Preliminary data implicate DBP as playing a role in regulating postnatal hepatic *FMO3* expression. Results from SNP discovery suggests a high degree of *FMO1* conservation, but did identify a functional, regulatory polymorphism in a basal promoter YY1 element that may account for up to a 2-fold variation in expression. Other novel genetic variants may partially explain interindividual variability in *FMO1 & 3*expression (Supported by PHS CA53106).

1096 HUMAN CYP2E1 DEVELOPMENTAL EXPRESSION: A ROLE IN FETAL & PEDIATRIC SUSCEPTIBILITY TO TOXICANTS?

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CYP2E1 is important in the bioactivation of small molecular weight toxicants such as toluene, benzene & trichloroethylene. Developmental changes in human CYP2E1 expression likely impact susceptibility of the fetus and young child to these compounds. Two previous studies of human CYP2E1 ontogeny yielded conflicting results. To define human hepatic CYP2E1 developmental expression pattern, microsomes were prepared from human liver samples [N=72 fetal (8-37 wks);

166 postnatal (1d-18 y)]. Samples from subjects likely to have liver disease were excluded. Microsomal CYP2E1 was quantitated by western blot analysis (LOD=1.0 fmol CYP2E1). Measurable expression was seen in 18/49 2nd trimester and 12/15 3rd trimester fetal samples [medians=0.35; 6.7 pmol/mg protein, respectively]. CYP2E1 increased after birth but was relatively low in the neonatal period. CYP2E1 in neonatal samples was less than that of infants from 1-3 months of age which was less than that of older infants, children and young adults [median (range)= 8.8 (0-70); 23.8 (10-43); 41.4 (18-95), respectively; each $p<0.001$, ANOVA, post-hoc]. Beyond 3 months of age, CYP2E1 protein values did not vary by age. At every age, 4-fold or greater intersubject variation was observed. Increasing postnatal age was associated with increasing protein [N=29, $p=0.001$, linear regression], whereas the relationship with increasing GA was less striking [$p=0.07$]. These data suggest that infants < 3 months of age may have significantly lower metabolic clearance of CYP2E1 substrates compared to older age groups. This may lead to a protective effect among very young infants exposed to ubiquitous toxicants such as toluene, benzene and trichloroethylene that are bioactivated by this enzyme. We suggest that the 4-fold variation in CYP2E1 protein among all pediatric patients is consistent with either genetic differences or variable protein stabilization by an unknown endogenous substrate.

1097 DEVELOPMENTAL EXPRESSION OF HUMAN HEPATIC CYP3A AND 2B ENZYMES.

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Within the human cytochrome P450 family, specific hepatic forms can show developmental expression patterns that may affect drug clearance, efficacy and safety. The objective of these studies was to use P450-specific enzyme activities or immunoquantitation to identify developmental expression patterns in a large (n~250) and developmentally diverse set of pediatric liver samples. CYP2B6 levels in fetal samples were found to be low, with few exceptions. Activities increased with birth, with an average activity of 0.07 nmol/min/mg observed for the birth to 3-month age category. CYP2C9-dependent activity was clearly shown in fetal samples, with a dramatic increase in activity associated with birth. CYP2C19 levels peaked at 6-12 months, with males showing a significant increase vs. females for this category. Different approaches were used to characterize changes in expression of CYP3A forms. Using an antibody specific to 3A5, protein levels showed no change in expression with age, however, ethnicity was a determinant with higher 3A5 levels observed for African American subjects in 8 of 9 age categories. The differentiation of 3A7 (fetal form) from 3A4 (primarily an adult form) within the same sample required method development. Specifically, the use of dehydroepiandrosterone (DHEA) metabolite profiles to simultaneously quantitate 3A4 and 3A7 in pediatric liver microsomes was studied. First, 7β-OH-DHEA was positively identified as the major metabolite of 3A4, based on co-chromatography and MS/MS analysis. The major metabolite of 3A7 has been reported as 16α-OH DHEA, but it is also formed by 3A4. Therefore the contributions of 3A4 and 3A7 were determined in mixtures of expressed isoforms by fitting the metabolite profiles to a multiple response model. Preliminary data suggest that DHEA metabolite profiles can differentiate 3A4 and 3A7 levels for adult and pediatric liver microsome samples.

1098 DEVELOPMENTAL CHANGES IN N-ACETYLTRANSFERASES: IMPLICATIONS FOR 4-AMINOBIPHENYL (4ABP) TOXICITY.

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Developmental changes in the expression of biotransformation enzymes affect the susceptibility of fetuses, infants and children to the therapeutic and toxic effects of chemicals. Arylamine N-acetyltransferases (NAT1 and NAT2) catalyze reactions involved in activation and detoxification of drugs and arylamine carcinogens including 4ABP. Several studies show that these enzymes are present early in human development. Interestingly, there is no apparent genotype-phenotype correlation in young children. When phenotypic determinations in children are done with the metabolic ratios used for adults, phenotype changes with age. This suggests that expression of the NAT genes is changing. Preliminary data with human postmortem samples from neonates and children show these samples have lower sulfamethazine NAT activity than that in adult liver. C57Bl/6J mice have been used to more completely assess the expression of NAT1 and NAT2 from birth to sexual maturity. Hepatic expression of NAT1 and NAT2, determined by quantitative RT PCR, increased with age. Hepatic N-acetylation of p-aminobenzoic acid, a murine NAT2 selective substrate, isoniazid, a murine NAT1 selective substrate, as well as the carcinogenic aromatic amines 2-aminofluorene and 4ABP, substrates for both isoforms, also increased with age. Toxicologic effects of this developmental pattern were evaluated with 4ABP. One reaction catalyzed by NATs is a step in the activation of 4ABP to DNA reactive products. Interaction of these products with DNA was evaluated by microarray analysis of hepatic gene expression and adduct formation in neonates and adults following exposure to 4ABP. Expression ratios of genes involved in cell cycle control were among those affected; however, the number of genes as well as the expression ratios was lower in neonates than in adults. A similar pattern was seen with 4ABP-DNA adduct levels. These results suggest that the level of expression of NAT1 and NAT2 occurring in neonates limits acetylation and contributes to decreased 4ABP toxicity compared to adults. (Supported by ES 09812 and ES 10047).

1099 OCCUPATIONAL LUNG DISEASE IN RESPONSE TO MIXED EXPOSURES: APPROACHES TO IDENTIFY THE TOXICITY OF PROCESS - DEPENDENT CONTAMINANTS.

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To date, the majority of exposure limits set by OSHA or EPA have been for individual particulate agents or chemical compounds. However, modern industrial operations generate complex mixed aerosols, the components of which are often process dependent. For example, the chemical composition of welding fume varies with the type of shielding and electrode used, while microbial contamination of organic dusts or used metal working fluids can dramatically alter the biological response upon exposure to these materials. In addition, the types and amounts of organic chemicals adsorbed onto the carbon core of particles generated by diesel engines can depend on engine speed, load, and the type of fuel consumed. There is increasing awareness that the toxicity of a mixed exposure may not simply be the additive effects of its components. Indeed, synergistic effects, involving soluble metals, adsorbed organics, surface acidity, microbial contamination and particle size or surface area may occur. For this reason, NIOSH has listed "mixed exposures" as a priority area in its National Occupational Research Agenda that must be addressed to allow appropriate and complete risk assessment in complex occupational settings. The objective of this symposium is to elucidate the various interactions among components of mixed exposures that are possible and to characterize the mechanisms involved in these interactions, using real-world occupational aerosols as examples.

1100 ISSUES THAT MUST BE ADDRESSED FOR RISK ASSESSMENT OF MIXED EXPOSURES: EPA EXPERIENCE WITH AMBIENT AIR QUALITY.

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Epidemiological studies have linked elevated levels of ambient particulate matter to increased morbidity and mortality from both cardiovascular and pulmonary diseases. Risk assessment efforts concerning ambient air quality must address the issue of mixed exposures, since in ambient air both gaseous and particulate fractions exhibit toxic potentials which may result in synergistic interactions. Making risk assessment even more difficult is the increasing awareness of the complex nature of ambient particulate matter itself. Recent investigations have been directed toward the elucidation of the toxic potential and adverse interactions of the various components of ambient particles. Research efforts have found several characteristics of ambient particles to be important factors for consideration. These characteristics include the unique toxic potential of soluble metals, absorbed organic chemicals, surface acidity and microbial contaminants on ambient particles. In addition, the unique toxicity of the ultrafine vs the fine fraction of ambient particles has become an issue of increasing importance. This presentation will review evidence which evaluates the relative importance of these toxic components of ambient particulate matter and mechanisms by which these components adversely affect the lungs. In addition, mechanisms by which pulmonary exposure to ambient particles can result in pathological cardiovascular reactions will be discussed.

1101 METAL WORKING FLUIDS AS COMPLEX MIXTURES.

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Exposure to aerosols of metal working fluids is associated with a variety of adverse respiratory effects. The identity and proportion of chemical species in these mixtures are dependent on several factors, including the manufacturer and the cooling and lubrication requirements of the machining process. In addition, a number of additives, such as biocides and anti-foaming agents, are added to the metal working fluids to enhance their operational lifespan. Also of considerable concern, an unknown number and amount of contaminants become part of the complex mixture during the machining process. These contaminants can include tramp oil, reaction products such as nitrosamines, metal fragments, and microbial products from contaminating bacteria and fungi. Only a limited amount of data in the published lit-

erature has addressed the issue of identifying the individual component(s) responsible for the adverse effect of inhaled metal working fluid aerosols. The approaches of these toxicology studies have included: 1) the comparison of used versus unused fluids, 2) the addition of individual components/contaminants to the unused fluid, 3) association studies which attempt to correlate and adverse effect with a biomarker such as specific antibodies, and 4) principal component analysis. Each of these approaches has been useful in identifying potential toxic components, but definitive answers to the complex issue of metal working fluid toxicity are still needed.

1102 PULMONARY RESPONSES TO WELDING FUMES: ROLE OF METAL CONSTITUENTS.

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It is estimated that more than one million workers worldwide perform some type of welding as part of their work duties. Epidemiology studies have shown that a large number of welders experience some type of respiratory illness. Respiratory effects seen in full-time welders have included bronchitis, airway irritation, lung function changes, and a possible increase in the incidence of lung cancer. Pulmonary infections are increased in terms of severity, duration, and frequency among welders. Inhalation exposure to welding fumes may vary due to differences in materials used and methods employed. The chemical properties of welding fumes can be quite complex. Most welding materials are alloy mixtures of metals characterized by different steels that may contain iron, manganese, silica, chromium, nickel, zinc, and fluorides. Animal studies have indicated that the presence and combination of different metal constituents is an important determinant in the potential pneumotoxic responses associated with welding fumes. Animal models have demonstrated that stainless steel welding fumes which contain significant levels of nickel and chromium induce more lung injury and inflammation and are retained in the lungs longer than mild steel welding fumes which contain mostly iron. In addition, stainless steel fumes generated from welding processes using fluxes to protect the resulting weld contain elevated levels of soluble metals. These soluble welding fumes have been shown to suppress lung macrophage function and significantly slow the clearance of bacterial pathogens from the lungs after infection. The presence of soluble metals, such as Cr, Ni, and Mn, and the complexes formed by these different metals, as well as fluoride compounds present in fluxes, are likely important in the pulmonary responses observed after welding fume inhalation.

1103 THE ROLE OF BACTERIAL AND FUNGAL CONTAMINANTS IN AGRICULTURAL RESPIRATORY DISEASES.

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Farmers and other agricultural workers face a variety of respiratory diseases including organic dust toxic syndrome (ODTS), farmer's hypersensitivity pneumonitis (HP), asthma-like syndrome, asthma, and pulmonary infections. These diseases arise from complex mixtures of bioaerosols and aeroallergens including pathogenic and non-pathogenic organisms; microbial constituents such as endotoxins and glucans; and allergens from arthropods, animals, plants and molds. ODTS is an acute inflammatory response to inhalation of dusts of organic origin, such as silage, grain dust, cotton dust, and compost. It is characterized by fever, headache, chest tightness, pulmonary infiltrates, and airway obstruction. The bacterial product, endotoxin, and the fungal product, beta-glucan, have been proposed as important etiologic agents in ODTS. Farmer's HP has a similar clinical presentation to ODTS but is an allergic disease caused principally by antigens from thermophilic bacteria. However, proinflammatory constitutents in organic dust may play a role in the disease. Asthma-like syndrome may arise from work in animal confinement units, such as those for intensive poultry and swine production, or from grain handling. This syndrome is an acute non-allergic airway obstruction caused by inflammatory responses to components of the organic dust. It differs from asthma in that it rarely leads to persistent airway hyperreactivity. Pulmonary infections may be zoonotic or may arise from a variety of niche organisms. Human and animal studies elucidating the mechanisms for initiation of pulmonary reactions to organic dusts will be presented. Risk assessment strategies for these mixed exposure environments will also be addressed. Supported by NIEHS P30 ES05605.

1104 EFFECT OF DIESEL EXHAUST PARTICLES (DEP) ON IMMUNE RESPONSES: CONTRIBUTION OF THE ORGANIC COMPONENT.

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The effect of DEP exposure on innate, cellular and humoral pulmonary immunity has been studied using rat, mouse and cell culture models. DEP consist of a complex mixture of petrochemical-derived organics on a carbon core and are regarded as major components of particulate urban air pollution. The alveolar macrophage is considered a key cellular component in pulmonary innate immunity. DEP and DEP organic extracts have been found to suppress alveolar macrophage cytokine (IL-1, TNF-α) and reactive oxygen species (ROS) responses to lipopolysaccharide. DEP depressed clearance of *Listeria monocytogenes* and INFγ-dependent clearance of BCG in a mouse model. INFγ-stimulated nitric oxide (NO) production was suppressed by DEP and DEP organic extract *in vitro*. Further fractionation of the DEP extract suggests that this activity was predominately in polyaromatic containing and more polar (resin) fractions. Organic-stripped DEP did not alter these innate pulmonary immune responses. The contribution of the organic component of DEP is less well defined with respect to acquired and humoral immunity. Indeed, both DEP and carbon black enhanced humoral immune responses (specific IgE and IgG) in an ovalbumin sensitized rat model. It is concluded from a review of the literature and the present work that both the particulate and adsorbed organics may contribute to DEP mediated immune alterations.

1105 EVALUATION OF MCASE-ES USING A TEST PANEL OF PHARMACEUTICAL STRUCTURES.

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Computational models such as the MultiCASE (MCASE) predictive toxicology system are gaining widespread use in academic, industrial and regulatory organizations to assess the potential mutagenicity of new chemical entities. Expanded databases that are the foundation of the expert system (MCASE-ES) have recently become available but the majority of the chemicals within the training set are largely from non-pharmaceutical sources. This system was evaluated using a test panel of 127 structures with known mutagenicity results against the 15 mutagenicity databases available. The evaluation is based on structures within the training set that are reduced to fragments of 2 to 10 atoms in length. MCASE activity values are assigned on the basis of this training set. Predictions based upon the activity value, or CASE unit, quantify the potential activity of the test molecule. We have found the MCASE-ES system to have a sensitivity and specificity of 70% and 60%, respectively, for our test panel. There are several ways in which these results may be improved. For example, we have found that the CASE unit values from true positive predictions exhibited a normal distribution while the CASE unit values from false positive predictions had a multi-modal distribution. This suggests that changes to the logic which link mutagenic activity to the CASE unit may reduce the number of false positive predictions, therefore improving specificity. It was observed that by increasing the CASE unit value associated with mutagenic activity we were able to improve specificity to 88% but resulted in a decrease in sensitivity to 44%. Increasing the number of pharmaceutical compounds present in the training sets, both positive and negative for mutagenicity, and/or appropriately weighing their relevance may improve the sensitivity of this program. This could provide better coverage of pharmaceutical compounds and decrease the number of false predictions.

1106 A YEAST RAD54-GFP GENOTOXICITY ASSAY, IS EFFECTIVE IN IDENTIFYING DIRECT ACTING MUTAGENS IN ADDITION TO CLASTOGENS NOT DETECTED BY BACTERIAL TESTS.

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A eukaroyotic (yeast) genotoxicity assay has been developed which detects increases in the activity of the DNA repair system. It uses a reporter system consisting of the DNA damage inducible promoter of the RAD54 gene fused to a GFP gene. A large study has been carried out to validate the utility of the assay in routine genotoxicity assessment. A single 96 well microplate is used to assess 4 compounds at 9 dilutions, and can be set up by a simple manual protocol for low throughput (<60 compounds/day) or with commercial robotic systems for higher throughput. There are three steps: yeast cells are added to compound dilutions, microplates are incubated overnight, spectrometric data is collected. Optical denisity is used to estimate reduced cell proliferation (toxicity). Fluorescence, normalised for cell density, is used to estimate activity of the repair system (genotoxicity). Data handling protocols are simple and transparent, leading to clear conclusions. Inspection of the data in graphical form allows direct assessment. Less than 0.5ml of compound (1mM) is required and over 250 toxic and non-toxic compounds have been tested, without S9 addition. The results to be presented include the following. Many of 'the usual suspects' are genotox positive in the test: these include EMS, MMS, NQO, MNNG, Cisplatin. Many clastogens are positive in the assay: these include phleomycin, daunorubicin, bleomycin. Expected oxidising agents (methyl viologen, hydrogen peroxide, bleomycin) and aldehydes (crotonaldehyde, benzaldehyde,

acetaldehyde) are positives. Several compounds that would require S9 in bacterial tests (benzo[a]pyrene, N-nitroso-N-ethyl urea, safrole) are also positive. There are some photomutagens (psoralen, 8-MOP) detected, though no attempt was made to exclude exposure to normal laboratory lighting. It was also interesting to detect colchicine as positive, suggesting a clastogenic as well as aneugenic mechanism for this compound.

1107 POSSIBLE ROLE FOR CHEMOTHERAPEUTIC AGENTS IN THE INDUCTION OF MITOCHONDRIAL DNA MUTATIONS AND INCREASED FREE RADICAL GENERATION.

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Mitochondrial DNA (mtDNA) mutations/deletions and altered expression levels of mtDNA-encoded respiratory chain subunits have been reported in various types of solid tumors by a number of laboratories. However, the cause of mtDNA mutations and their roles in drug resistance and disease progression remain to be elucidated. We hypothesized that certain chemotherapeutic agents may cause mtDNA mutations, which contribute to drug resistance and disease progression by altering respiratory function, reactive oxygen species generation, and sensitivity to therapeutic agents. We evaluated mtDNA mutation frequency, free radical generation and clinical outcomes using chronic lymphocytic leukemia (CLL) as a model system. Primary leukemia cells were isolated from 10 untreated and 10 previously treated CLL patients. We found that CLL cells from previously treated patients had significantly higher levels of cellular superoxide than their untreated counterparts ($p = 0.0085$). Six regions of the mitochondrial genome were sequenced using DNA isolated from primary CLL cells in order to determine the mtDNA mutation frequency. We found that untreated patients had similarly low rates of heteroplasmic and homoplasmic mutations. In contrast, previously treated patients had a significantly higher heteroplasmic mutation frequency ($p = 0.0140$), but a comparable frequency of homoplasmic mutations ($p = 0.7128$). The higher mutation frequency in patients receiving prior treatment correlated with an increased number of amino acid changes in the regions we sequenced ($p = 0.0468$). Our results demonstrate that CLL patients who have been previously treated produce higher levels of cellular superoxide have a significantly higher heteroplasmic mtDNA mutation frequency, and possess more functional mtDNA sequence variations than untreated patients. Thus, we have shown for the first time a relationship between chemotherapeutic treatment and mtDNA alterations.

1108 MUTAGENICITY AT THE *HPRT* LOCUS OF T-CELLS FOLLOWING EXPOSURE OF WILD-TYPE AND CYTOCHROME P4502E1-NULL MICE TO ACRYLONITRILE.

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Acrylonitrile (AN), an important industrial chemical, is carcinogenic in rats and mice, and CYP2E1 is the only enzyme responsible for oxidative metabolism of AN in the mouse. To investigate the potential role of CYP2E1 in AN-induced mutagenesis and carcinogenesis, we compared the relative mutagenic responses at the *Hprt* locus of T-cells isolated from spleens of female wild-type (WT) versus CYP2E1-null mice exposed (beginning at 6 wk of age) to AN at 0, 2.5 (WT mice only), 10, 20 or 60 (null mice only) mg/kg by gavage daily for 6 wk (5 days/wk) (note that 60 mg AN/kg is lethal to WT mice). The dose-response for *Hprt* MFs were determined *via* the T-cell cloning assay for cells from mice necropsied 24 h after the last day of dosing (n = 11-13/group for each genotype). In WT mice, MFs increased with increasing dose of AN [i.e., MFs were 2.3 ± 1.9 (SD) $\times 10^{-6}$, $3.1 \pm 1.2 \times 10^{-6}$, and $5.4 \pm 2.4 \times 10^{-6}$ for 2.5, 10, and 20 mg AN/kg, with respective P-values of 0.6, 0.063, and 0.016 when compared with a mean control MF value of $1.7 \pm 0.9 \times 10^{-6}$]; however, only exposures to 20 mg AN/kg led to a significant increase over the control mouse MF values. In CYP2E1-null mice, *Hprt* MFs were significantly increased over background ($1.7 \pm 0.9 \times 10^{-6}$) in the 60 mg AN/kg ($5.3 \pm 2.3 \times 10^{-6}$; $P = 0.002$) but not the 20 mg AN/kg treatment group ($1.6 \pm 0.7 \times 10^{-6}$). The occurrence of a significant mutagenic response in WT mice but not in CYP2E1-null mice exposed to 20 mg AN/kg (a carcinogenic dose-level in the cancer bioassay in WT B6C3F1 mice), suggests that the process(es) leading to mutation at the high dose level of 60 mg AN/kg in CYP2E1-null mice contribute little to the mutagenic effect measured in AN-treated WT mice. On the other hand, the significant mutagenic response in WT mice given 20 mg AN/kg, but not in CYP2E1-null mice given the same dose, indicates that oxidative metabolism, presumably to the DNA-reactive metabolite cyanoethylene oxide, is essential to AN-induced mutagenicity at dose levels leading to cancer in conventional mice.

1109 EFFECTS OF BENZENE ON HEMATOPOIETIC STEM CELLS.

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Benzene, a carcinogen that induces chromosomal breaks as a primary mode of genotoxicity in the bone marrow (BM), is associated with leukemia in humans. Genetic consequences and misrepair of DNA lesions resulting from benzene metabolites interacting with molecular targets may lead to changes in hematopoietic stem cells (HSC) that give rise to the leukemic clones. To investigate benzene-induced DNA damage responses in HSC, we studied the effects of 1, 4-benzoquinone (BQ) treatment on murine HSC *in vitro*. We also compared effects of benzene inhalation on male and female 129/Sv and 129/SvJ mice that differ at the *Prkdc* locus encoding the catalytic subunit of DNA-PK, which has a primary role in nonhomologous end joining. HSC were enriched from BM by negative selection then purified HSC were obtained by fluorescence activated cell sorting. HSC cultured in the presence of BQ for 24 h showed a dose-dependent cytotoxic response. For the inhalation study, mice were exposed to 0 or 100 ppm benzene for 6 h/day, 5 days/week for 2 weeks. Liver microsomal CYP2E1 activity was measured. Male mice of both strains had decreased white blood cell counts following exposure to benzene while female mice showed no change. Genotoxicity *in vivo* was assessed using flow cytometry to enumerate micronucleated mature normochromatic erythrocytes (MN-NCE) and micronucleated reticulocytes (MN-PCE) in blood. Comparable increases in MN-NCE and MN-PCE populations in both strains of mice following benzene exposure were seen, indicating that the difference in *Prkdc* alleles does not affect benzene-induced genotoxicity. However, male mice were significantly more susceptible to benzene-induced genotoxicity than female mice. There was no significant change in the fraction of apoptotic cells in BM or in the percentage of BM HSC from exposed mice compared to unexposed mice. RNA was isolated from BQ-treated HSC and HSC of exposed and unexposed mice for quantitative RT-PCR analysis of DNA repair genes. DNA repair pathway(s) that act on benzene-induced DNA lesions may partially explain the observed gender bias.

1110 MECHANISTIC DIFFERENCES OF BENZENE-INDUCED LEUKEMOGENESIS: GENOTOXIC IN P53-DEFICIENCY *VS.* EPIGENETIC IN THE WILD TYPE.

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The mechanistic background of benzene-induced leukemia had long been an enigma until recently, when the discovery of the peculiar cell kinetics of the stem/progenitor cells during benzene exposure was elucidated. Leukemia induction by benzene inhalation was first reported in 1887, when Le Noire described multiple cases of leukemia among Parisian cobblers. In the present study, a newly developed measure for cell-cycle of CFUs, the BUUV method, disclosed not only the underlining reason of the inconsistency but also defined the separate mechanism of leukemogenicity in wild type mice and p53 deficiency. In p53+/+ mice, p53 dependent- oscillational changes in marrow cellularity were seen during/after benzene exposure, with concurrent suppression/expression of p21. This oscillation was not observed in p53 deficient mice. cDNA micro-array examined along with the benzene exposure showed a compatible gene expressions pertinent to the cell cycle, including cyclin-dependent kinases. Taken together, our present data suggest that the leukemogenicity between mice carrying wild-type p53 and mice lacking p53 seem to differ from one another. In the case of p53 knockout mouse, DNA damage such as weak mutagenicity and or chromosomal damages are retained, and those damages participated in the induction of a consequent activation of proto-oncogenes and the like, which led cells to further neoplastic changes. In contrast, in the case of p53 knockout mice, a dramatic oscillational change in the cell cycle of the stem cell compartment seems to be an important factor for mice carrying the p53 gene.

1111 *IN VIVO* ANALYSIS OF ALACHLOR MUTAGENESIS.

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Several lines of evidence suggest that the olfactory mucosal carcinogenicity of the herbicide alachlor (2-chloro-2', 6'-diethyl-N-(methoxymethyl)acetanilide [15972-60-8]) is dependent upon target tissue bioactivation to a mutagenic metabolite. To substantiate this hypothesis *in vivo*, male Big Blue rats (F344 background, Taconic) were administered alachlor at the dietary equivalent of 126 mg/kg/d for 30, 60, or 90 days, with concurrent controls receiving powdered diet only (n=7 treated and 7

control rats per time point). Following three months of alachlor exposure, the mutant frequency (ratio of confirmed mutant plaques to total plaques screened) in the olfactory mucosa of treated rats was approximately 4 times higher than that of the concurrent control (1.3E-4 vs. 3.1E-5, p=0.002; [913, 000 plaques screened]). There was similarly a trend toward an elevated olfactory mucosal mutant fraction following two months of exposure, although statistical significance was not achieved. In contrast, nasal respiratory mucosa, which is not a target tissue for alachlor carcinogenesis and is located adjacent to the olfactory mucosa in the nasal cavity, did not display an elevated mutant frequency in alachlor-treated rats compared to concurrent (3 mo) controls. DNA sequence analysis will be performed to determine the nature of alachlor-induced olfactory mucosal mutations. These observations lend further support to the hypothesis that olfactory mucosal bioactivation to a mutagenic metabolite is pivotal to the carcinogenic mechanism in this tissue.

1112 IDENTIFICATION OF CARCINOGENS USING TRP53 HETEROZYGOUS NULL MICE AND LOSS OF HETEROZYGOSITY AT THE TRP53 LOCUS.

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Mice heterozygous for a Trp53 null and wild type allele are p53 haploinsufficient and dysfunctional for cell cycle checkpoint control and induction of apoptosis under genotoxic stress. Exposure of p53 deficient mice to mutagenic carcinogens induced neoplasia during the period in which unexposed co-isogenic haploinsufficient and homozygous wild type mice were free from neoplasia. Maximum tolerated doses (MTD) have been, in general, effective in inducing neoplasia with reduced latency after 26-week exposure to p53 haploinsufficient mice. Latency was shortened by requiring only an additional genetic alteration(s) at the functional Trp53 locus (mutation or loss of heterozygosity) and/or inactivation of another tumor suppressor gene. Based on a chemical database of published studies, an analysis was carried out to determine the predictability of this model to identify known or suspected human carcinogens (IARC Group 1 or 2) versus those least likely to be carcinogens (IARC Group 3). Under the conditions of the short-term cancer bioassays in this model in p53 deficient mice, the model showed an accuracy of 80% (47/59 Group 1/2 vs. 3 correctly predicted). Accuracy improved to 88% (23/26) if comparison was restricted to genotoxic Group 1 and 2 carcinogens. Coupling genotoxicity and induction of loss of heterozygosity (LOH) at the Trp53 locus improved accuracy to 100% (17/17). Overall, the conventional 2-year cancer bioassay in rats and mice showed an overall accuracy of 72% (26/36) and 100% (16/16) for genotoxic carcinogens (Group 1/2 vs. 3). Interspecies extrapolation between rodents and humans is difficult due to the possibility of species differences, but demonstration of an operational mechanism similar between rodents and humans, e.g. mutation or loss of p53 function through LOH may reduce uncertainity.

1113 A PROSPECTIVE CLINICAL EVALUATION OF TYPE I SENSITIZATION AND DERMAL COMPATIBILITY OF A BACILLUS SERINE PROTEASE IN A BODY LOTION.

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A protease enzyme in a prototype beauty bar has been shown to induce allergic antibody (sensitization) in clinic subjects1. Skin compatibility and potential for protease-induced sensitization from use of enzyme in leave-on body moisturizers is not understood. This study tested potential for a body lotion containing Bacillus spp. subtilisin protease, used intermittently over 18 months to induce allergic antibody, as measured by skin prick test (SPT) and serum measurement of enzyme-specific IgE. 864 healthy atopic women, 18-60 years of age from the US, Canada, Germany and France used product that contained 100ppm of protease 5 consecutive days per month, for 18 months. Regular lotion was used the remaining days of each month. Aerosol exposures during showering averaged 0.24ng/m3 of enzyme protein, measured 12 hrs after lotion application. Previous work showed that enzyme remained on the skin for 8 to 12 hours2. Skin evaluation and SPTs were conducted every 3 months. Capacitance and TEWL measures were made in a subset of subjects at 3 month intervals: skin biopsies occurred at baseline and at the first 3-month time point. The data showed increased hydration of the skin over time. Clinical evaluation and histopathology showed no skin irritation. One subject became sensitized after 6 months product use, but exposure to a cross-reacting protease in a carpet cleaner may have contributed to this response. This subject also became sensitized to a non-cross reacting protease from exposure to another carpet cleaning product. At 15 months two subjects became sensitized. These subjects did not experience any other known exposures that may have contributed to the reactions. None of the subjects had allergic symptoms. While this study showed favorable skin compatibility of the protease containing lotion, the occurrence of allergic antibody to the enzyme was unacceptable for product commercialization. 1J. All Clin. Immun. 1998 101:179-87 2 Human Exper Toxicology 1999. 18(8): 527.

1114 EVIDENCE OF ALLERGIC ANTIBODY TO A BACILLUS SERINE PROTEASE IN ATOPIC WOMEN: A RETROSPECTIVE EVALUATION.

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There has been increasing use of enzymes in consumer products. Effective safety evaluation and exposure control has minimized the risk of enzyme-induced Type I allergy in consumers. Prevalence of allergic antibody to Bacillus spp. enzymes in a clinic population of 2500 was assessed in the early 1970's after introduction of enzyme detergents1. No subject had allergic antibody to the enzymes. In the present study, subjects were tested as part of screening for clinical studies testing skin compatibility of consumer products. From 1999 to 2002, atopic women within the ages of 18 to 60 years were tested for the presence of allergic antibody to a Bacillus spp. subtilisin protease. Subsets were tested for allergic antibody to 2 other proteases and 2 Bacillus spp. amylases. Allergic antibody was detected by skin prick test (SPT) and serology for specific IgE or IgG antibody. A total of 2549 subjects were tested: 535 in France, 1009 in Germany, 279 in Canada, and 726 in the USA. Of the 2549 subjects tested, 4 subjects were confirmed SPT (+) (wheal at least 3mm greater than the negative control + flare). Two of the 4 had IgE and/or IgG antibody to the enzyme. A fifth subject was SPT (+) on initial testing but refused follow-up tests. There was no evidence of allergic symptoms from use of cleaning products. Four of the 5 did not use laundry products that contained this enzyme and there was no clear explanation as to why any of them developed antibody that recognized the protease. Earlier studies have demonstrated that use of laundry products containing similar enzymes is unlikely to cause sensitization in consumers. Two subjects were in Canada, 1 in the USA and 2 in Germany. These data indicate that there is a prevalence of sensitization to this Bacillus protease, in comparison to the previous study1. These data indicate a need for industry to continue to monitor the population to ensure expanded uses of enzymes in consumer products do not increase the risk of Type I sensitization. 1 Clin Allergy, 1973 3:143-160

1115 EVALUATION OF THE SENSITIZATION POTENTIAL OF TWO LUBRICANT ADDITIVES, PHENYL-ALPHA-NAPHTHYLAMINE AND ALKYLATED PHENYL-ALPHA-NAPHTHYLAMINE: A COMPARISON OF DATA FROM THE LOCAL LYMPH NODE ASSAY, THE BUEHLER GUINEA PIG ASSAY, AND HUMAN REPEAT INSULT PATCH TEST.

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Phenyl-alpha-naphthylamine (PAN) and alkylated phenyl-alpha-naphthylamine (APAN), two high temperature anti-oxidants commonly used in aviation turbine lubricants have been widely used for many years with relatively few associated cases of contact allergy reported. In these studies PAN and APAN were evaluated for sensitization potential and potency using the local lymph node assay (LLNA), and these results were compared with data previously generated using a modified Buehler guinea pig test and the human repeated insult patch test (HRIPT). Chemicals were tested using female CBA mice at concentrations raging from 1-100%. Both chemicals tested positive in the LLNA inducing dose responsive and statistically significant increases in lymph node cell proliferation reaching a stimulation index (SI) of ∝3. A series of non-linear regression models were applied to the data and the model which fit the data best based on likelihood ratio tests was subsequently utilized in a bootstrap analysis to obtain an uncertainty distribution around the calculated EC3 (the chemical concentration required to induce a SI of 3). Based on this analysis, the mean EC3 values and 90 % confidence bounds were 2.29% (0.01%, 6.4%) and 11.76% (4.4%, 24.6%) respectively for PAN and APAN. This data correlated positively with the guinea pig data where induction and challenge with equal concentrations of chemicals resulted in a greater percentage of animals responding and higher severity indices for PAN versus APAN. The LLNA data also correlated positively with the human data where HRIPT showed that use levels of both PAN and APAN failed to induce sensitization when applied in lubricants at concentrations of 1.5% and 2.5%, respectively, levels lower than their calculated EC3 values.

1116 FACTORS AFFECTING BINDING OF NATURAL RUBBER LATEX (NRL) PROTEINS TO GLOVE DUSTING POWDER.

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Adverse reactions to NRL proteins are caused by either topical, parenteral or respiratory exposures. NRL proteins bound to glove powder are the major source of the respiratory exposure and can cause serious reactions in frequent users of NRL

gloves. Binding of protein to glove powder has been documented in the laboratory and clinical studies. The specific factors that lead to allergen attachment and may affect the binding intensity have not been determined. We evaluated protein extracts from medical gloves with and without accompanying powder. The ratios of the amount of soluble protein in extracts and the protein remaining on powder differed among gloves tested. The ratios did not correlate with either the total protein on the glove, glove weight or total amount of powder, indicating that other factors may enhance or reduce the intensity of protein binding. To investigate the conditions that may affect the binding, we exposed several types of clean powder to NRL antigens under various conditions and evaluated protein levels on starches and respective protein solutions. Our data showed that protein binding was dose-dependent, with the saturation point at the concentrations above 0.2 mg/ml. The temperature and pH of media made no or only minor difference in the binding intensity, but marked differences were observed among the individual starch preparations. Two cross-linked corn starch powders showed a strong affinity to attach proteins, while the protein binding to cooking corn starch and oat starch was much lower. Those differences appeared more obvious if starches were exposed to NRL proteins in water than in pH-7.4 PBS. Our findings indicate that physico-chemical properties of glove dusting powder and some procedural factors may play a significant role in the protein binding affinity. With an appropriate selection of conditions and type of powder, it may be possible to reduce attachment of NRL proteins to glove powder, and consequently reduce the aeroallergen levels in hospital environments.

1117 EFFECT OF SULFUR DIOXIDE INHALATION ON THE POPULATION AND FUNCTION OF PERIPHERAL BLOOD LEUKOCYTES AND PULMONARY CELLS IN CATTLE.

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Cattle production sometimes occurs in areas adjacent to oil and gas developments with potential exposure to sulfur dioxide (SO_2) as one of the components of sour gas emissions. However, effects of known concentrations of SO_2 on cattle have not been described. In a series of experiments, 24 growing calves (steers) were sequentially exposed (head-only) to air amended to contain SO_2 concentrations from 0 to 20 ppm. Steers intermittently inhaled gases for 6 hours/day over periods of 23, 9, and 8 days (experiment one) and 10, 7, and 7 days (experiment two) at the concentrations of 1, 5 and 20 ppm of SO_2 respectively. Parallel to the experimental animals a group of 22 control calves was exposed to clean filtered air designated as 0 ppm. The data obtained from experimental and control groups of steers were compared by analysis of variance to determine the effect of SO_2 on the immune function of cattle. Several immunological parameters were altered by exposure steers to various concentrations of SO_2. The CD4/CD8 ratio of T lymphocytes ($P < 0.05$) was reduced suggesting potential impairment of cell-mediated immunity. The ratio of degenerative to healthy pulmonary cells increased ($P<0.05$) in steers exposed to SO_2 gas. The number of CD14 cells (marker for monocytes) in blood increased perhaps in response to cytokine signals arising from the damaged pulmonary cells. Changes in nitric oxide (NO) production by alveolar macrophages suggesting that SO_2 gas affected their phagocytic activity. Our findings suggest that SO_2, at a concentration as low as 1 ppm, had deleterious effects on cellular functions. The altered immune responses may have implications for production efficiency and health of cattle exposed to air polluted with SO_2. In conclusion, cattle are sensitive to sulfur dioxide as previously shown for humans and other animal species.

1118 ENDOTOXIN AND ALLERGY: LPS IS A STRONG ADJUVANT FOR CAT ALLERGEN Fel d 1-SPECIFIC IgE RESPONSE IN A MOUSE SUBCUTANEOUS IMMUNIZATION MODEL.

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The question whether LPS is beneficial or disease-promoting in relation to asthma and allergy has been raised in several recent epidemiological studies. We asked whether LPS would increase the antibody response and give an allergic (IgE), or non-allergic (IgG2a) antibody profile after immunization with the cat allergen Fel d 1 in a mouse model. Also, when LPS and Fel d 1 are introduced to the immune system, we asked whether the timing of the two agents relative to each other is crucial. Mice were injected subcutaneously with LPS and/or Fel d 1 four times in various temporal combinations. IgE, IgG1 and IgG2a antibodies specific to Fel d 1 were measured in serum using ELISA. A greatly increased specific antibody response was observed, both for IgE, IgG1 and IgG2a, when LPS was introduced to the immune system together with the cat allergen Fel d 1, and in particular after repeated injections. No such adjuvant effect was observed when LPS was introduced alone prior to or subsequent to the allergen in the present subcutaneous immunization model. The resulting antibody response was not polarized in terms of Th1- or Th2-dependence. The adjuvant effect from endotoxin for the supposedly Th2-dependent Fel d 1-specific IgE response was strong. The so-called 'hygiene hypothesis' postulates that microbial agents protect against the development of allergy, and that the increase in allergy observed in many countries is caused by reduced exposure to microbes or microbe-derived substances such as endotoxin in the home environment. The present results do not support the 'hygiene hypothesis'.

1119 RESPIRATORY ALLERGY ASSAY IN BROWN-NORWAY RATS EXPOSED TO DIPHENYLMETHANE-4, 4'-DIISOCYANATE (MDI).

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A rat bioassay has been developed for the identification and classification of respiratory allergy using trimellitic anhydride (TMA) and MDI. Emphasis was placed to study route-of-induction dependent effects and their progression upon inhalation challenge with the chemical, that included the analysis of specific and non-specific airway hyper-reactivity and pulmonary inflam-mation. Refinement of the bioassay focused on procedures to probe for changes occurring upon challenge with TMA or MDI and one day later with methacholine aerosols using physiological (Penh) and bronchoalveolar lavage endpoints, and the weights of bronchial (BLN) and auricular lymph nodes (ALN). Rats were either sensitized epicutaneously (area treated/rat: 10 cm2; TMA: day 0: 150 ul vehicle/50% TMA on each flank, day 7: booster administration to the skin of the dorsum of both ears using half the concen-tration and volume used on day 0; MDI: days 0/7: 100 ul/50 ul neat MDI as described for TMA) or by 5 x 3 hrs/day inhalation exposures (TMA: 25 and 120 mg/m3; MDI: 2 and 30 mg/m3). Following challenge with TMA, the rats sensitized to TMA elaborated marked changes in physiological endpoints and sustained pulmonary inflammation. Lung weights were significantly increased as were the weights of BLN following inhalation induction and ALN following topical induction. MDI challenged rats displayed changes in Penh when sensitized at 30 mg/m3 by inhalation. The weights of lungs and BLNs were sligthly increased in rats sensitized epicutaneously or at 30 mg/m3 whilst the ALNs were increased only following dermal induction. In summary, the findings support the conclusion that the BN rat model is suitable to identify TMA as agent causing both an immediate-type change of breathing patterns and a delayed-type, sustained pulmonary inflammatory response. The marked effects observed in rats topically sensitized to TMA could not be duplicated with MDI. It appears that endpoints originating from extrapulmonary tissues are poor substitutes for changes occurring at the pulmonary level.

1120 EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IMPAIRS MAMMARY GLAND DIFFERENTIATION IN PREGNANT C57BL/6 MICE AND PREVENTS PUP SURVIVAL.

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While conducting experiments designed to test the immune response of mice exposed to TCDD *in utero*, we observed that pups born to dams exposed to TCDD during pregnancy all died within 24 hours of birth. This was despite the fact that the amount of TCDD given (5 ug/kg/day on gestational day 0, 7, and 14) was below that reported to cause fetal mortality. There was no gross cleft palate or severe hydronephrosis in the exposed pups. Furthermore, in a preliminary experiment, pups from a TCDD-treated dam survived when moved to a vehicle-treated dam, while pups in the converse foster did not survive. This suggested that lactation in the TCDD treated dams may be impaired. The present study was therefore conducted to test the hypothesis that mammary gland differentiation in pregnant mice is impaired by TCDD exposure. Impregnated C57Bl/6 mice were treated with vehicle or TCDD as described above. Dams (n=7/group) were sacrificed on the day of parturition, and mammary glands and plasma were collected. Glands were weighed and whole mounts of the tissue were scored, in blind, based on gland development. Glands from TCDD-treated mice were substantially less differentiated than controls, with only 10 - 70% of the branched ductal structures exhibiting lobulo-alveoli. Lobulo alveoli which did develop in the TCDD-treated mice were small and poorly formed relative to controls. Gland weights were reduced by 25% in TCDD-treated mice. Serum prolactin levels were determined to address a potential mechanism for the suppressed gland development. Prolactin levels in the TCDD-treated mice were 50% lower than in vehicle treated mice. These results demonstrate that

mammary gland differentiation is impaired in pregnant mice treated with TCDD. This novel finding suggests that exposure to TCDD may impair milk production, which could account for neonatal pup mortality.

1121 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) INHIBITS PROSTATIC EPITHELIAL BUD FORMATION IN THE UROGENITAL SINUS (UGS) OF C57BL/6J MICE WITHOUT INHIBITING ANDROGEN SIGNALING.

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In utero and lactational TCDD exposure reduces ventral, dorsolateral, and anterior prostate weights. To elucidate the underlying mechanisms we examined the initial step in prostate development, epithelial budding from the UGS. Budding is an androgen-dependent process that TCDD inhibits both *in vivo* and *in vitro*. We tested the hypothesis that TCDD inhibits prostatic epithelial bud formation by inhibiting androgen signaling. Prostatic epithelial buds developed *in vitro* when gestation day (GD) 14 UGS was cultured for 5 days with 10^{-8} M 5α-dihydrotestosterone (DHT). DHT-dependent budding was inhibited by 10^{-5} M OH-flutamide, an androgen receptor (AR) antagonist, and by 10^{-9} M TCDD. Androgens are known to stimulate epithelial budding by binding to AR in mesenchymal cells. To determine whether TCDD inhibits the androgen signaling pathway, primary mesenchymal cells were prepared from GD 14 UGSs cultured for 3 days with 10^{-8} M DHT and either 0.1% DMSO or 10^{-9} M TCDD. Mesenchymal cells were transiently transfected with an androgen-responsive luciferase reporter, then treated with 0.1% EtOH (vehicle), 10^{-8} M DHT, 10^{-8} M DHT + 10^{-5} M OH-flutamide, or 10^{-8} M DHT + 10^{-9} M TCDD. In cells isolated from both DMSO- and TCDD-exposed UGS, 1) DHT increased luciferase activity about five-fold in comparison to vehicle-exposed cells, 2) OH-flutamide severely inhibited DHT-dependent luciferase activity, and 3) TCDD did not inhibit DHT-dependent luciferase activity. Androgen-dependent gene expression was also analyzed by real-time RT-PCR quantitation of mRNA for AR and 5α-reductase-type II. GD 14 UGSs were cultured for 3 days with 0.1% EtOH, 10^{-8} M DHT, 10^{-8} M DHT + 10^{-5} M OH-flutamide, or 10^{-8} M DHT + 10^{-9} M TCDD. OH-flutamide completely blocked the effect of DHT on both genes, whereas TCDD had no effect on mRNA expression for either. In conclusion, the inhibition of UGS prostatic epithelial bud formation by TCDD does not appear to result from an inhibition of androgen signaling. (Supported by NIH Grant ES 01332)

1122 EFFECT OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON THE IMMUNOLOGICAL STATUS OF C57BL/6 PREGNANT MICE.

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Whether transient immunosuppression seen during pregnancy when combined with exposure to environmental immunotoxicants could exacerbate immunosuppression leading to deleterious effects on maternal health has not been previously investigated. Therefore, the current study evaluated the immune status of C57BL/6 pregnant mice following exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). To this end, C57BL/6 pregnant (gestational day=14) and virgin female mice were injected with a single dose of 10 μg/kg of TCDD by the intraperitoneal route. TCDD treatment caused an early immunotoxic effect in the thymus of pregnant mice with the cellularity decreasing at 48 hr of exposure, but apparent in virgin mice only after 72hr. TCDD treatment also caused more marked alterations in the thymic T cell subpopulations of pregnant mice when compared to the virgin mice, with a decrease in double-positive T cells ($CD4^+CD8^+$) and an increase in single-positive ($CD4^+$, $CD8^+$) and double-negative ($CD4^-CD8^-$) T cells. Also, the thymic but not the splenic T cell proliferative responses to mitogens were more dramatically altered in TCDD-treated pregnant mice when compared to the virgin mice. Flow cytometric analysis demonstrated that no significant changes in apoptosis and the expression of CD4, CD8, B220, NK1.1 and Mac 3 markers were observed in splenocytes from dioxin-treated virgin and pregnant mice. Immunization of mice with Staphyloccocus enterotoxin A (SEA) caused a similar immunotoxic response in dioxin-treated pregnant and virgin mice with decreased draining LN cellularity, lower percentages of SEA-responsive Vβ3+ and Vβ11+ T cells and a reduced *in vitro* proliferative response to the recall antigen. Together, our findings indicate that pregnancy augments the sensitivity to dioxin-induced immunotoxicity in the thymus, but not in secondary lymphoid organs. (Supported by NIH grants R01ES09098, F31ES11562, R21DA014885 and R01HL058641)

1123 THE EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN ON TRIGGERED- AFTERDEPOLARIZATIONS IN ISOLATED RAT VENTRICULAR MYOCYTES.

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Dioxin TCDD (2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin) exposure can cause immune system dysfunction, carcinogenesis and cardiotoxicity. The effects of TCDD on action potentials and afterdepolarizations were studied in 132 rat ventricular myocytes using Nystatin-perforated patch-clamp techniques. The animal protocol was approved by Animal Care and Use Committee of the University of South Carolina (Protocol# 1091). The action potential duration measured at 90% of repolarization (APD90) was significantly prolonged by TCDD treatment. The effects of TCDD were concentration-dependent (1 to 100 nM) and time-independent (5 to 30 min). The transpiring frequency of triggered delayed-afterdepolarizations (DADs) was increased from 12.5% (1/8 cells) in control to 75% (6/8 cells, $P<0.01$) after TCDD exposure. In the presence of either isoproterenol (Iso, 10 nM) or Kay K 8644 (1 uM), TCDD (10 nM) markedly augmented the amplitude and frequency of the triggered-DADs and initiated sustained-spontaneous action potentials in majority of the experiments. Corresponding to the increase of DADs, TCDD (10 nM) significantly enhanced the transient inward current (Iti) recorded immediately after the end of a +40 mV depolarizing pulse. In the presence of Iso or Bay K 8644, triggered-early afterdepolarizations (EADs) were observed only in cells co-exposed to TCDD (10 nM). These results indicate that TCDD itself can prolong action potential duration and cause abnormal triggered-afterdepolarizations. These effects of TCDD may lead to clinical cardiac arrhythmia especially when the susceptible subjects are stressed by elevated sympathetic activity or suffering from other cardiomyopathies coincided with Ca2+ -overload.

1124 DIOXIN INDUCES GROWTH ARREST AND REDUCES CELL CYCLE GENE EXPRESSION IN THE FETAL MURINE HEART.

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Although one of the earliest and most sensitive effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in the avian embryo is cardiomyocyte growth arrest (Teratology 64:201 2001), little is known about TCDDs effects on the fetal murine heart. Our objectives were to determine whether TCDD induces similar effects in the fetal murine heart and whether growth arrest is associated with changes in cell cycle gene expression. Pregnant C57Bl6N mice were dosed by oral gavage with 0 (corn oil), 1.5, 3, 6, 12, or 24 ug/kg TCDD on gestation day (GD) 14.5 (n=6-16 litters/Tx). On GD17.5 fetal hearts were fixed or frozen. From the 0-6 ug/kg groups, fixed hearts were sectioned and stained with anti-proliferating cell nuclear antigen (PCNA), while RNA was isolated from frozen hearts and analyzed using microarrays. Intensity values were scaled by Affymetrix Microarray Suite 5 and normalized by GeneSpring. Fold changes in gene expression were determined using GeneSpring and dose-related changes by ANOVA ($p<0.05$). Although no maternal toxicity or fetal mortality was observed in TCDD-treated animals, dioxin treatment decreased fetal heart-to-body weight ratio in a dose-dependent manner ($p<0.008$) with a maximal reduction of 14 ± 3%, relative to controls. Of the 1.5-6 ug/kg doses analyzed, only 6 ug/kg significantly reduced the percentage of proliferating cardiomyocytes as assessed by PCNA staining (control, 26 ± 4%, 6 ug/kg, 5 ± 1%; $p<0.001$). Growth arrest was associated with reduced expression of genes involved in cardiomyocyte proliferation. TCDD induced dose-related reductions in G1/S-related, CDK2, cyclin A1; and G2/M-related, Cdc2, Cdc25C, cyclin B1. Ongoing efforts have focused on cluster analysis to determine patterns of gene expression and identify genomic networks that mediate premature cardiomyocyte growth arrest induced by TCDD. (Supported by ES09804 and 10433 to MKW and ES09106 to KSR).

1125 TCDD-INDUCED CHANGES IN GENE EXPRESSION PROFILES IN DEVELOPING PAWS OF 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-tetrachlorodibenzo-p-dioxin (TCDD). These compounds are potent reproductive and developmental toxicants due to their ability to modulate gene expression and cellular differentiation/proliferation. Previous studies have identified developing paws as a putative target in embryos exposed to TCDD. In characterizing this

response, gene expression profiles by microarray genechip technology was employed. Based on preliminary studies in the developing paws, we hypothesize that TCDD induces changes in genes involved in development, cell cycle regulation and mesenchymal epithelial interactions. Pregnant C57Bl/6 mice were injected with TCDD (30 μg/kg, i.p.) on gestational day 14, and fore and hind paws collected from embryos 24 hours later. Comparisons of relative gene expression profiles were conducted between vehicle control and TCDD exposed embryos, fore and hind paws, and embryo uterine position. Genes involved in development, cell cycle regulation (i.e. apoptosis, proliferation and differentiation), and mesenchymal epithelial interactions were closely monitored. AhR responsive genes were examined and used as internal experimental controls. Microarray analysis, using GeneSpring software, identified TCDD altered expression of genes involved with development, e.g. hox genes, apoptosis, e.g. caspase 14, and mesenchymal epithelial interactions, e.g. fibroblast growth factor binding protein. AhR responsive genes, Cyp1a1 and Cyp1b1, were induced in all TCDD exposed embryos. These results indicate that TCDD-induced changes in gene expression during development may cause alterations in paw development. These findings are consistent with those in an AhR responsive reporter gene transgenic model and provide further evidence of developing paws as a target of TCDD. (Funded by NIH grant ES09430, Training grant ES07026, and Center grant ES01247).

1126 EFFECT OF TCDD AND RETINOIC ACID ON MATRIX METALLOPROTEINASE EXPRESSION IN NORMAL HUMAN KERATINOCYTES.

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Exposure to the environmental contaminant 2, 3, 7, 8-tetrachlordibenzo-p-dioxin (TCDD) results in a number of pathological lesions that require matrix metabolism and tissue remodeling, including prostate and mammary tubule morphogenesis, palatal development, and tumor promotion. We have found that TCDD exposure of normal human keratinocytes results in increased expression and activity of several matrix metalloproteinases (MMPs). MMPs degrade the protein components of the extracellular matrix and basement membrane, and their activity is necessary for physiological processes that require tissue remodeling and cell migration. Our hypothesis is that TCDD mediates some of its biological effects by directly altering the expression of genes involved in matrix metabolism and cellular migration as well as indirectly through interactions with the retinoic acid (RA) signaling pathway. Retinoids are powerful regulators of cell growth and differentiation and are widely used in the prevention and treatment of a variety of cancers in humans. RA binds to two types of nuclear receptors, the retinoic acid receptors (RARs) and retinoic x receptors (RXRs). Using quantitative real time RT-PCR, we found that TCDD exposure of normal human keratinocytes results in increased mRNA levels of RARγ and RXRα, the predominant retinoid receptors expressed in skin. Northern analysis of mRNA isolated from TCDD, all-trans RA (atRA) and TCDD + atRA show that TCDD-induced MMP mRNA expression is further increased upon co-treatment with all-trans retinoic acid. Transient transfections using constructs containing up to 4000 bp of the MMP-1 promoter linked to the luciferase reporter suggest that TCDD and atRA induction of MMP-1 is mediated, at least in part, through transcriptional activation. Further, we find that different regions of the 5′ flanking sequences are involved in mediating TCDD and atRA activation. These data suggest that interactions between these two pathways may result in aberrant matrix remodeling and contribute to TCDD-induced phenotypes.

1127 LACTATIONAL NOT *IN UTERO* EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN DISRUPTS THYROID HORMONE HOMEOSTASIS IN HOLTZMAN RATS.

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We have previously reported that even a single oral administration of low dose of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) given on gestational day 15 (GD15) caused a reduction in the serum thyroxin (T4) level in pups on postnatal days (PND)21, followed by the occurrence of thyroid hyperplasia on PND49 in Holtzman rats. Using arylhydrocarbon receptor (AhR) null mice, we have also confirmed that the T4 suppressing effect of TCDD is mediated by AhR and is due to the enhanced biliary excretion of T4-glucuronide, which is conjugated by UDP-glucuronosyltransferase (UGT)-1, a TCDD inducible enzyme. The present study was conducted to clarify a relative impact of lactational exposure on the disruption of thyroid hormone homeostasis in pups compared to *in utero* exposure by using cross-fostering protocol. Pregnant Holtzman rats were given an oral dose of 1000 ng TCDD/kg bw on GD15 or an equivalent volume of corn oil as vehicle-control. Half of the TCDD-treated litters and half of the control litters were cross-fostered on PND1, which turned out to comprise the following groups: C/C (control), T/T (perinatal exposure), T/C (prenatal exposure only), and C/T (postnatal exposure only). Male and female pups were sacrificed on PND21 (at weaning) and sera and tissues were collected. We found that serum total T4 levels of male and female pups were significantly decreased in the C/T and T/T group but not in the T/C group compared to the C/C group on PND21 (n=4-6). In addition, mRNA levels of UGT1A6, UGT1A7 and cytochrome P450 (CYP) 1A1 in the liver were highly elevated in the C/T and T/T group, but not in the T/C group compared to the C/C group. An induction of UGT1A6 and CYP1A1 in the liver of the C/T and T/T groups but only a trace in the T/C group was visualized immunohistochemically, supporting the molecular biological data on PND21. The present results led us to conclude that TCDD-induced disruption of thyroid hormone homeostasis observed in pups were mainly due to lactational exposure.

1128 SPECIES-SPECIFIC TRANSCRIPTIONAL ACTIVITY OF SYNTHETIC FLAVONOIDS IN GUINEA PIG AND MOUSE CELLS AS A RESULT OF DIFFERENTIAL INTERACTION OF ARYL HYDROCARBON RECEPTORS WITH DIOXIN RESPONSIVE ELEMENTS.

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To investigate possible species-specificity of aryl hydrocarbon receptor (AhR)-mediated signal transduction pathways, activities of TCDD and six synthetic flavonoids were evaluated in mouse hepatoma and guinea pig adenocarcinoma cells transfected with an AhR-responsive luciferase reporter. These investigations demonstrated similar rank order potency for the ability of these flavonoids to antagonize the TCDD-induced reporter gene expression in both cell lines. However, in the presence of the flavone alone, a species-specific difference in agonist activity was observed. In guinea pig cells, several flavonoids demonstrated agonist activity up to 50% of the maximum TCDD response. In mouse cells, however, no significant agonist activity was observed at the same concentrations based on luciferase enzyme activity, protein expression, and mRNA analysis. Moreover, the competitive ligand-binding assay, using [^{3}H]-TCDD in cytosolic fractions, demonstrated that 3'-methoxy-4'-nitroflavone had a similar IC_{50} in both recombinant cell lines, suggesting that the flavone has similar binding affinity to receptors from both species. However, electrophoretic mobility shift assay using the cytosolic fractions demonstrated that this flavone elicited binding to the DRE by guinea pig but not mouse AhR complex. The AhR's dependency in this differential interaction was further demonstrated using *in vitro* synthesized receptor and Arnt. Together these data suggest that the species-specific agonist/antagonist activity of these flavone derivatives is due to the efficacy of these flavonoids to elicit, in a species-specific manner, an AhR conformation that recognizes regulatory response elements. (Supported by NIH grant ES09702 and Center grant ES01247)

1129 EFFECTS OF THE DOSE OF DIMETHYLARSINIC ACID (DMA) ON THE URINARY CONCENTRATION OF DIMETHYLARSINOUS ACID (DMA^{III}).

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Long-term administration of DMA in the diet causes an increase in hyperplastic lesions and tumors of the rat urinary bladder epithelium at doses of 40 and 100 ppm. There is no effect on the urinary bladder at 2 and 10 ppm. The female rat appears to be more sensitive to the effects of DMA than the male. Long-term treatment of male rats with similar doses of DMA in the drinking water is also carcinogenic to the urinary bladder. In short term studies, a dietary dose of 100 ppm DMA rapidly produces urothelial cytotoxicity followed by a regenerative hyperplasia. We have determined that the reactive intermediate DMA^{III} is present in the urine of DMA-treated rats at micromolar concentrations as early as 24 hr after the start of treatment. *In vitro* studies in our lab have shown that DMA^{III} is cytotoxic to rat (MYP3) and human (1T1) cells at 0.5-0.8 μM concentrations. To determine if the urinary concentration of DMA^{III} is affected by the dose of dietary DMA, groups of 7 female 5-week old Fischer F344 rats were treated with 0, 2, 10, 40, or 100 ppm DMA for 4 wk. Fresh void urine was collected during treatment weeks 2 and 3 and analyzed by ion pair chromatography with hydride generation atomic fluorescence (HPLC-HGAFD) detection. The results of the analyses for DMA^{III} are as follows: Wk 2, 2 ppm-0.02±0.00 μM; 10 ppm-0.05±0.01 μM; 40 ppm-0.25±0.07 μM; 100 ppm-0.92±0.15 μM. Wk 3, 2 ppm-0.03±0.01 μM; 10 ppm-0.12±0.02 μM; 40 ppm-0.28±0.09 μM; 100 ppm-0.55±0.15 μM. These results show that the concentration of urinary DMA^{III} is affected by the concentration of dietary DMA in a dose-responsive manner. Also, the concentration of urinary DMA^{III} in the 40 and 100 ppm DMA groups is similar to or above the concentrations which cause cytotoxicity *in vitro* while the urinary concentration of DMA^{III} at the no-effect doses of 2 and 10 ppm is well below the concentrations which cause *in vitro* cytotoxicity.

1130 PERSISTENT INDUCTION OF HEPATIC AND PULMONARY PHASE II ENZYME EXPRESSION BY THE CARCINOGEN 3-METHYLCHOLANTHRENE IN THE RAT.

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Cytochromes P450 (CYP)1A1 and 1A2 enzymes play pivotal roles in the bioactivation of chemical carcinogens. We reported earlier that exposure of rats to 3-methylcholanthrene (MC) leads to persistent induction of hepatic CYP1A1/1A2 enzymes for up to 45 days after MC withdrawal. CYP1A1/1A2 and phase II enzymes of drug metabolism such as glutathione S-transferase (GST-α), NAD(P)H quinone reductase (NQO1), aldehyde dehydrogenase (ADH), and UDP glucoronosyl transferase (UDPGT) are members of the *Ah* battery and are regulated by the Ah receptor (AHR). In this investigation, we tested the hypothesis that MC treatment of rats would lead to persistent induction of these enzymes, but not epoxide hydrolase (EH), which is not a member of *Ah* battery. Adult female Sprague-Dawley rats were treated with MC (93 μmol/kg) once daily for 4 days and sacrificed at 1, 15, or 28 days after MC withdrawal. Hepatic and pulmonary expression of the AHR and phase II enzymes was studied. MC elicited increases in hepatic and pulmonary expression of the AHR mRNA at 1 day, but the induction declined by day 15. On the other hand, MC caused persistent induction of hepatic GST-α, NQO1, and EH activities for up to 15 days, with induction declining by day 28. In contrast, MC-mediated induction of ADH and UDPGT activities persisted for 28 days. In the lung, MC elicited sustained induction of ADH, NQO1, and EH, but not GST-α and UDPGT, for up to 15 days. Western blot experiments yielded data that correlated with those of enzymatic activities. Taken together, our findings indicate that MC differentially regulates the expression of hepatic and pulmonary phase II enzymes, and that AHR-independent mechanisms may have contributed to the sustained induction of these enzymes by MC. Since phase II enzymes play important roles in the detoxification of reactive electrophiles to non-carcinogenic metabolites, persistent induction of these enzymes in humans could alleviate cancer risk in individuals exposed to environmental chemicals. (Supported in part by a NIEHS grant 09132 to BM.)

1131 *IN VITRO* CHARACTERIZATION OF A RECOMBINANT ADENOVIRUS EXPRESSING UDP-GLUCURONOSYL TRANSFERASE 1A7.

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UDP-glucuronosyl transferase 1A7 (UGT1A7) is a phase II enzyme that usually results in increased elimination of xenobiotic substances and drugs, thereby reducing toxicity. UGT1A7 is expressed at low basal levels in the liver but is easily induced by several agents, including oltipraz, a dithiole thione, and 3-methylcholantrene, a polycylic aromatic hydrocarbon (PAH). Work in our laboratory has demonstrated that UGT1A7 has an *in vitro* substrate specificity for bulky planar and non-planar phenols, such as benzo[a]pyrene (BaP) metabolites. Therefore UGT1A7 may play an important role *in vivo* in the elimination of BaP, a PAH, thus reducing the carcinogenic effects associated with BaP exposure. A recombinant adenovirus expressing rat UGT1A7 under control of a cytomegalovirus promoter (Ad-CMV-r1A7) was developed to aid in the determination of the role of UGT1A7 *in vivo*. Western blot analysis of primary rat hepatocytes from a strain of rat devoid of UGT1A expression (Gunn) infected with Ad-CMV-r1A7 resulted in detectable UGT1A7 levels *in vitro*. Expression of UGT1A7 increased as a function of time (24-72 hours) and multiplicity of infection (MOI 0-4.0). Infection of Gunn rat hepatocytes with a β-galactosidase expressing adenovirus (Ad-CMV-βgal) served as the control. Furthermore, media analyzed by high performance liquid chormatography from Gunn rat hepatocytes infected with Ad-CMV-r1A7 and treated with acetaminophen (APAP), a substrate of UGT1A7, was found to have increased amounts of APAP-glucuronide formed as compared to control. It is concluded that Ad-CMV-r1A7 encodes for functional UGT1A7 in a time and MOI-dependent manner. Further utilization of Ad-CMV-r1A7 will allow for characterization of UGT1A7 in an *in vivo* model.(Supported by 1R01ES07763 from the National Institute of Environmental Health Sciences.)

1132 REGIOSPECIFICITY AND GLUCURONIDATING ACTIVITIES OF MAJOR RAT LIVER UGT1A FORMS TOWARD BENZO(a)PYRENE METABOLITES.

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Benzo[a]pyrene (B[a]P), a prototype polycyclic aromatic hydrocarbon (PAH) and procarcinogen, undergoes extensive metabolism to form various phenol, quinone, and diol/tetrol metabolites. These metabolites are thought to be prevented from undergoing further bioactivation by UGT-catalyzed glucuronidation. UGT1A7, a low constitutive but PAH- and oltipraz-inducible UGT in rat liver, was reported previously by our laboratory to be highly active towards B[a]P metabolites. The purpose of this study was to compare the regiospecificities and activities of UGT1A7 and the three other major UGT1 isoforms expressed in Sprague-Dawley rat liver, UGT1A1, UGT1A5, and UGT1A6, towards a panel of 14 B[a]P metabolites. Complementary DNAs coding for UGTs were expressed in HEK293 cells, and crude membrane fractions were tested for activity at 100 μM B[a]P metabolite and 1.5 mM UDP-[^{14}C]glucuronic acid. Relative glucuronidating activities were determined by normalizing the data for the level of UGT1A determined by immunoblot analysis. Of the four forms tested, UGT1A7 showed the broadest specificity and highest overall activities with 13 out of 14 substrates glucuronidated at rates $\rangle$ 0.2 nmol/mg/min (1-, 2-, 3-, 4-, 5, 6, 7, 9, 10, 11, and 12- and the 4, 5- and 7, 8-dihydrodiols). UGT1A1, the major bilirubin UGT, was the only other form with significant activities. Eleven out of 14 substrates were glucuronidated by UGT1A1 at rates $\rangle$ 0.2 nmol/mg/min) including the 3- and 9-monophenols and B[a]P-4, 5-dihydrodiol. Surprisingly, UGT1A6, a well known PAH-responsive isoform, was active towards only two minor metabolites (5- and 12-monophenols). Immunoblot analysis revealed high constitutive expression of UGT1A1 in rat liver that was not further induced by either PAHs or oltipraz. These findings suggest that UGT1A1 may be a principal constitutive UGT contributing to the detoxification and elimination of B[a]P metabolites. (Supported by 1R01ES07763 from the National Institute of Environmental Health Sciences).

1133 GLUTATHIONE REDOX POTENTIAL ALTERS CARCINOGEN METABOLISM ENZYME EXPRESSION AS MEASURED BY SELDI ANALYSIS OF TRANSCRIPTIONAL PROTEINS.

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Toxicity of chemical carcinogens is dependent on expression of bioactivation and detoxification enzymes. Carcinogen metabolism and exposure to oxidative stress alters the intracellular levels of glutathione (GSH) and cysteine, which shifts the intracellular redox potential and may affect transcriptional regulation of carcinogen metabolizing enzymes. Treatment of human cell lines with N-acetyl-L-cysteine or buthionine sulfoximine reduces or oxidizes the GSH pool, respectively, and quantitative PCR indicates changes in mRNA levels of both the activating enzyme cytochrome P450 1A1 (CYP1A1) and the detoxification enzyme NAD(P)H:quinone oxidoreductase (NQO1). To determine whether these changes are due to effects on transcriptional protein interactions with DNA response elements, a strategy was developed to detect proteins bound to DNA-regulatory elements covalently coupled to an epoxide protein chip array. Protein binding to the oligonucleotide sequence is determined by surface-enhanced laser desorption/ionization (SELDI-MS). To test this technique, recombinant human c-jun was expressed in E. coli BL21:DE3 and incubated on the chip surface containing the bound activator protein 1 (AP-1) oligonucleotide sequence. SELDI analysis demonstrated that 42 pmoles c-jun (35.5 kDa) saturated 250 pmoles of AP-1 oligomer. An increase in c-jun from nuclear extracts of phorbol ester-treated HeLa and HT29 cell lines was readily detected. Regulation of NQO1expression is partially controlled by the antioxidant response element containing an AP-1 consensus sequence and is responsive to xenobiotics, antioxidants, oxidants, and environmental stresses resulting in coordinate induction of many detoxification enzymes. Similarly, CYP1A1 is regulated by protein interactions with a xenobiotic response element also affected by oxidative stress. SELDI technology can provide a powerful means to investigate protein-DNA interactions in response to induction of competing pathways for carcinogen biotransformation. (Support: RCMI RR03032; MBRS/GM-028248).

1134 ALTERED METHYLATION AS A POSSIBLE BIOMARKER OF TOXICITY.

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DNA methylation (ME) is an epigenetic mechanism that plays an important role in mammalian transcriptional regulation. Aberrant patterns of ME are implicated in carcinogenesis and in certain neurological, immune and developmental disorders. To determine if altered ME is a precursor to the toxic effects of a variety of chemicals, and if understanding ME changes might provide an enhanced assessment of the toxic potential of a chemical, preliminary experiments were conducted on the effects of model toxic compounds on global and GC-specific ME in H4IIE rat hepatoma cells. Results were compared to those generated from a "Tox Cluster" battery of *in vitro* assays (cell number, 3-(4, 5-dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) for mitochondrial function, glutathione-S-transferase

leakage for cell membrane integrity, and ATP levels). Model compounds include: 5-aza-2'deoxycytitine (dAzaC), a demethylating agent; rotenone, an inhibitor of oxidative phosphorylation; staurosporine, a PKC inhibitor; and camptothecin, which causes double strand DNA breaks. Cells were treated with several doses of each compound for 24 or 72 h. Global levels of ME were decreased and GC-rich ME were altered by non-toxic doses of dAzaC. Toxic doses of camptothecin and rotenone did not alter levels of global ME at 72 h, possibly because the acute lethality of these compounds preceded any effect on ME. In contrast, 24 h treatment of a toxic dose of staurosporine led to global hypomethylation. Studies are currently being performed to more thoroughly determine dose-response relationships so that alterations in ME may be closely matched to doses shown to be toxic and non-toxic. The preliminary findings regarding dAzaC- and staurosporine-induced altered ME indicate that the incorporation of a ME assessment into toxicity testing protocols may provide a more complete picture of the toxic potential of the compound of interest and might substitute for some traditional parameters.

1135 EFFECT OF BROMODICHLOROMETHANE AND DIBROMOACETIC ACID ON THE METHYLATION OF DNA AND THE IGF-II GENE IN MICE AND RATS.

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The disinfection by-products (DBPs) are a diverse group of chemicals found in chlorine treated drinking water of which the trihalomethanes (THM) and haloacetic acids (HAA) are found in highest concentration. Some THM and HAA have demonstrated carcinogenic activity in the liver and kidney of rodents. DNA hypomethylation, demonstrated by a general decrease in the 5-methylcytosine (5-MeC) content of DNA, is an early event in carcinogenesis and an epigenetic mechanism for chemical carcinogens, including the THM and HAA. The ability of the THM, bromodichloromethane (BDCM) and the HAA, dibromoacetic acid (DBA) to cause DNA hypomethylation was determined in the liver and kidney. BDCM was administered in the drinking water and by oral gavage to female B6C3F1 mice and male F344 rats. DBA was administered in the drinking water to male and female mice and rats. DNA methylation was determined by high-performance liquid chromatography (HPLC), by Dot-blot analysis using a monoclonal antibody specific for 5-MeC and by bisulfite treatment of DNA followed by PCR and sequencing to determine the methylation of the insulin-like growth factor-2 (IGF-II) gene. BDCM (50-100 mg/kg by gavage and 350-700 mg/L) and DBA (1000-2000 mg/L) decreased the 5-MeC content in liver and kidney DNA by 25-46% in both mice and rats. In normal mouse liver and kidney, the region of the IGF-II gene evaluated had 14-20 of 24 CpG sites methylated. DBCM and DBA reduced the number of CpG sites that were methylated to 0-8. Hypomethylation was found after exposure to BDCM or DBA for 4 to 5 days and was maintained for 28 days. Thus, the two DBPs caused DNA hypomethylation that is consistent for an epigenetic mechanism of carcinogenesis. Supported in part by grant 1R03 ES10537 from NIEHS.

1136 CHROMATE INDUCES THE HGPRT GENE SILENCING BY DNA METHYLATION: A NEW EPIGENETIC MECHANISM FOR CHROMATE CARCINOGENESIS.

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Hexvalent chromate (Cr 6+), recognized as a carcinogen by IARC, has widespread exposure both occupationally and to the general public. Epidemiological studies showed that Cr6+ compounds could induce respiratory tumors following inhalation exposure. Although a large number of studies focused on the mechanism of Cr 6+ carcinogenesis, it still remains a topic for intense research investigation. In this study, the human osteoblastic cell line (HOS) was exposed to Cr 6+, selected by 6-thioguanine (6-TG), and the HGPRT gene expression was measured by Northern blot. The results showed that the number of 6-TG resistant colonies was 7 fold higher in the cells treated with Cr 6+ 8 times than those treated only once. There was a dose-related increase in the 6-TG resistant frequency and 90% of 6-TG resistant colonies expressed the HGPRT gene when treated once. However, only 20% of the colonies expressed this gene when treated 8 times. Furthermore, when those 6-TG resistant colonies which lacked the HGPRT gene expression were treated with 5-Aza-2-deoxyctidine, then selected in HAT medium, the expression of HGPRT gene was turned on again. Additonally numerous methylation sites were found in those resistant colonies by sodium bisulfite treatment followed by sequencing. Our results demonstrate that Cr 6+ induces a high 6-TG resistant frequency and HGPRT gene silencing in HOS cells and such gene silencing is mediated by DNA methylation. Supported by grants ES 05512, ES 10344, ES 0260 and CA 16087 from NIH.

1137 THE ANALYSIS OF TRANSCRIPTIONAL REGULATION OF NICKEL-INDUCIBLE GENE CAP43.

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Cap43 gene has been cloned in our laboratory from human lung A549 cells as highly inducible by both soluble and insoluble nickel compounds (Zhou et al., 1998). Since nickel mimics hypoxic condition in cells subsequent studies have revealed that Cap43 mRNA is indeed induced by hypoxic conditions (Salnikow et al., 2000). Further, the importance of hypoxia in the induction of Cap43 was shown in studies using mouse cells with the knock-out of HIF-1α transcription factor. Thus, transcriptional upregulation of the Cap43 mRNA is highly dependent upon the HIF-1 transcription factor. In addition to hypoxia Cap43 mRNA can be induced by the elevation of intracellular calcium, however, this upregulation is dependent on another transcription factor, AP-1 (Salnikow et al., 2002). High inducibility by hypoxia makes Cap43 an important marker for cancer cells since most tumor cells experience hypoxia due to limited vasculature in the growing tumor body. Cap43 RNA and protein is remarkably stable and immunohistochemical staining have demonstrated strong overexpression of Cap43 protein in lung, breast, prostate, esophageal and melanoma cancers relative to normal tissues. Thus our preliminary studies suggest that Cap43 protein is an excellent cancer marker. In order to better understand transcriptional regulation of this new tumor marker we have cloned a 3.0 kbp fragment of the Cap43 promoter. The sequence identity was verified and a computer analysis of the predicted transcription factors binding sites has been performed. Based on the map of the predicted transcription factor binding sites we created a number of deletion fragments of the promoter. The full size promoter and all deletion fragments were inserted into a pGL3 plasmid with a Luciferase reporter gene. These constructs were used for the analyses of the transcriptional regulation of Cap43 expression in normal and transformed cells.

1138 DOWNREGULATION OF A ZINC FINGER PROTEIN-ZFP61 BY Ni IN MOUSE CELLS *IN VITRO* AND *IN VIVO.*

P. Zhang and M. Costa. *NYU Medical Center, New York, NY.*

We studied the gene expression pattern in the mouse PW cell line following nickel treatment. We found that a gene zinc finger protein 61 from a pool of downregulated genes was suppressed by Ni exposure and this effect was reversed by trichostatin A . We have confirmed that zpf61 (L28167) was downregulated by Ni, Co., and hypoxia in Northern Blot. The zfp61 RNA level was downregulated in PW cells (a mouse cell line) after Ni and Co treatment for 24 hrs, this effect was partially reverted by pretreatment with Trichostatin A (TSA) a histone deacetylase Inhibitor, which raised the overall levels of Histone acetylation in the cell. *In vivo* data also support this result, as IP injection of nickel could downregulate zfp61 transcription in various tissues as lung, liver.. Meanwhile, as Cobalt and hypoxia can induce zfp61 downregulation as well, we proposed that this parallel effect may be due to a mechanism involving the HIF dependent pathway. We further confirmed this idea by showing that in HIF defective cell lines, this zfp61 transcription depression by Ni was absent. Futher studies will reveal the possible function of this gene and the toxicological significance of its downregulation by Ni.

1139 DOWN REGULATION OF A SERINE PROTEINASE INHIBITOR BY NI AND CO IS DEPENDENT ON HYPOXIA INDUCIBLE FACTOR SIGNALLING.

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We have utilized the mouse Affymetrix chip to study the gene expression pattern in mouse fibroblast cells following nickel treatment. Spi2.1, a member of the mouse Serpin (serine protease inhibitor) family, was the most down-regulated (32-fold) among all of the genes studied. We have confirmed that Serpin was down-regulated by Ni and also investigated which signalling pathways may be involved in this down-regulation of spi2.1 gene expression. PW cells (a mouse cell line) were treated with Ni and Co for 24 hrs, then total RNA was isolated and a 480 bp DNA fragment was amplified from spi2.1 full-length cDNA and used in Northern hybridization. Ni and Co exposure silenced Serpin expression, and this effect was suppressed by trichostatin A, a histone deacetylase inhibitor that raised the overall levels of histone acetylation in the cell. This down-regulation appeared to be operating *via* a hypoxia inducible factor mechanism (HIF), since exposure to hypoxia, Ni and Co caused the suppression of gene expression and the affect was greatly diminished in Hif Knock out cells. Calcium ionophore also down-regulated Serpin. We are furthering our studies of Serpin gene suppression by Ni to other species and to its role in Ni-induced carcinogenesis. (Supported by grant numbers ES05512, ES10344, ES00260 from the NIEHS).

1140 SCREENING NICKEL CARCINOGENS FOR INDUCTION OF MITOTIC RECOMBINATION IN D MELANOGASTER.

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Nickel compounds were tested for genotoxicity in the wing spot assay of D melanogaster. Compounds assayed included nickel oxide black, nickel oxide green, nickel monosulfide and nickel arsenide. Third instar larvae were exposed to tested compounds *via* 6 hour acute feeding treatments. Both nickel oxide green and nickel monosulfide were evaluated as negative in yielding mutant clones of any type. However, nickel oxide black was found to be a positive inducer of small single spots and total spots at 80 mM. Significant ($p<0.05$) dose response relationships also existed for nickel oxide black for induction of small single spots and total spots. About 77% of single spots induced by nickel oxide black in trans-heterozygous wings involved mitotic recombination based on the inhibition in inversion heterozygous wings. Nickel oxide black was ineffective at inducing twin spots, which arise exclusively from mitotic recombination between centromere and flr gene. Uptake of nickel particles and surface zeta potential affect acetylation and condensation of histone leading to the failure of twin spot occurrence

1141 MOLECULAR PROFILING OF GENES SHOWING ALTERED EXPRESSION IN THE LIVERS OF RATS TREATED WITH NON-GENOTOXIC CARCINOGENS FOR 28 DAYS.

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Sponsor: T. Shirai.

We performed global gene expression analysis in the rat livers to clarify common molecular events that appeared in response to repeated oral administration of representative non-genotoxic liver carcinogens for 28 days. Six-week-old male SD:IGS/DuCrj rats were fed diet containing 600 ppm-phenobarbital (PB), 600 ppm-thioacetamide (TAA), or 20, 000 ppm-di(2-ethylhexyl)phthalate (DEHP) for up to 1 year. As a negative control for carcinogenicity, a hepatotoxic dose (10, 000 ppm) of non-carcinogenic acetaminophen (APAP) was selected. After 28 days of feeding, mRNA expression analysis was performed in the livers using GeneChip Rat Genome U34A Array (Affymetrix Inc.). Among genes those expression levels were unchanged by APAP-treatment, a group of genes showing common expression patterns (up- or down-regulated) between PB, DEHP, and TAA were selected for expression analysis at 1 year of treatment by real-time RT-PCR. Among 7, 000 genes included in the chip, genes up-regulated twice or more by treatment with PB, TAA, DEHP, or APAP as compared to the control levels were 43, 477, 179, and 39, respectively. Genes down-regulated twice or less were 74, 315, 245, and 63, respectively. In every chemical examined, majority of up-regulated genes were classified into the category of Metabolism, and down-regulated genes involved diverse functional clusters including Cell signaling/Communication, Metabolism, and Cell/Organism defense. Furthermore, majority of down-regulated genes were different between chemicals, and therefore this difference may determine the phenotype of biological responses induced by each chemical. A total of 12 genes were found to show similar expression pattern (up- or down-regulated) common to the carcinogens. However, many of these genes did not follow similar expression pattern at 1 year of treatment. On the other hand, one gene (EST) showed an inverse expression pattern from 28 days to 1 year, and this change may reflect the altered cellular function caused by long-term exposure to non-genotoxic carcinogens.

1142 EXPRESSION OF MUTANT K-RASV12 IN MOUSE LUNG EPITHELIAL CELLS INCREASES GENERATION OF PEROXIDES THROUGH COX-2, RESULTING IN DNA DAMAGE.

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The K-ras gene is frequently mutated in lung adenocarcinoma. However, wild-type K-ras is a tumor suppressor in lung [1]. This raises a question: how is mutant K-ras protein so aggressively oncogenic? Several studies demonstrate that expression of a constitutively active mutant of ras leads to increase in reactive oxygen species (ROS) generation [1, 2]. Fibroblasts or keratinocytes stably transfected with a constitutively active H-ras produced large amounts of superoxide [2, 3, 4]. In this report we demonstrate that stable transfection of constitutively active human K-ras V12 into nontransformed E10 cells from peripheral mouse lung epithelium leads to increased level of peroxides, as indicated by FACS analysis of intracellular oxidation of CM-H2DCF-DA. Furthermore, we observed significant increase in DNA strand-break damage measured by Comet assay in the cells expressing K-rasV12 compared with parental line E10 and vector control. This damage was reduced by 50% by pretreatment of the cells with catalase, confirming hydrogen peroxide involvement. Both peroxide generation and DNA single strand breaks were completely blocked by pre-treatment with cyclooxygenase-2 specific inhibitor SC 58125, suggesting COX-2 as the source of reactive oxygen species. COX-2 has been repeatedly implicated in lung cancer. ROS generated as an effect of RasV12 activity through COX-2 could contribute to transformation by DNA damage and/or by alterations in intracellular signaling. This may explain the active oncogenicity of mutant K-ras. [1] Zhang Z. et al. Nature Genet 29(1): 25-33, 2001; [2] Sundaresan M, et al. Biochem. J. 318 (Pt.2): 379-382, 1996; [3] Irani K, et al. Science 275: 1649-1652, 1997; [4] Lee A.C, et al. JBC 274 (12): 7936-7940, 1999; [5] Yang, J-Q, et al. Mol. Carcinogen. 26: 180-188, 1999.

1143 ESTROGEN-INDUCED STIMULATION OF MACROPHAGES LEADING TO THE GENERATION OF REACTIVE OXYGEN SPECIES IN THE TARGET ORGAN OF CANCER.

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The sex hormones-estrogen, progesterone, and testosterone have a profound influence on macrophage cell biology and function. Macrophages are located throughout the female and male reproductive tracts including the ovary, uterus, and testis. The expression of aromatase in macrophages suggests that estrogen may be produced in these cells through the conversion of androgen to estrogen. In addition, the estrogen receptor is known to be present in macrophages which suggests that estrogen may modulate the activity of these cells. It is not clear whether the growth of macrophage cells is regulated by estrogen. Recently, we reported that estrogen exposure of J744A1 cells increased the rate of BrdU incorporation. In this study, we investigated the effects of synthetic estrogen, diethylstilbestrol (DES) and natural estrogen, 17-beta-estradiol (E2) on the cell growth and rate of proliferation in the presence/absence of the antiestrogen tamoxifen. We found that J774A1 show a dose dependent increase in cell growth when treated with E2 at concentrations from 1 ug/ml up to 1 pg/ml, after 48h and 72h of treatment, with 1pg/ml showing more than a 2-fold increase in growth when compared to the control after 72 hrs. Co-treatments with E2 and tamoxifen (at doses 10 fold higher than E2) showed inhibition of E2 induced growth. Our results suggest that the growth of macrophage cells is estrogen sensitive. In addition to the immunogenic functions, macrophages play a key role in cell signaling due to their ability to produce cytokines and reactive oxygen species. Estrogen-induced stimulation of macrophages leading to the hyperproduction of the proinflammatory cytokines, such as IL-1, IL-6, and TNF, in the target organ of cancer, may be involved in the induction of instability in the genome, including oxidative damages to genes leading to gene mutations. Supported by the NIH grant ES10851.

1144 ESTROGEN REGULATES AH RESPONSIVENESS IN MCF-7 BREAST CANCER CELLS.

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Cytochromes P450 1A1 (CYP1A1) and 1B1 (CYP1B1) catalyze the metabolic activation of numerous procarcinogens and the hydroxylation of 17beta-estradiol (E2) at the C-2 and C-4 positions, respectively. Both are under the transcriptional control of the aryl hydrocarbon (Ah) receptor, and there are indications of cross-talk between estrogen receptor-alpha- and Ah receptor-mediated signaling in breast and endometrial cells. We examined the effects of short- and long-term E2 exposure on Ah responsiveness in MCF-7 human breast cancer cells to investigate this interaction. Short-term exposure to 1 nM E2 elevated the ratio of the 4- to 2-hydroxylation pathways of E2 metabolism in 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD)-treated cells. Cells maintained long-term (9 to 12 mo.) in low-estrogen medium progressively lost Ah responsiveness as indicated by the diminution of TCDD-induced E2 metabolism and the expression of CYP1A1 and CYP1B1 mRNAs. These E2-deprived cells showed elevated levels of estrogen receptor-alpha mRNA, but depressed levels of Ah receptor mRNA. The reduction in Ah-responsiveness was reversed by culture for four passages in medium supplemented with 1 nM E2. Expression from a transiently transfected CYP1B1-promoter-luciferase reporter construct was reduced in the long-term, E2-deprived cells in a manner similar to the endogenous gene. Activity from CYP1B1 promoter-reporter construct was restored by co-transfection with an Ah receptor expression construct. This indicates that AhR expression was limiting in the estrogen-deprived MCF-7 cells. These studies suggest an additional role of estrogen in carcinogenesis may involve enhanced Ah responsiveness leading to elevated expression of the procarcinogen-bioactivating enzymes, CYP1A1 and CYP1B1.

1145 PCB METABOLITES AS INITIATING AGENTS IN HEPATOCARCINOGENESIS.

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PCB commercial mixtures are complete rodent carcinogens. Mixtures and congeneric PCBs are efficacious promoters in rat two-stage hepatocarcinogenesis, but the question of PCB's initiating activity remains controversial. Congeneric PCBs are metabolized both *in vitro* and *in vivo* to strongly electrophilic species that bind to proteins and to DNA. We have reported previously that 4-chlorobiphenyl (PCB-3) shows initiating activity by inducing gamma-glutamyl transpeptidase (GGT)-positive foci in a modified Solt-Farber protocol. To investigate the mechanism of this activation, we examined two metabolites of PCB-3: 3-OH and 4-OH PCB-3. Fisher 344 male rats were fasted for 4 days, then 24 hrs after refeeding were injected i.p. with 400 umol/kg PCB metabolites, or injected p.o. with diethylnitrosamine (DEN) (20mg/kg) as a positive control. After a two-week recovery period, all animals were treated with selection agents: 3 daily doses of 2-acetylaminofluorene (2-AAF, 30 mg/kg), followed by a single dose of carbon tetrachloride (2 ml/kg) and by 3 additional daily doses of 2-AAF. Rats were killed two weeks after the last treatment. Although treatment with each metabolite increased the number of GGT-positive foci per cc and per liver, this increase was statistically significant only for the 4-OH-PCB-3 metabolite when compared to control rats. All DEN-treated rats developed visible nodules as well as GGT-positive foci in the liver. These data support our hypothesis that the metabolic activation of 4-chlorobiphenyl occurs *via* para hydroxylation, most likely leading to the 3, 4-catechol. (Supported by ES 07380)

1146 EFFECT OF MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS ON HUMAN CELLS IN CULTURE.

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Human exposure to polycyclic aromatic hydrocarbons (PAH) occurs through complex mixtures of PAH, some of which are carcinogenic. Standard Reference Material (SRM) derived from coal tar, diesel particulate matter, and urban dust obtained from the National Institute of Standards, contained a mixture of PAH including carcinogenic, non-carcinogenic and weakly carcinogenic compounds. In order to study the effect of these PAH in complex mixtures with different levels of carcinogenicity (both weakly carcinogenic and non-carcinogenic) on DNA-binding and induction of cytochrome P450 (CYP) enzymes, artificial mixtures (1597H-coal tar, 1649H-urban dust, and 1650H-diesel exhaust) of PAH were procured in our laboratory. Human mammary carcinoma derived cell line MCF-7 was treated with the artificial mixtures of PAH, and analyzed for PAH-DNA adduct formation and induction of CYP enzymes. Results indicate that the artificial mixtures tested formed DNA adducts 24 and 48 hrs after treatment, like those treated with benzo[a]pyrene (B[a]P). Induction of CYP enzymes was also observed as measured by ethoxyresorufin O-deethylase (EROD) assay. These results demonstrate that both non-carcinogenic and weakly carcinogenic PAH in the complex mixture affect the DNA binding and the induction of CYP enzymes. Supported in part by grant CA28825, NCI, DHHS.

1147 EXPLORATION OF TOXICOLOGICAL INTERACTIONS BETWEEN JET A FUEL AND BENZO(a)PYRENE (BaP) ON MALIGNANT TRANSFORMATION OF HUMAN KERATINOCYTES.

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Due to its widespread use, occupational and environmental exposures to Jet A fuel have become a source of public and regulatory concern. However, it is still unclear how the interactions among the components of these complex mixtures affect their carcinogenic potential in humans. Benzo(a)pyrene (BaP) is the one of the most potent carcinogens present in petroleum mixtures. It was of interest, therefore, to evaluate how the transforming potential of BaP was affected by the presence of low levels of Jet A. To address this issue, we used the immortalized human epidermal keratinocyte cell line, RHEK-1. As a first step of this project, the toxicity of Jet A and BaP on RHEK-1 was determined by clonogenic assay. The LC10 and LC50 for Jet A were 0.004% and 0.03%, respectively; whereas the LC5 and LC50 of BaP were 0.4μM and 2.3μM, respectively. In subsequent studies, the transforming efficiency of BaP with or without Jet A in RHEK-1 cells was evaluated. RHEK-1 cells were treated repetitively (every 4 passages) with the following chemicals or controls: Culture medium, S9 alone, acetone solvent control, MNNG positive control, BaP Low (0.4μM), BaP High (2.3μM), Jet A (0.004%) alone, BaP Low + Jet A, and BaP High + Jet A. Morphological analysis, Anchorage Independent Growth (AIG), saturation density and cell cycle kinetic analysis were the endpoints studied to determine malignant progression of RHEK-1 cells following BaP and/or Jet A treatments. At passage 13, the following was observed: 1) cultures treated with BaP or MNNG showed low, but detectable, AIG as compared to negative controls and 2) cells exposed to BaP High + Jet A exhibited higher colony forming efficiency than did those exposed to BaP High alone. Although preliminary, this finding suggests that Jet A may enhance the malignant transformation of BaP. This study was supported by NIEHS R01 ES-09655.

1148 EFFECT OF 2, 2', 4, 4', 5, 5'-HEXACHLOROBIPHENYL (PCB-153) ON GENE EXPRESSION IN MICE DEFICIENT IN THE P50 SUBUNIT OF NF-κB.

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Polychlorinated biphenyls have been shown to be hepatic tumor promoters and to induce liver tumors in experimental animals. The exact mechanism of the tumor promoting activity of PCBs is not well understood, but alterations in expression of genes that regulate cell proliferation and apoptosis have been proposed. Our previous studies showed that a single dose of PCB-153, a non-coplanar PCB congener, causes transient increases in the hepatic DNA binding activity of NF-κB and cell proliferation in rats and mice. Using a transgenic mouse model that is deficient in the p50 subunit of NF-κB (p50 -/-), we have demonstrated that the activation of NF-κB contributes to changes in cell proliferation and apoptosis in response to PCB-153 treatment. In the present study, we used gene microarrays to examine changes in gene expression following a single dose of PCB-153 in p50 -/- mice as well as wild type mice. Mice received a single i.p. injection of corn oil or PCB-153 (300 μmol/kg) and were euthanized 2 days later. Hepatocyte proliferation was increased by PCB-153 in wild type mice, but not in p50 -/- mice. The p50 -/- mice had more spontaneous apoptosis than wild types, and PCB-153 inhibited apoptosis in p50 -/- mice. Total RNA was collected from liver tissues and used for Affymetrix microarray analysis. Identified in the microarray analysis were 30 genes altered by PCB-153 in both wild type and p50 -/- mice, 93 genes altered by absence of p50 in both control and PCB-treated mice, 25 genes altered by PCB-153 in wild type mice, but not in p50 -/- mice, and 33 genes altered by PCB-153 only in p50 -/- mice, not in wild type mice. This study will lead to a better understanding of the mechanisms of hepatocarcinogenesis by PCBs. (Supported by ES07380)

1149 BENZO(a)PYRENE, DISTILLATE MARINE DIESEL FUEL AND A MIXTURE OF 5 POLYAROMATIC HYDROCARBONS, SINGLY AND IN COMPLEX MIXTURES, INDUCED CYTOTOXICITY AND MALIGNANT TRANSFORMATION IN HUMAN KEROTINOCYTES, RHEK-1.

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Exposure to petroleum-based mixtures of polycyclic aromatic hydrocarbons (PAH's) has been associated with an increase in cancer incidence. However, little is currently known about the interactions among PAH components in these mixtures and risk assessment is difficult. To explore this issue, we have begun to study the effects of distillate marine diesel fuel (DMA) and a defined mixture of 5PAH's (anthracene, chrysene, fluoranthene, pyrene and phenanthrene) on the carcinogenicity of benzo(a)pyrene (BaP) in the immortalized human keratinocyte cell line, RHEK-1. Cytotoxicity analysis using the clonogenic assay was performed to determine concentration ranges for each of the chemicals/mixtures used in subsequent transformation studies. To test the carcinogenic potency of BaP alone and in the presence of either DMA or the 5PAH's, RHEK-1 cells were treated repetitively with the following: 1) BaP LC5 [0.4 and 0.5 μM in acetone or DMSO, respectively]; 2) BaP LC50 [2.3 and 2.75 μM in acetone and DMSO, respectively]; 2) BaP LC5 + LC10 DMA [0.0025% in acetone]; 3) BaP LC5 + LC10 5PAH's [2 μM in DMSO]; 4) BaP LC50 + LC10 DMA; 5) BaP LC50 + LC10 5PAH's; 6) 5PAH's alone [2 μM]; or 7) the solvent controls. All treatments were carried out in the presence of rat S9 fraction. As an indicator of malignant transformation, anchorage-independent growth (AIG) in methylcellulose (MC) was measured in each culture as a function of time. Although preliminary, the results of this analysis through passage 16-20 showed that in contrast to the control (S9, solvent, DMA, or 5PAH's alone), cultures exposed to BaP have begun to acquire slight, but detectable, AIG. Interestingly, the culture with the highest cloning efficiency in MC is currently the BaP LC5 + 5PAH mix (7.5% at passage 16). Treatment of RHEK-1 and evaluation of the cells for AIG and, eventually, tumorigenicity in mice will continue. Funded by NIEHS Grant RO1 ES09655.

1150 POLYCYCLIC AROMATIC HYDROCARBONS WITH BAY-LIKE STRUCTURES INHIBITED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN IMMORTALIZED HUMAN PANCREATIC DUCTAL EPITHELIAL CELLS.

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Smoking is a well-documented risk factor for the development of pancreatic adenocarcinoma. Polycyclic aromatic hydrocarbons (PAHs) are one class of compounds in this complex mixture of combusted by-products of tobacco. The most abundant PAHs in cigarette smoke are methylated anthracenes and phenanthrenes. Several reports showed that methyl- or chloro-anthracenes, which possess a bay-like structure affect epigenetic events such as an induced release of arachidonic acid, inhibition of gap junctional intercellular communication (GJIC), and induction of mitogen activated protein kinases in a pleuripotent rat liver epithelial stem cell line. Anthracenes with no bay-like structures were inactive. These biological effects are all molecular events associated with the promotional phases of cancer. To understand the epigenetic toxicity of PAHs related to pancreatic cancer, a human immortalized pancreatic ductal epithelial cell line, H6c7, was used to examine the epigenetic effects of PAHs. H6c7 cells were GJIC-incompentent and did not express detectable connexin messages when grown in medium containing hormones and growth factors. Telomerase activity was high in these immortalized H6c7 cells. In the presence of the c-AMP elevating drugs (forskolin and IBMX) in basal medium without growth factors, the cells became GJIC competent and expressed connexins. Telomerase activity was also decreased by c-AMP elevating drug treatment. After induction of c-AMP, 1-methylanthrance with bay-like structures inhibited GJIC, whereas the 2-methylanthracene lacking a bay-like structure had no effect on GJIC. Telomerase activity remained high in 1-methylanthrance treatment but not with 2-methylanthrance. These results indicate that a prominent component of cigarette smoke, namely methylantracenes with distinct structural configurations, could be a potential etiological agent contributing to the epigenetic events of pancreatic cancer. Funded by the NIEHS Superfund grant #P42 ES04911-07.

1151 EVALUATION OF EVIDENCE TO EXPLAIN THE LUNG TUMORIGENESIS IN A/J MICE INDUCED BY EXPOSURE TO AN ENVIRONMENTAL TOBACCO SMOKE SURROGATE.

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Recently, lung tumors were reproducibly induced in A/J mice exposed to an experimental surrogate of environmental tobacco smoke ("ETS") using a protocol that requires a 5-month "ETS" inhalation period followed by a 4-month post-inhalation period. The present study was conducted to identify potential mechanisms underlying this tumor response. Male A/J mice were exposed to "ETS" (88% sidestream smoke, 12% mainstream smoke) at 100 mg total particulate matter/m3 or to fresh air (sham control) for 6 h/d, 5 d/w, for 5 months or were kept under one of two food restriction regimens to mimic or exceed the body weight reduction seen with "ETS" exposure. Half the mice were kept for an additional 4-month post-treatment period. At both time points, K-ras mutations in codons 12, 13, and 61 were analyzed by DNA sequencing after PCR amplification of DNA extracted from lung tumor material, and micronuclei in reticulocytes and normochromatic erythrocytes in peripheral blood were counted by a flow cytometric method. Pulmonary inflammation was assessed by differential counting of lavaged polymorphonuclear neutrophils, alveolar macrophages, and lymphocytes at the end of the treatment period. No pulmonary inflammation was observed. Urethane pre-treatment induced the signature mutation pattern in codon 61 expected from literature. At the end of the treatment period, micronuclei and mutation frequency in codon 12 were slightly higher in "ETS"-exposed mice, similar to the levels seen in food-restricted mice. These findings were not seen at the end of the post-treatment period, when "ETS"-induced tumor multiplicity was increased by approximately 2-fold over sham control. No change in the K-ras mutation pattern was detected. Thus, the mechanisms studied here did not explain the observed increase in lung tumor multiplicity by "ETS" inhalation.

1152 EFFECT OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) EXPOSURE ON NORMAL HUMAN MELANOCYTES AND MELANOMA CELL LINES.

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Our hypothesis is that the environmental contaminant 2, 3, 7, 8-tetrachlordibenzo-p-dioxin (TCDD) alters tumor promotion by altering the expression of genes involved in cell-cell interactions and matrix remodeling. Although the molecular mechanisms of tumor promotion by TCDD are not clearly understood, it is believed that TCDD exerts its effects through activation of the Aryl Hydrocarbon Receptor (AhR)/Arnt transcription pathway. Studies of AhR/Arnt have focused on the heterodimers ability to activate transcription of xenobiotic metabolizing genes including members of the cytochrome (CYP450) family of monooxygenase enzymes. Our data demonstrates that TCDD treatment of normal human keratinocytes results in increased expression and activity of the matrix metalloproteinases (MMPs), enzymes that degrade the protein components of the extracellular matrix and basement membrane. The goal of this study is to characterize the TCDD-responsiveness of primary melanocytes and melanoma cell lines. We investigated TCDD-responsiveness by investigating induction of mRNA expression of two known targets for TCDD, cytochrome p450s (CYP) 1A1 and 1B1. We also examined the effect of TCDD on expression of genes involved in melanoma progression, including the MMPs, plasminogen activator inhibitor-2 (PAI-2), E-cadherin, and EMMPRIN. We used both northern analysis as well as quantitative real-time RT-PCR to examine expression of CYP1A1, CY1B1 and MMP-1 in normal human melanocytes, two invasive melanoma cell lines (A2058, HT-144) and two less invasive cell lines (Bowes, SK-Mel-2). Our data demonstrates that while normal melanocytes do not appear to be responsive to TCDD exposure, melanoma cell lines display varying degrees of TCDD-responsiveness. This suggests that responsiveness to TCDD and activation of the AhR receptor pathway is associated with melanoma, and that TCDD-activation of this pathway during melanoma progression alters expression of genes critical to metastasis.

1153 REGULATION OF EGR-1 BY TCDD IN HUMAN LUNG EPITHELIAL CELLS.

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In humans exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), there is an increased risk of lung cancer and development of chronic obstructive pulmonary disease. Because relatively little is known about the role of TCDD in pulmonary diseases, our objective was to investigate the mechanism of toxicity of TCDD using an *in vitro* human lung model. Using toxicogenomic analysis, we previously have shown that a plethora of genes are altered by TCDD in human lung cells. For the present study we investigated the regulation of early growth response 1 (Egr-1) gene since it was detected to be significantly altered by microarray analysis in both malignant (A549) and nonmalignant (HPL1A) cell lines exposed to TCDD for 24 hours. Egr-1 is a transcription factor that is a negative regulator of cell growth with antiapoptotic activity and can also enhance growth related to malignancy. Therefore, elevation of Egr-1 protein levels may be linked to tumor promotion activity of TCDD. To confirm that altered Egr-1 RNA levels lead to altered translation, we exposed HPL1A cells to 10 nM TCDD for 1, 3, 6, 24 or 48 hrs. By western blot analysis we found that levels of Egr-1 protein are higher in TCDD treated cells compared to vehicle treated cells at all time points tested. Using real time RT-PCR sybergreen detection, the relative fold increase of steady state mRNA for Egr-1 from TCDD treated HPL1A cells paralleled the protein expression profile. Our data show that there was a decreasing amount of both Egr-1 mRNA and protein over time, yet always higher in TCDD-treated cells. To investigate transcriptional activity of the Egr-1 promoter, cells were transfected with a plasmid containing the Egr-1 binding sites. A minimal increase in luciferase activity was detected in TCDD-treated cells. This modulation of Egr-1 expression by TCDD is likely through a post-transcriptional mechanism, such as mRNA stabilization that has previously been shown for other known TCDD altered genes.

1154 OVEREXPRESSION OF THE INTEGRIN-LINKED-KINASE PATHWAY IN HCB-TREATED FEMALE RATS.

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Hexachlorobenzene (HCB), a widespread environmental contaminant, causes gender-specific liver tumor promotion in the rat. Modulation of gene expression is the hallmark of epigenetic carcinogens such as HCB. We have previously shown that in female rats HCB causes significant decreases in mRNA and protein levels of the liver gap junction subunits, connexin 26 and connexin 32 (Cxs), and in gap junctional intercellular communication (GJIC). This study aim to assess the mechanistic basis for HCB-induced cell-cell changes by looking at modulation of signaling cascades. The Integrin-Linked-Kinase (ILK) phosphorylation pathway, which is overexpressed in many tumors, causes transformation of epithelial cells by disrupting cell-cell junctions. We hypothesize that HCB modulates the expression of the ILK pathway in female rats. Rats were exposed to HCB by gavage for five consecutive days and killed 45 days after the last dose, i.e. when Cxs are decreased. Western blot analysis showed a significant ($p \leq 0.05$) increase of ILK in treated rats compared to controls. Protein levels of Akt, a signaling target for ILK, and its phosphorylation

were significantly ($p \leq 0.05$) decreased; this suggests that the phosphorylation signal in HCB-treated females is relayed through another target protein, such as GSK3. ILK is known to phosphorylate GSK3 leading to translocation of beta-catenin from the cytoplasm into the nucleus. Assessment of nuclear beta-catenin showed higher levels in HCB-treated females compared to controls. In the nucleus the beta-catenin-Lef1 complex can down-regulate the expression of E-cadherin, a key cell-cell adhesion molecule. Western blot analysis showed that E-cadherin is significantly ($p \leq 0.05$) lower in HCB-treated rats compared to controls. Overall, results show that the ILK pathway is overexpressed in HCB-treated female rats. We propose that HCB promotes liver tumor formation by modulating GJIC and cell-cell junctions *via* an activation of the ILK pathway. (supported by the TSRI, Government of Canada)

1155 SPECIES DIFFERENCES IN THE INDUCTION OF HEPATOCELLULAR DNA SYNTHESIS BY DIETHANOLAMINE.

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Diethanolamine increased the incidence and multiplicity of liver tumors in the mouse following chronic exposure. Diethanolamine inhibits cellular choline uptake. Choline deficiency produces tumors in rodents, therefore, diethanolamine, through depletion of choline, may result in tumor development in rodents. The potential for diethanolamine to function through this mode of action in humans is not known. The present study examined the effect of diethanolamine and choline depletion on DNA synthesis in primary cultured mouse, rat, and human hepatocytes. Hepatocytes were cultured with sublethal concentrations of diethanolamine (0 to 500 μg/ml) for 24 hours and DNA synthesis measured. In mouse and rat hepatocytes DNA synthesis was increased following treatment with 10 μg/ml diethanolamine and higher (3 to 4-fold over control). In contrast, diethanolamine failed to increase DNA synthesis in human hepatocytes. Incubation of hepatocytes in medium containing reduced choline (1/10 to 1/100 of normal medium; 0.898 mg/L to 0.0898 vs. 8.98 mg/L) for 24 hours increased DNA synthesis (1.6- and 1.8-fold of control in mouse and rat hepatocytes, respectively), however, choline depletion did not induce DNA synthesis in human hepatocytes. Mouse and rat hepatocytes incubated in medium supplemented with 2 to 50-fold excess choline reduced diethanolamine-induced DNA synthesis to control levels or below. In addition, gene expression analysis of mouse and rat hepatocytes following diethanolamine treatment revealed an increase in genes associated with cell growth and a decrease in expression of apoptotic genes. These results showed that the induction of DNA synthesis by diethanolamine was species specific and appears to be related to hepatocellular choline depletion. This mode of action for rodent hepatocarcinogenicity of diethanolamine may therefore not be relevant to humans.

1156 INHIBITION OF PANCREATIC CANCER CELL GROWTH BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ AGONISTS.

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Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated nuclear receptor that belongs to the nuclear receptor superfamily of transcription factors. PPARγ is widely expressed in tumors from diverse tissue/organs, and PPARγ agonists such as prostaglandin J2 (PGJ2) inhibit growth of cancer cells through inhibition of cell cycle progression, induction of differentiation, and apoptosis. Treatment of Panc-1, Panc-3 and Panc-28 pancreatic cancer cells with 1 - 20 μM PGJ2 or the C-substituted diindolylmethane (DIM), 1, 1-bis(3'-diindolyl)-1-(*p*-trifluoromethylphenyl)methane (DIM-C-pPhCF_3) inhibited pancreatic cancer cell growth, and similar results were observed with ciglitazone. The growth-inhibitory effects observed for ciglitazone and DIM-C-pPhCF_3 were accompanied by decreased expression of cyclin D1 in Panc1 which could be inhibited by the proteasome inhibitor MG132 but not by the protease inhibitor calpain II. In contrast, PGJ2 did not affect levels of cyclin D1 protein in Panc1 cells, whereas in other cancer cell lines, all three PPARγ agonists (PGJ2, ciglitazone and DIM-C-pPhCF_3) induced proteasome-dependent degradation of cyclin D1. The mechanisms of action of these PPARγ agonists in other pancreatic cancer cell lines are currently being investigated to determine the contributions of cell context and PPARγ ligand structure on cyclin D1 expression and other pathways required for inhibition of pancreatic cancer cell growth.

1157 IPRODIONE-INDUCED RAT LEYDIG CELL TUMORS ARE MEDIATED BY TRANSIENT DECREASES IN PLASMA TESTOSTERONE.

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Iprodione is a non-mutagenic fungicide that, following lifetime exposure at 70 mg/kg/day, is associated with benign Leydig cell (LC) tumors in the testes of rats. The rat is sensitive to the development of LC tumors by compounds that cause perturbations to the hypothalamic-pituitary-gonadal axis and so this mode of action was investigated. Following a single oral gavage administration of iprodione (0, 70, 300 mg/kg) a rapid, transient, and dose-dependent change in plasma testosterone (TST) and luteinizing hormone (LH) levels was observed. The greatest effect on TST was observed 2 h after dosing when 60 and 75% decreases in TST were detected at 70 and 300 mg/kg, respectively. The effects on LH were most marked 4 h post dosing when 36% and 55% increases in LH were noted at 70 and 300 mg/kg, respectively. TST and LH levels returned to normal 6-10 h following the acute iprodione exposure. To confirm that these effects occur following repeated exposure, iprodione was administered to male rats by oral gavage (6, 70, 300 mg/kg) for 14 days. TST levels on day 14 were significantly reduced at the high dose at up to 4 h post-dosing, as well as at 70 mg/kg/day at 2 h post-dosing. LH levels were significantly elevated in the 300 and 70 mg/kg dose groups at 4 h post-exposure. Both TST and LH levels returned to control levels within 24 h. In a separate 14-day study, assessment of LC proliferation using BrdU labeling revealed a dose-dependent increase in LC proliferation in animals treated with iprodione at 300 and 70 mg/kg/day (+74% and +36%, respectively). These data indicate that oral administration of iprodione results in a rapidly reversible hormonal imbalance that, upon repeated administration, is associated with an increase in LC proliferation. A clear threshold for hormonal change and LC proliferation occurs at 6 mg/kg/day. It is postulated that LC proliferation provoked by lifetime exposure to high levels of iprodione resulted in the tumors observed in the rat chronic study.

1158 IPRODIONE INHIBITS TESTOSTERONE BIOSYNTHESIS IN LEYDIG CELLS THROUGH A RAPID AND REVERSIBLE MECHANISM.

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Iprodione is a non-mutagenic fungicide that, following lifetime exposure, is associated with an increased incidence of benign Leydig cell (LC) tumors in the testes of male rats. Explanatory studies in the rat have demonstrated that iprodione administration results in decreased plasma testosterone (TST) and increased plasma luteinizing hormone (LH) levels. Experiments were undertaken to elucidate the mechanism of this iprodione-induced hormonal imbalance. Iprodione and its major metabolites did not bind to the androgen receptor or inhibit 5-alpha-reductase and addition of iprodione to culture media reduced TST secretion by rat testicular sections in a concentration-dependent manner. To evaluate effects on steroidogenic activity, LC were isolated from porcine testes and TST secretion evaluated following iprodione exposure. Iprodione-mediated inhibition of TST release was detected as early as 3 hours following exposure and occurred at a concentration of 1 ug/ml and above but not at lower concentrations. Two metabolites of iprodione demonstrated a similar inhibitory effect. Removal of iprodione from the culture medium reversed this effect. The iprodione-induced inhibition of TST secretion was observed when LC were stimulated with LH, agents that enhance cAMP production (i.e., cholera toxin, forskolin), or a cAMP analog. However, no effects of iprodione were observed on gonadotrophin-stimulated cAMP production indicating that the inhibition of TST secretion by iprodione is located downstream of cAMP production. The incubation of LC with 22-R hydroxycholesterol, a cholesterol substrate that does not require active transport to the mitochondria, antagonized the inhibitory effect of iprodione on TST synthesis, suggesting that iprodione prevents the active transport of cholesterol into the mitochondria. Thus, the results indicate that iprodione modulates LC steroidogenesis through a reversible inhibition of active cholesterol transport.

1159 THE EFFECTS OF THE BENZENE METABOLITES PHENOL AND CATECHOL ON C-MYB AND PIM-1 SIGNALLING IN HD-3 CELLS.

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Exposure to the environmental toxicant benzene has been proposed to lead to carcinogenesis. Benzene is a known leukemogen and bone marrow suppressant in humans and in animals. While the mechanism of how benzene mediates leukemogen-

sis remains unknown, it is generally accepted that, in order for benzene to exert its toxicity, it first must be metabolized in the body by cytochromes P450 to metabolites such as phenol and catechol. During blood cell development the transcription factor c-Myb, plays a role in cell differentiation such that overexpression of the protein inhibits differentiation of induced erythrocytes. While little is known about the upstream activators that regulate c-Myb protein activity, c-Myb can be regulated by the serine/threonine kinase Pim-1. Mutated or overexpressed versions of the c-myb and pim-1 oncogenes are believed to play a pivotal role in the development of a wide variety of leukemias and other tumours. The objective of this study was to investigate the effects of the benzene metabolites phenol and catechol on the c-Myb and Pim-1 signalling pathway as a possible mechanism of benzene mediated leukemogenesis. To evaluate this objective, HD-3 cells were transfected with a Myb-responsive luciferase reporter plasmid derived from the chicken mim-1 promoter, along with control vector DNA or a plasmid expressing Pim-1. Cells were then exposed to phenol or catechol 1-24 hr before non-proprietary dual luciferase activities were measured in cell lysates. Results from this study revealed a time and concentration dependent increase in c-Myb activity after exposure to either phenol or catechol. Increased c-Myb activation upon exposure to catechol appeared to be Pim-1 independent, while the role of Pim-1 is currently being investigated in phenol activation of c-Myb. This study supports the hypothesis that benzene metabolites can mediate the signalling pathway involving the c-myb and pim-1 oncogenes. (Support: Canadian Institute of Health Research and Queen's University ARC)

1160 THE EFFECT OF PHENOBARBITAL ON CELL PROLIFERATION IN MICE DEFICIENT IN THE P50 SUBUNIT OF NF-κB.

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Phenobarbital (PB) is a nongenotoxic tumor promoter in the liver. One mechanism by which PB may exert its tumor promoting activity is by inducing oxidative stress. We previously found that PB administration increased hepatic NF-κB DNA binding activity. In this study we examined whether NF-κB is necessary for the induction of cell proliferation by PB. We used a mouse model that is deficient in the p50 subunit of NF-κB; previous studies had found that hepatic cell proliferation induced by PCBs or peroxisome proliferators was decreased in p50 -/- mice. Mice (p50 -/- and wild-type B6129) were fed a control diet or one containing 0.05% PB for 3, 10 or 34 days. At the end of the experiment, the mice were euthanized and livers removed and processed. At the 3 day time point, cell proliferation was significantly increased by PB in the p50 knockout mice but not in the wild-type mice. After 10 days, cell proliferation was significantly increased by PB in both p50 -/- and wild-type mice; genotype had no significant effect. At 34 days, cell proliferation was significantly increased in the p50 -/- mice compared to wild-type mice; phenobarbital slightly, but not significantly ($p = 0.17$), increased cell proliferation. These findings imply that the p50 subunit of NF-κB is not required for the induction of cell proliferation by PB at these time points. (Supported by ES11480)

1161 DIETHYLSTILBESTROL INDUCES CASPASE 6 ACTIVITY IN FEMALE ACI RATS.

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Diallyl sulfide (DAS) is a component of garlic that prevents cancer in several animal models. Its chemopreventive effects are attributed to modulation of enzymes. Diethylstilbestrol (DES) is a synthetic estrogen that causes breast cancer in female ACI rats following metabolism to reactive oxygen species (ROS). We previously showed that DAS inhibits DES-induced ROS. Caspase activation is associated with ROS in some models, yet there is little information on their role in DES-induced carcinogenesis. Caspases are aspartic acid-specific proteins constitutively expressed in the inactive form that are activated after proteolytic cleavage. Caspases 8 and 9 are upstream, and caspases 3 and 6 are downstream caspases that cleave critical cellular proteins resulting in apoptosis. Our purpose was to study the roles of caspases in the early stages of DES-induced carcinogenesis and the impact of coadministering DAS. Using female ACI rats, Group 1 was a control. Group 2 received 50 mg/kg DAS for 4 weeks. Group 3 received corn oil for 1 month with 15 mg of DES implanted after 2 weeks. Group 4 received 50 mg/kg DAS for 4 weeks with 15 mg of DES implanted after 2 weeks. 125-ug protein from breast tissue were incubated with caspase-specific fluorophores and analyzed by fluorescent assay. Relative fluorescence was measured by comparison to an uninduced control per sample. Exposure to DES resulted in a significant increase in mean caspase 6 activity relative to the controls; caspases 3, 8 and 9 activities were also significantly higher. The magnitude of caspase 6 and 8 activity was a factor of 10 greater than 3 and 9. Exposure to DAS induced caspase 3 activity and inhibited caspase 9 activity with no effects on either caspases 6 or 8. Coadministration of DAS/DES potentiated caspase 8 activation relative to the DES group. We showed for the first time that caspase 6 induction occurs early in DES-induced breast carcinogenesis in a mechanism not mediated by ROS. We conclude that manipulation of caspase 6 may provide opportunities for chemoprevention in estrogen-induced breast cancer.

1162 ALTERED RESPONSE OF TSC2-NULL RENAL EPITHELIAL CELLS TO THE PHORBOL ESTER TPA.

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Loss of tumor suppressor protein expression or function dramatically alters cellular responses to chemicals. The phorbol ester tumor promoter, 12-O-tetradecanoylphorbol 13-acetate (TPA), stimulates cell proliferation through a rapid activation of protein kinase C (PKC), followed by gradual degradation of the kinase. TPA also activates the GTPase Rap1 in some cell types. Tsc2 has a proposed GTPase activating protein (GAP) function for Rap1, and we have observed an increase in PKC expression in Tsc2-null cells. Therefore, we wanted to compare the cellular response of Tsc2-null (ERC-18) and Tsc2-competent (NRK-52E) cells to this tumor promoter. Morphologic changes in ERC-18 cells treated with 100 ng/ml TPA for 24 hours were consistent with apoptosis (shrinkage, rounding, some evidence of blebbing). NRK-52E cells showed no morphologic change after 24 hours of TPA treatment. Cyclin D1 expression and MPM2 phosphorylation were used as indicators of proliferation, and neither increased during treatment. Although no substantial increases in Rap1 activity (GTP-bound Rap1) or PKC phosphorylation were observed after 5, 15, or 60 minutes of TPA treatment in either cell type, phosphorylation of ERK, a reported downstream effector of both PKC and Rap1, was markedly increased in ERC-18 cells after 5 minutes of TPA treatment, and the increase was sustained over the first hour of treatment. ERK phosphorylation also increased in NRK-52E cells after 5 minutes, but rapidly returned to control levels within 1 hour. After 24 hours of TPA treatment, PKC phosphorylation was decreased only in ERC-18 cells. The specific PKC inhibitor bisindolylmaleimide VIII acetate also induced morphologic changes (shrinkage, rounding) in ERC-18, but not NRK-52E cells. These results imply that the response of Tsc2-null renal epithelial cells to TPA appears to be dependent on changes in PKC phosphorylation, not Rap1 activation.

1163 NNK RESTORED THE CAP-DEPENDENT PROTEIN TRANSLATION BLOCKED BY RAPAMYCIN.

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Eukaryotic translation initiation factors play an important role in the control of protein translation and regulated by distinct factors. Among the regulatory factors, eIF4E and its binding protein 4E-BP1 have focused the most attention. The reversible binding between eIF4E and 4E-BP1 can be maintained through phosphorylation state of 4E-BP1. Rapamycin has shown to affect translation by changing state of phosphorylation of 4B-BP1. In this study, the effect of NNK on protein translation was examined on human bronchial epithelial cells (NL-20 cells). Following incubation with rapamycin, various concentrations of NNK were treated on NL-20 cells. The concentration of NNK was determined by MTT assay and the results showed the normal cell viability during the test period. De novo protein synthesis was measured through the incorporation of 35S-Met into NL-20 cells and the result revealed that NNK increased protein synthesis compared to rapamycin alone. In Western blot, rapamycin blocked phosphorylation of 4E-BP1, and decreased the eIF-4E protein. However, NNK restored the rapamycin-induced phosphorylation of 4E-BP1 and increased eIF4E level in a concentration-dependent manner. The alterations of the protein expression level of cyclin D1, cyclin E, cyclin A, cyclin B, cyclin G, p53, p21 and other related protein (c-myc, Erk1) were analyzed. Our results showed that cyclin D1, cyclin E, cyclin G and Erk1 were over-expressed in all groups of NNK with rapamycin, while cyclin A, cyclin B, p53, p21 and c-myc had no crucial change in NNK with and without rapamycin. Therefore, rapamycin blocked cap-dependent protein translation, decreased de novo protein synthesis, however, NNK restored phosphorylation of 4E-BP1, and increased eIF4E, cyclinD1, cyclinE, cyclinG and Erk1. These results suggest that NNK can pertubate the protein translation, and cell cycle control through cap-dependent pathway in NL-20 cells. Supported by BK21

1164 DISSOCIATION OF PRIMARY PATHOLOGIC CHANGES FROM SECONDARY INFLAMMATORY EFFECTS IN PHOSPHODIESTERASE INHIBITOR-INDUCED VASCULOPATHY.

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This study was conducted to discern primary pathologic events from secondary inflammatory changes associated with Phosphodiesterase (PDE) inhibitor-induced vasculopathy in rats. We also aim to ascertain whether metabonomic changes associated with this vasculopathy reflect secondary inflammatory changes or primary

pathologic effects. Mesenteric vasculopathy was induced in rats by 3 daily oral doses of the PDE IV inhibitor CI-1018 at 750 mg/kg. The inflammatory component of the lesions was diminished by administration of the glucocorticoid dexamethasone or by depleting peripheral blood neutrophils with rabbit anti-rat PMN antiserum (ANS). Metabonomic analysis was conducted on urine samples and select mediators, thought to be involved in PDE inhibitor-induced vasculopathy, were assayed. Serum interleukin-6 was measured by enzyme immunoassay, plasma endothelin-1 (ET-1) was determined by radioimmunoassay, and inducible nitric oxide synthase (iNOS) and the cleaved form of caspase-3 was determined by immunohistochemistry. DEX or ANS decreased the incidence and severity of vascular lesions induced by CI-1018, but a characteristic minimal vascular lesion remained in the absence of inflammatory infiltrates. In rats given CI-1018 alone, increases in serum IL-6 were observed, accompanied by increased immunoreactivity of medial smooth muscle cell and endothelium/inflammatory cell infiltrates for caspase-3 and iNOS, respectively. Plasma levels of ET-1 were decreased. Inhibition of the inflammatory response by DEX attenuated changes in IL-6, ET-1, iNOS, and caspase-3. In metabonomic studies, principal component pattern separation between control samples and samples from rats given CI-1018 in the presence or absence of DEX or ANS were evident. These data suggest that metabonomic profile shifts induced by CI-1018 may be associated with primary pathologic changes while changes in IL-6, ET-1, iNOS, and caspase-3 reflect secondary inflammatory responses.

1165 IDENTIFICATION OF BIOMARKERS FOR WITHERING SYNDROME IN RED ABALONE USING NMR-BASED METABONOMICS.

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Physical, chemical and biological stressors can induce adverse biochemical effects within marine organisms, including alterations in gene expression, protein concentrations, and metabolic status. Whereas transcriptomics and proteomics have emerged as powerful tools for assessing the effects of stressors in the aquatic environment, metabonomics has yet to receive attention. High-resolution nuclear magnetic resonance (NMR) spectroscopy is particularly appropriate for investigating metabolic status, since potentially hundreds of endogenous metabolites can be quantified rapidly in tissues or biofluids, with minimal sample preparation. This approach will be illustrated by our study of withering syndrome (WS) in red abalone (*Haliotis rufescens*), an important aquaculture species along the Californian coast. Although of bacterial origin, recent studies suggest that the presence of the pathogen in combination with elevated seawater temperatures can synergistically stimulate WS pathogenesis. Before investigating the potential roles of environmental stressors, including pollutants, biomarkers of WS must first be identified. As such, one-dimensional ^{1}H NMR spectra of hemolymph, digestive juice, and perchloric acid extracts of foot muscle and digestive gland, from both healthy and diseased abalone have been recorded. Many metabolites have been identified and subsequently confirmed by two-dimensional NMR methods. Preliminary principal component analyses of the foot muscle data have revealed several differences in metabolite levels between healthy and diseased animals. These include decreased levels of valine, alanine, carnitine, glycine, tyrosine, phenylalanine and glycogen, and elevated lactate, betaine and taurine in withered abalone. Analyses of the other tissues and biofluids are underway. The identification of biomarkers will provide a powerful tool for characterizing the effects of stressors on WS pathogenesis and for determining the efficacy of feed-based antibiotics that are currently under development.

1166 GLOBAL PROTEOMICS FOR HUMAN BLOOD SERUM I: MULTIDIMENSIONAL SEPARATION OF PEPTIDES COUPLED WITH MASS SPECTROMETRY.

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The importance of early detection in many human diseases has been well documented. New methods taking advantage of the rapid advancements in mass spectrometry are now being used to identify new biomarkers linked to human disease. Proteins in human serum range in concentration over at least 7 orders of magnitude, while the most advanced mass spectrometry technologies allow measurement of proteins with concentration ranges up to about 5 orders of magnitude. One method to reduce the concentration range would be to remove or deplete high abundance proteins, such as albumin. This study uses a combination of powerful mass spectrometry technologies with improvements in sample preparation to perform a global proteomic analysis of the proteins found in human serum. This analysis can then be used as a foundation for measuring differences in protein content and concentrations of serum samples with onset and progression of disease. Two complementary sample preparation methods were compared to ascertain differences. The first method depleted immunoglobulin content from serum and used strong cation exchange chromatography (SCX) of the trypsin-digested serum peptides for analysis by capillary reversed-phase liquid chromatography (LC) with ion trap tandem mass spectrometry. This method successfully identified 500 serum proteins in about 80 fractions. A complementary second method removes immunoglobulins and albumin, and selects for cysteine containing peptides. 87 proteins were identified with this method, in a single fraction. 70% of these proteins were identified in the previous analysis.

1167 IDENTIFICATION OF DOXORUBICIN-INDUCED CHANGES IN PROTEIN FEATURES IN RAT SERA USING CLUSTER ANALYSIS AND EXPRESSION PROFILING.

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Two-dimensional gel electrophoresis is a powerful technology that can be used to discover biomarkers associated with drug toxicity. The present study was initiated to determine whether specific proteins associated with doxorubicin (DOX)-induced cardiotoxicity could be identified using this methodology. Serum samples were collected from male spontaneously hypertensive rats (SHR) treated with DOX, with/without pretreatment with the cardioprotectant dexrazoxane (DZR). Protein features from the 2-D gels were measured using quantitative image analysis to create protein expression maps (PEMs). To compare PEMs, the signal intensities for each feature were assigned to an index of corresponding pI and relative molecular mobility (RMM) coordinates. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to test the ability of the PEMs to discriminate between treatments. Expression profile matching was used to identify protein features that displayed a particular pattern of interest. Animals that were treated with either saline or DZR clustered together, indicating that the cardioprotectant had little effect on serum proteins by itself. The animals treated with DZR-DOX clustered together but fell between the controls and the DOX cluster of animals, consistent with the partial attenuation seen in the myocardial lesion scores and cardiac troponin T levels observed with DZR pretreatment. Several protein features that correlated with a pattern of complete or partial protection by DZR pretreatment could be identified using expression profile matching. Some of those features that have been identified and annotated include: alpha-1-antiproteinase, fibrinogen, anti-thrombin III, and several apolipoproteins, suggesting the presence of a chronic underlying systemic immune response to the cardiac tissue injury. These data indicate that 2-D gel analysis of serum identified a consistent set of protein features correlating with both myocardial injury and cardioprotectant activity.

1168 MEASUREMENT OF BLOOD BREVETOXIN LEVELS BY RADIOIMMUNOASSAY OF BLOOD COLLECTION CARDS AFTER ACUTE, LONG-TERM AND LOW DOSE EXPOSURE IN MICE.

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Blood collection cards provides an efficient sample collection, storage and extraction format for biomonitoring toxins in living animals. One potential application of this method is to assess exposure to brevetoxin during red tide events. This report evaluates a radioimmunoassay (RIA) using a sheep anti-brevetoxin serum to detect brevetoxin extracted from blood dried on cotton fiber filter cards from mice treated with the brevetoxin congener (PbTx-3). The RIA has high affinity for PbTx-3 (EC50= 1.2 nM (n=10)) and recognizes both type 1 and type 2 brevetoxins. We first analyzed a sample set from an acute time course exposure, using a maximal non lethal dose (180 μg/kg) for 0.5, 1, 2, 4 and 24 h. Blood brevetoxin levels were at mean levels of 36 nM at 30 minutes and maintained levels above 25 nM during the 1-4 h time points. We next analyzed blood brevetoxin exposure after longer durations (0.5, 1, 2, 3, 4 or 7 days). Mean blood brevetoxin levels were measured at 26.0 nM at 12 h decreased to 8.2 nM at 1 day and 1.3 nM at day 2. Values at days 2-7 were higher but not significantly different from vehicle treated animals. We next determined the lowest measurable dose using increasing concentrations of PbTx-3 (10-300 μg/kg). Analysis of the blood samples at 60 min, revealed a linear relationship between external and internal dose (r2 = 0.993). All doses of brevetoxin administered were detectable at 1 h with the significant levels found for the lowest administered dose of 10 μg/kg, a dose that was ten fold lower than that which caused observable symptoms. Radioimmunoassay provided sensitive detection with minimal interference of residual matrix following extraction of dried blood from

the cards and allowed detection of brevetoxin at doses ten times lower than the lowest observable effect level and at times up to two days after exposure. This work does not necessarily reflect NOAA or EPA policy.

1169 COMPARATIVE ANALYSIS OF REACTIVE OXYGEN SPECIES IN HUMAN PLASMA AND BLOOD.

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Reactive oxygen species (ROS) are commonly associated with diseased states (including asthma, cardiovascular disease, cancer) infections, and exposure to various toxicants in humans. It is of interest in epidemiology studies to characterize the association of oxidative stress in relation to health/exposure status to ascertain which biological media, plasma or whole blood, is a more sensitive measure of ROS. In our study we evaluated differences between plasma and whole blood in the levels of ROS-induced chemiluminescence (using lucigenin as a chemiluminescence agent) measured simultaneously from the same individuals (n=194 human subjects) over a 3-week study. Mean ROS-induced chemiluminescence measured in plasma (590.8 ± 193.6; mean ± SD; Relative Luminescence Units (RLU)) was approximately 2-fold greater (significant at $p \leq 0.001$) in comparison to whole blood (280.7 ± 204.6; RLU). Although whole blood ROS levels were less than plasma ROS levels, we did observe that the inter-individual variation was greater for whole blood compared to plasma. These data suggest that human plasma is a more sensitive biological matrix to measure the levels of ROS-induced chemiluminescence. We have an opportunity to assess associations between ROS levels and modulators of ROS including diet, age, gender, and smoking status. This abstract does not necessarily represent USEPA policy.

1170 BIOAVAILABILITY OF BISPHENOL A: PREDICTION OF ESTROGEN DISRUPTION IN HUMANS.

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We are concerned over the potential low dose and long term exposure of the general population to exogenous dietary xenoestrogens like bisphenol A (4, 4' -isopropylidene-2-diphenol, BPA). BPA exposure may occur in humans since BPA is found in the lining of food cans and dental sealants. We developed a PBPK/PD model to simulate the distribution of BPA and endogenous estrogen (E2) with serum hormone binding globulins (SHBG) and albumin (ALB). The model used blood and tissues concentrations following oral low and high doses of 14C-BPA in female F-344 rats to estimate tissue distribution coefficients, metabolism and excretion parameters. The model was used subsequently to predict the plasma E2 bound to SHBG (E2-SHBG), ALB (E2 -ALB) and free fractions of E2 of British and Japanese women since we hypothesize that BPA could indirectly alter free E2 level, and a good correlation was reported to exist between estrogen bound (E2-SHBG and E2-ALB)and rates of breast cancer in these women. We propose using the model predictions with the plasma SHBG/ALB bindings as alternative tests to predict the effects of endocrine disruptors.

1171 BIOLOGICAL MONITORING OF BISPHENOL A IN A KOREAN POPULATION.

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To conduct biological monitoring of environmental exposure to bisphenol A (BPA), which has potential of endocrine disruptor, we analyzed conjugated BPA in blood and urine in a Korean population (N = 60) using reverse phase- HPLC/FD (average levels of serum- and urinary BPA, 1.5 ng/ml (range, 0-36 ng/ml) and 5.63 ng/ml (range, 0-147 ng/ml), respectively). After geometric transformation of the levels, we found a positive association between serum- and urinary BPA levels (p = 0.03). The level of serum-BPA was approx. 3-fold lower than that of urinary BPA. For proper BPA biological monitoring, host susceptibility need to be investigated. For this purpose, we further studied effects of genetic polymorphism, R184S occurred in exon 1, in UDP-glucuronosyltransferase (UGT) 1A6, which are involved in BPA metabolism. Genotyping was done by real time PCR with our designed primers and probes. As results, allele frequency of wild (w) and mutant (m) was 0.83 and 0.17, respectively and followed Hardy-Weinberg's law. Average levels of serum- and urinary BPA were higher in w/w subjects (N = 40) than others (N = 20: w/m, 18; m/m, 2). However, it was not significant (p = 0.10 and 0.32 in serum- and urinary BPA, respectively). As UGT1A6 genetic polymorphism affects its activity, metabolism of BPA can be affected by UGT1A6 genetic polymorphism. Further enlarged study is required to clarify effects of UGT1A6 genetic polymorphism on BPA.

1172 PITFALLS OF BROAD-BASED CHEMICAL SCREENING OF FIRE RESPONDERS IN CALIFORNIA: A FALSE ALARM ON CYANAZINE HERBICIDE.

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This case study describes a medical toxicology review on chemical screening tests administered to fire fighters, police, and others who responded to a 2-week long smoldering fire at a greenwaste processing facility in southern California. A few of the nearly 200 fire responders reported health complaints about 4 weeks after fire ended. Blood testing for an herbicide panel revealed presumably high amounts of blood cyanazine, a triazine herbicide that is carcinogenic in animals. Normal range data were not available on blood cyanazine due to its relatively limited applications. Based on the initial findings, other fire responders were offered screening that again revealed frequently elevated values. Several patients were advised that their elevated blood cyanazine levels may indicate appreciable risks of cancer. More focused evaluation of the cyanazine data revealed high blood cyanazine levels in control subjects and others with little or no contact with the site or fire emissions. Repeat samples in some individuals showed higher cyanazine levels weeks after initial blood sampling. No dose-response trends were apparent, even among individuals with expected high exposure duration and magnitude. Also, cyanazine was not registered for use in southern California, and would be readily destroyed by fire. Subsequent investigations revealed the cyanazine findings were anomalous; the clinical assay for this rarely tested pesticide exhibited an interference from blood caffeine (and therefore frequent false positives). Moreover, cyanazine exhibits relatively rapid excretion (within days), and hence would not be expected to remain elevated from an exposure that ended several weeks earlier. We conclude that broad-based chemical screening, in the absence of well founded study design and cautious interpretation and validation relative to exposure factors, can adversely affect the monitored population by inducing unwarranted fears and anxiety and by generating unnecessary medical screening and treatment costs.

1173 VALIDATION OF FUMONISIN BIOMARKERS IN HIGH-RISK POPULATION OF LIVER CANCER.

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Fumonisins (FNs), especially fumonisin B1 (FB1), are carcinogens and strong tumor promoters in animal models. Human populations in certain areas of world are exposed to higher levels of FNs through contaminated dietary components. FB1 has been linked to high incidence of primary liver cancer in certain areas of China. FB1- induced biochemical alterations in animal and human cell models, such as disruption of sphingolipids metabolism, has been proposed to serve as potential biomarkers; however, validation of these biomarkers in humans, especially in high-risk populations has not been done. In this study, we collected a total of 155 urine and blood samples from residents of three villages in Fusui County, Guangxi, China. These areas have very high liver cancer incidence and mortality rates. Metabolites of sphingolipids, sphinganine (Sa) and sphingosine (So), were measured using the method of HPLC-fluorescent detection with D-erythrosphingosine (C20) as the internal standard. The measured levels of Sa and So were normalized with the urinary creatinine level. The average ratio of Sa/So urine samples (n=155) is 0.29 with 95% of confident limit (CI) at 0.24-0.34. Female samples (n=55) had higher average ratio of Sa/So (0.37, 95% CI: 0.27-0.49) than the ratio obtained from males (n=100), which average ratio is 0.25 with 95% CI: 0.20-0.31. Dietary intake of FB1 was monitored for 1 week in a sub-study group that consisted of 19 males and 15 females from different households among the studied population. FB1 was detectable in 30 out of 34 (88.2%) food samples collected from household of study participants. Daily intake of FB1 was greatly varied among participants and ranged from 25 ug/person to 1, 083 ug/person. The association between dietary exposure to FB1 and metabolites of sphingolipids in blood and urine was further examined. (Supported by the NCI grant CA94683).

1174 ANALYSIS OF URINARY 1, 1, 2, 2-TETRACHLOROETHYLENE (PERC) METABOLITES BY HPLC ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) AS POTENTIAL EXPOSURE BIOMARKERS.

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The industrial solvent and dry cleaning agent, PERC (CAS 127-18-4), produces liver tumors in mice and nephrotoxicity and renal tumors in male rats. This toxicity may involve reactive intermediates which could induce similar toxicity in workers. Bioactivation of PERC is reported to occur by oxidation by CYP2E1 and GSH. Trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are reported PERC urinary metabolites and the GSH conjugate is cleaved to S-(1, 2, 2-trichlorovinyl)-L-cysteine (TCVC) and acetylated to N-ac-TCVC. A biomonitoring method was developed to measure urinary levels of TCA, DCA, TCVC and N-ac-TCVC with deuterated TCVC, N-ac-TCVC and DCA as internal standards. Samples were loaded onto SPE columns and PERC metabolites were eluted with acetone, dried and diluted in MeOH for HPLC ESI-MS/MS analysis on a Phenomenex Jupiter C18 column. A 10-min linear gradient (55:45 H2O:MeOH 1% acetic acid to MeOH 1% acetic acid) at 300 µL/min eluted the compounds of interest within 12 min. The mass spectrometer was operated using ESI-MS/MS, 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. Some urine samples from laundry workers with no demonstrated PERC exposure and dry cleaning workers that were potentially exposed to PERC during loading or unloading of garments contained detectable amounts of PERC metabolites in urine collected during pre or post-shift periods. The limit of detection (LOD) was 0.47 pmol for TCVC, 0.8 pmol for N-ac-TCVC, 11.45 pmol for DCA, and 7.17 pmol for TCA. A small number of samples contained amounts of PERC metabolites that exceeded the limit of quantification (LOQ) - 4.68 pmol for TCVC, 8.03 pmol for N-ac-TCVC, 114.5 pmol for DCA, and 71.73 pmol for TCA. The analysis appears to offer significant advantages over typical extraction and derivatization procedures required for GC-MS analysis of these compounds. Thus, PERC internal exposure levels may be quantified by HPLC ESI-MS/MS analysis of these metabolites.

1175 LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS IN AMNIOTIC FLUID SAMPLES FROM SMOKERS AND NONSMOKERS.

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Previous studies from this laboratory have focused on the characterization of blood protein adducts formed *in utero* as a result of maternal smoking during pregnancy. These biological samples, obtained during the third trimester of pregnancy, at delivery, have clearly shown a correlation between maternal smoking and exposure of the fetus to tobacco smoke carcinogens, including 4-aminobiphenyl and benzo(a)pyrene. In the present study, we examined exposure of the fetus during the first trimester of development to various environmental carcinogens, particularly those found in tobacco smoke. Amniotic fluid samples were obtained from women undergoing routine amniocentesis at between 16 and 20 weeks gestational age. Amniotic fluid, produced by the fetal lungs and kidneys, is an important part of pregnancy and fetal development and this fluid surrounds the fetus throughout pregnancy. In these studies, samples of amniotic fluid were obtained from nonsmokers as well as 0.5 pk/da smokers through >2pk/da smokers. Amniotic fluid samples were extracted and analyzed by HPLC and GC/MS for the presence of polycyclic aromatic hydrocarbons (PAHs). Amniotic fluid levels of PAHs were found in almost all samples analyzed. However, there was a clear correlation between levels of maternal smoking and PAHs in the amniotic fluid. 1-hydroxypyrene levels ranged from 1.54 ± 0.12 µg/ L in nonsmokers to 11.72 ± 0.67 µg/L in women smoking >2 pks/da, indicating approximately a 10X increase over nonsmokers. Similar results were found with more widely established carcinogens, including hydroxylated benzo(a)pyrene derivatives, which ranged from 1.41 ± 0.13 µg/L in nonsmokers to 11.56 ±0.59 µg/L in >2pk/da smokers. These results indicate that exposure to harmful environmental carcinogens can occur during early gestational periods and may place the fetus at a risk of genotoxic as well as teratogenic events.

1176 HYPOURICOSURIA, A BIOMARKER OF INORGANIC ARSENIC EXPOSURE.

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Arsenic (As) is a human carcinogen, affecting million of people worldwide. Most ingested As is excreted in the urine within a few days, making measurements of urine As a good biomarker of recent As exposure. However, other biomarkers for assessing continue As exposure or toxic As effect are necessary. The catabolic steps that generate uric acid from nucleic acids and free purine nucleotides involve degradation through purine nucleoside intermediates to hypoxanthine and xanthine. The latter are ultimately oxidized to uric acid in man using sequential reactions catalyzed by the enzyme xanthine oxidase. In rats, the presence of uricase transforms uric acid to allantoin. Arsenite (AsIII) complexed xanthine oxidase preventing reduction of the enzyme by purines. This effect causes xanthinuria and hypouricosuria. Urine is the preferential material for screening of abnormal metabolism of purines. Three different models of arsenite (AsIII) exposure were evaluated, human or rats exposed chronically and mice exposed to 0, 3, 6 and 10mg/kg daily during 9 days. Urinary uric acid from 97 individuals chronically exposed to water naturally contaminated with As content (129 ppb) were significantly lower (2.4 mg/L) than those found in 28 control people (3.2 mg/L). Urinary levels of uric acid in rats treated daily with 1.2 mg As/kg v.o during six weeks were evaluated. A significant decrease of uric acid concentrations in the urine was observed during the first 3 weeks of As exposure. Significant dose-response decrement of urinary uric acid was observed in mice treated orally during 9 days with arsenite. These results suggest that uric acid may be a useful easy biomarker for assessing continue As exposure.

1178 ANALYSIS OF PLASMA AND URINE FOR METABOLITES FOLLOWING INHALATION EXPOSURE OF FEMALE AND MALE MICE AND RATS TO 1, 3-BUTADIENE OR 1, 2-DIHYDROXY-3-BUTENE.

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1, 2-Dihydroxy-3-butene (ene-diol) is a marker for the flux through the hydrolysis detoxication pathway of butadiene (BD) that leads to the major human urinary metabolite, M1 (diolmercapturate). Ene-diol is also the precursor to 1, 2-dihydroxy-3, 4-epoxybutane (EBD), which is the major source of BD-induced protein and DNA adducts in all species studied. To characterize the impact of ene-diol formation during BD metabolism *in vivo*, we evaluated the levels of ene-diol as well as the urinary metabolites MI and MII (monohydroxy mercapurate) in mice and rats following inhalation exposure to BD or directly to ene-diol. Metabolites were analyzed by gas chromatography equipped with mass selection detection, with both the cis- and trans-isomers measured as total ene-diol. In mice and rats necropsied immediately after nose-only exposure to 0, 62.5, 200, or 625 BD for 6 h, the dose-response curves for ene-diol in plasma had positive curvature (e.g., in control and BD-exposed female mice, the respective plasma levels were 0, 0, 171 ± 6 ng/ml, and 10, 480 ± 3, 320 ng/ml). MI and MII in 24 h urine samples were increased above background after BD exposures, but only MI was increased after exposure of animals to 6 or 18 ppm ene-diol for either 6 h or for 4 weeks (6 h/day, 5 days/wk). Lower levels of ene-diol, compared with MI, were also detected in urine samples from animals exposed to BD or ene-diol (e.g., in female rats exposed to 200 ppm BD, the levels of ene-diol and MI were 41 ± 2 ng/mL plasma and 44 ± 20 mg/mL urine, respectively). Repeated exposures to these levels of ene-diol led to increased frequencies of *Hprt* mutations in mice and rats (see companion abstract by Q. Meng *et al.*). The study indicates that ene-diol is rapidly cleared after low-level (< 200 ppm) BD exposures but accumulates following high-level exposures. Further studies are needed to determine the fraction of ene-diol that is cleared *via* formation of the EBD compared to excretion of ene-diol and M-I.

1179 URINARY (2-METHOXYETHOXY)ACETIC ACID: AN EFFECTIVE GAS CHROMATOGRAPHIC TEST METHOD FOR QUANTIFICATION.

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(2-Methoxyethoxy)acetic acid (MEAA) is a metabolite and biomarker for exposure to 2-(2-methoxyethoxy)ethanol (diethylene glycol monomethyl ether, DEGME, or DiEGME) and bis(2-methoxyethyl) ether (diglyme); both are glycol ethers and are of concern because of the general toxicity of these compounds. Glycol ethers have been frequently reported to damage the male reproductive system, hemaopietic system, and fetal/embryonic development. Occupational exposure by these widely used glycol ethers is likely, since they are readily absorbed through the skin. Specifically, 2-(2-methoxyethoxy)ethanol is used as an anti-icing additive to the military jet fuel JP-8, and bis(2-methoxyethyl) ether is an aprotonic solvent with industrial uses and is a component of some hydraulic fluids including brake fluid. A simple and effective general test method for MEAA in urine samples was developed to monitor any exposed population. Urine specimens were first spiked with deuterated (2-butoxy)acetic acid, which was used as a procedural internal standard. The samples were extracted with ethyl acetate, concentrated, and treated by acid catalyzed esterification to produce the corresponding ethyl esters of MEAA and the internal standard. Subsequently, the ethyl ester derivatives were extracted using meth-

ylene chloride and concentrated to produce the final solution for gas chromatographic analysis. A mass selective detector (MSD) using a 50-m X 0.20-mm (id) HP-1 capillary column and a temperature program of 50 to 230C was used for the gas chromatographic measurement. Ion m/z 59 was monitored for the ethyl ester of MEAA and ion m/z 66 was monitored for the internal standard. A recovery study using 2, 10 and 20 micrograms/mL MEAA spiked urine samples demonstrated good accuracy and precision; recovery varied between 95-103%. The limit of detection (LOD) was found to be approximately 0.1 micrograms/mL (0.8 micromoles/L) for this analysis method.

1180 DETERMINATION OF PLATELET ACTIVATION LEVELS AND TIME COURSE IN CYNOMOLGUS MONKEYS.

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Blood platelets are essential for normal blood clotting; however, excessive platelet activation can contribute to thromboses that can lead to disseminated intravascular coagulation, heart attacks and strokes. Evaluation of platelet activation is an important part of safety assessment of therapeutics and several markers have been described for clinical evaluation of platelet activation. This study examines platelet activation assays for potential use in preclinical safety evaluation studies performed in Cynomolgus monkeys (Macaca fascicularis). Platelet activation levels and kinetics were tested using ELISA and flow cytometry methods for measurement of plasma levels of b-thromboglobulin, P-selectin, platelet factor-4, and cell-surface expression of P-selectin (CD62P) on platelets. Essential to measurement of platelet activation was the minimization of non-specific activation during blood collection using syringes containing CTAD (citrate, theophylline, adenosine, and dipyridamole) anticoagulant. Upon determination of antibody crossreactivity in Cynomologus monkey samples, the degree and time course of platelet activation were examined. The b-thromboglobulin average baseline level in CTAD anticoagulant samples was 13.2 +/- 4.6 IU/mL (SEM, n=4). In comparison, b-thromboglobulin levels in samples obtained using 3.2% w/v sodium citrate anticoagulant had an average b-thromboglobulin level of 179.6 +/- 3.3 IU/mL (n=4). Sodium citrate plasma samples with *in vitro* platelet activation using 20 mM adenosine diphosphate (ADP) had an average b-thromboglobulin level of 175.2 +/- 3.7 IU/mL (n=4). Similar results were seen for CD62P levels detected by flow cytometry. This study should identify reliable sample collection and analytical methods to be used for the determination of platelet activation in the Cynomolgus monkey as part of safety assessment of therapeutics.

1181 SENSITIVITY OF SPECIFIC BIOCHEMICAL MARKERS TO PREDICT CATECHOLAMINE INDUCED CARDIOMYOPATHY IN CYNOMOLGUS MONKEYS.

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In the last 10 years relatively sensitive and specific peripheral biochemical markers of cardiomyopathy have been characterized. These biochemical markers, creatine phosphokinase (CPK) and its isoenzymes, myoglobin, and more recently, the troponin T and troponin I subunits of the troponin protein complex permit identification of cardiac injury. This study evaluated the feasibility of using of CPK and its isoenzymes, myoglobin, and troponins T and I for the detection of myocardial injury in cynomolgus monkeys (*Macaca fascicularis*). Myocardial injury was induced chemically by slow, short-term infusion of norepinephrine bitartrate. Ten animals were implanted with telemetry transmitters configured to provide lead II electrocardiograms (ECG) and measurements of central arterial pressure and body temperature. A two-hour continuous intravenous infusion of norepinephrine (0.0625 mL/kg/min) was used to induce cardiomyopathy. Cardiovascular parameters were collected by radiotelemetry, continuously between prestudy and 7 days post-dose. Blood samples for measurement of cardiac biomarkers were collected predose and at various time points, beginning 2 hours after the end of infusion and continuing until necropsy and gross and histological evaluation, 7 days after treatment. Postmortem examination confirmed cardiac injury ranging in severity from minimal to marked. The severity of myocardial lesions was well correlated with serum troponin levels and ECG abnormalities. This data indicated that, of the biochemical markers evaluated in this study, troponin T and troponin I were the most specific and sensitive indicators of cardiac muscle injury. The results of this study indicate that serum troponin levels rise quickly and persist long after cardiac injury making them a more reliable indicator of cardiac muscle injury by permitting confirmation of cardiac injury well after other biochemical markers cease to be increased. Furthermore with coexistent skeletal muscle injury, troponins were near normal while the other biochemical markers were generally increased.

1182 MECHANISM-BASED URINARY BIOMARKERS OF RENAL PHOSPHOLIPIDOSIS AND INJURY.

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In animals treated with aminoglycoside antibiotics renal phospholipidosis causes nephrotoxicity and limits the dose and length of drug therapy. Therefore, a biomarker that can be used to monitor phospholipidosis before the onset of nephrotoxicity would be extremely valuable for preclinical and clinical safety studies and could be used for candidate screening. Megalin, a glycoprotein receptor, is expressed in numerous tissues (renal proximal tubule (RPT), lung, testis and macrophages)that are sensitive to the development of phospholipidosis. Although the physiologic role of megalin in the RPT brush border membrane is to mediate the uptake of proteins (e.g. albumin) and receptor-vitamin complexes (e.g. retinol binding protein-retinol) from the glomerular filtrate, it also mediates the uptake of polybasic drugs such as gentamicin by the RPT, a process necessary for the development of RPT phospholipidosis, injury and cell death. In theory, toxicants that are substrates for megalin binding should decrease megalin-mediated uptake of endogenous substrates by competing for megalin binding and preventing the recycling of megalin. The result should be an increase in excretion of endogenous substrates (e.g. retinol, albumin, and cubulin) in the urine in an amount that is proportional to degree of competition. In male Sprague-Dawley rats treated with gentamicin we show that urinary retinol and albumin excretion (ca. 2-fold increase) are early and sensitive markers of renal phospholipidosis that precedes both intracellular accumulation of phospholipids (sudan black staining), formation of myeloid bodies (confirmed by electron microscopy), and histologic and serum chemistry evidence of renal injury. This work supports the usefulness of monitoring urinary retinol and albumin excretion for early detection and monitoring of renal phospholipidosis and resultant injury.

1183 ELECTROENCEPHALOGRAPHIC RESPONSE TO ACUTE 3-NITROPROPIONIC ACID (3-NPA) EXPOSURE.

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3-NPA is a suicide inhibitor of succinate dehydrogenase (SDH) and has been implicated in food poisoning in China following ingestion of moldy sugarcane. SDH is the TCA cycle, as well as oxidative phosphorylation Complex II, enzyme. Its inhibition leads to cellular energy deficits. Studies have shown that energy deficit in GABAergic neurons after 3-NPA exposure led to an increase in GABAergic neurotransmission. This effect may be associated with the depression of motor activity and somnolence in the acute stage of 3-NPA neurotoxicity. However, after the acute phase, interference with GABAergic neurotransmission may increase excitability and lower threshold for seizures as well. In order to assess the effect of 3-NPA on electrocerebral activity, the ECoG was recorded in conscious, adult, male Sprague-Dawley rats *via* bipolar, epidural electrodes implanted at the level of the somatosensory cortex. Following baseline recording (saline injection), rats were injected s.c. with 3-NPA at 30 mg/kg or pretreated with L-carnitine (LC), an enhancer of mitochondrial energy metabolism, administered i.p. at 100 mg/kg prior to 3-NPA. The power spectra obtained by use of Fast Fourier Transformations were divided into 1.25-4.50 Hz (delta), 4.75-6.75 Hz (theta), 7.00-9.50 Hz (alpha-1), 9.75-12.50 Hz (alpha-2), 12.75-18.50 Hz (beta-1), and 18.75-35.00 Hz (beta-2). Treatment with 3-NPA was associated with a trend toward a power decrease in the delta frequency band. Following pretreatment with LC prior to 3-NPA, ECoG power in the right hemisphere was elevated relative to the left hemisphere in all frequency bands. Results indicate dominance of inhibitory neurotransmission during the acute stage of 3-NPA-induced neurotoxicity and a stimulatory action of LC on mitochondrial energy metabolism.

1184 A COMPARATIVE RELIABILITY STUDY OF THREE TEST BATTERIES: THE BEHAVIORAL EVALUATION FOR EPIDEMIOLOGY STUDIES (BEES), THE NEUROBEHAVIORAL EVALUATION SYSTEM2 (NES2) AND THE BEHAVIORAL ASSESSMENT AND RESEARCH SYSTEM (BARS).

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The BEES is distinguished from other batteries by its focus on re-engineering traditional tests on a touch-screen. Psychometric properties are compared with those for the NES2 and BARS. The tests include Finger Tapping, Digit, Spatial, and Auditory Span, Pattern Discrimination, Pattern Memory, Simple and Choice

Reaction Time, Switching, Tracking, Symbol-Digit, Trails A/B, and Vigilance. Adaptive vocabulary estimates pre-morbid intelligence. Test selection was based on sensitivity to insults, age, and recommendations by WHO and the ATSDR. A test-retest study was conducted among 20 subjects who were administered the three test batteries. Comparisons were based on pairwise cross-correlations, and attenuation adjusted correlations. Mean stability was evaluated using linear and non-linear components. Unadjusted cross-correlations were modest. The only test that measured the same performance across batteries was Symbol-Digit. Reaction Time was correlated between the BARS and NES, but not with the BEES. After adjustment for attenuation, the correlations were markedly stronger between the BARS and NES and between the BEES and NES, but not between the BEES and BARS. Tests from the BEES were shorter, had the highest unadjusted reliability, and had the highest 3 min. adjusted reliabilities (>.80). BEES test scores also achieved linearity with repetition and homoscedasticity of variance across trials. Six factors were identified that assess memory, decision time, hand speed, pattern analysis, and hand-eye flexibility with respective reliabilities of 0.90, 0.92, 0.90, 0.95, 0.90, and 0.89. The profile across batteries was altered by unequal reliabilities and differences in what-is-being-measured which is influenced by the interface. The psychometric properties of the BEES supports recommendation for repeated-measures assessments of pre-clinical performance where first trial stability is desired. Supported by ES04696 and ES07033.

1185 LIVER TOXICITY PREDICTION AND CLASSIFICATION USING MICROARRAY DATA: APPLICATION OF REFERENCE DATA-TRAINED MODELS TO CUSTOMERS' DATA SETS.

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The promise of toxicogenomics in identification of the general toxic response lies in its ability to assay the entire transcriptome for toxicity-related changes relative to a baseline. To that end, we have built a large reference database of gene expression data that contains thousands of liver samples from rats treated with hepatotoxicants, non-hepatotoxicants, and multiple vehicle controls. We have identified predictive gene markers and have built multiple predictive models that can discriminate general toxic responses, multiple pathologies, species specificity, and individual compounds solely based on gene expression data. These models are validated not only by cross-validation techniques, but also by using compound-treated sample data from multiple pharmaceutical companies worldwide. In some cases, these are proprietary compounds that were part of the pharmaceutical company's drug development pipeline. For the majority of the gene expression data we received from external companies, our models are insensitive to differences associated with chip processing and biological (e.g., strain) parameters. Results pertaining to the cross-validation of internal training set data and external data from pharmaceutical companies will be presented and compared. Our results show that the high success rates associated with external data are approximately the same as rates associated with our own internal data, indicating that variability across sample generation and chip processing sites does not preclude the successful identification of liver toxicity. Our models have also shown that gene expression data can be a more sensitive indicator of human-specific effects than classical measures.

1186 COMPARISON OF MICROARRAY DATA GENERATED FROM THE SAME RNA AT 15 DIFFERENT PROCESSING SITES.

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Differences in microarray processing protocols, reagent stocks, and production lots are just a few of the many parameters that cause a yet undetermined amount of process variability ultimately reflected in resultant signal intensity data. There are many components necessary to effectively characterize baseline differences in data due to these factors. One of these is the assessment of a common batch of total RNA at each site to separate processing variability from biological variability. In order to estimate data compatibility between our large reference gene expression database of over 1000 rat liver samples and data from external sources, we shipped eight total RNA samples to 15 different sites representing six pharmaceutical companies worldwide. Four biological replicates each of samples treated with chloroform and samples treated with vehicle were submitted. Each facility processed the RNA using their own protocols, reagents, QC metrics, personnel, and chip lots and ran the resultant cRNA over the Affymetrix RGU34A chip. Data sets were sent to Gene Logic for analysis. Statistical analyses such as ANOVA, PCA, correlation, and pair-wise scatterplots are being used to determine overall data compatibility. The fragment probe sets being used for the assessment include all tiled genes, genes known to be expressed in the liver, genes known to be invariant within the liver across treated samples, and predictive markers of a general toxic response in the liver. The results to date show far more gene changes due to site than toxic effects when using all tiled probe sets. However, liver-expressed genes and predictive toxicity markers characterize the toxic response over and above the site differences. In addition, the predictive markers of liver toxicity are reliable predictors across data sets from different sites of chip processing. The predictive models are able to predict vehicle controls and their toxin-treated counterparts correctly despite differences in chip processing protocols.

1187 USING GENE MARKERS IDENTIFIED FROM A LARGE DATABASE BUILT WITH PRIMARY RAT HEPATOCYTES FOR PREDICTION OF HUMAN HEPATOTOXICITY.

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To prioritize lead compounds early in development, companies need an assay that requires little compound, and is higher throughput than animal studies, yet provides a prediction of a compound's potential human hepatotoxicity. To produce such an assay, we have built a large reference database of gene expression data that contains thousands of primary rat hepatocyte cultures treated with hepatotoxicants and non-hepatotoxicants. The compounds represent all types of pathologies induced in humans and/or animals, including liver necrosis, cholestasis, hepatitis, and non-genotoxic carcinogenesis. The majority of the compounds used to build this database are marketed pharmaceuticals with well-known human safety profiles, including some that induce hepatotoxicity in humans but not rats. We have identified marker genes and built predictive models that classify samples into general toxicity, species specificity, and *in vivo* pathology categories. These models predict toxicity on a biological sample by sample basis, allowing individual replicate prediction in order to provide a more accurate result. Preliminary testing has shown these models to have a high true positive rate and an acceptable false positive rate on a sample by sample and compound by compound basis. These models are validated using 1) statistical cross-validation approaches, 2) tests with data generated with new compounds at our site, and 3) customer-generated data supplied to us in a blinded fashion. Our models have shown the ability to predict drugs that induce toxicity across species as well as some that are human-specific hepatotoxicants. To date we have received one data set from a customer. Our prediction revealed no false positives on a sample by sample basis and accurate prediction of 4 peroxisome proliferators. These included WY-14643, a recognized compound, and 3 proprietary drugs, none of which had been used to build our predictive models. These preliminary results suggest that primary rat hepatocytes can be used to predict human hepatotoxicity.

1188 CHANGES OF GENE EXPRESSION PROFILES IN STABLE RENAL TUBULE EPITHELIAL CELL LINES AS BIOMARKERS OF DRUG-SPECIFIC TOXICITIES.

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The therapeutic utility of many drugs is limited by their toxicity. Renal toxicity, a major reason for drug failures during development, has been observed with diverse drugs such as immunosuppressants, e.g. cyclosporine, and aminoglycoside antibiotics, e.g. gentamicin. Animal models for predicting nephrotoxicity are expensive, not very accurate. A human renal epithelial cell culture model system for evaluating drug toxicities would be highly desirable. Stable cell lines have been developed that represent the three major segments of the human renal tubule, i.e. proximal tubule (PT), thick ascending limb of Henles loop (TAL) and collecting tubule (CT). These cell lines retain their normal segment specific characteristics such as cytological structure, functional markers, characteristic responses to hormones and drugs. This study seeks to evaluate the applicability of these cell lines for identifying gene expression profiles as biomarkers of renal toxic drugs. The three cell lines were cultured in serum free, segment specific growth factor supplemented media with or without drugs with known renal toxicities *in vivo*, including cyclosporine, cisplatinum; gentamicin, tobramycin, amikacin and kanamycin. Appropriate low to high doeses of each drug were incubated with confluent cultures for 24 hours at 37oC prior to collection for RNA and protein extraction. The effect of these drugs was evaluated by gene expression microarrays using the Amersham CodeLink Expression Bioarrays. The results showed that human renal epithelia treated with these drugs responed in a dose-dependent fashion, and with the same segment-specific sensitivities (PT>TAL>CT) as seen *in vivo*. Specific gene biomarkers will be presented. These data suggest that human renal epithelia cultures represent a good model for *in vitro* testing of drugs for nephrotoxicity.

1189 MICROARRAY ANALYSIS OF NRF2 PATHWAY AND NOVEL CO-REGULATED GENES INDUCED BY ACETAMINOPHEN.

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Nrf2 regulates the expression of drug metabolizing enzymes and antioxidant genes essential for the detoxification of acetaminophen (APAP). Microarray analysis was employed to examine the global gene expression alterations in the Nrf2 pathway

and to discover novel co-regulated genes that may have potential functions in the Nrf2 pathway and/or APAP detoxification. Rats were treated with APAP and liver samples were collected 3, 6, and 24 hrs post dose. Gene expression changes were monitored using the Affymetrix RGU_34 GeneChip®. Data analysis revealed a cluster of phase II and glutathione pathway associated genes 24 hrs following APAP exposure. Many of these genes, such as Heme Oxygenase, UGT1A6, γ-Glutamylcysteine synthase, Aflatoxin B1 aldehyde reductase, and GSTa2, have been described as being transcriptionally regulated by Nrf2. Two interesting co-regulated genes were Protein Kinase C delta binding protein and UGT1A7, neither of which have been identified in the literature as being induced by APAP in rats and/or implicated as Nrf2-regulated at the transcription level. Clearly, the novel co-regulated genes UGT1A7 and protein kinase C delta binding protein will require further molecular characterization to determine the extent of their respective roles in APAP detoxification and/or the Nrf2 pathway. QRTPCR analysis corroborated the microarray results, indicating that APAP induced the gene expression levels of UGT1A7, Protein Kinase C delta binding protein, Heme Oxygenase, γ-Glutamylcysteine synthase, Aflatoxin B1 aldehyde reductase, and GSTa2. Furthermore, the induction of Nrf2 pathway components and phase II enzymes, such as members of the glutathione synthesis pathway were evaluated in both *in vivo* (rat) and *in vitro* (primary rat hepatocytes) models. Similarities and differences between the two models will be presented. Overall, the microarray analysis provided a comprehensive gene expression fingerprint of the changes in both the Nrf2 and glutathione pathways, allowing for the elucidation of novel co-regulated genes that may be intimately involved in APAP detoxification.

1190 IDIOSYNCRATIC DRUG REACTIONS: INVESTIGATING THE ROLE OF THE DANGER SIGNAL.

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BACKGROUND: Idiosyncratic drug reactions (IDR) occur rarely, but there are a great number of drugs which can induce these reactions in humans. Mechanisms of IDRs are unknown, but it is believed that most are immune-mediated. For an immune response to be induced, Poly Matzinger proposed in her Danger Hypothesis that host cells should be stressed. In an initial test of this hypothesis we treated animals with drugs known to induced IDRs and monitored two markers of cell stress. METHODS: Seventy-two female Balb/C mice were treated with one of the five drugs associated with IDRs: felbamate, clozapine, carbamazepine, phenytoin or lamotrigine. Mice were put in groups of four, and treated with the drug for 1, 3 or 5 days. Levels of NADPH quinone oxidoreductase 1 (NQO1) and glutathione-S-tranferase (GST) were analyzed in the liver and bone marrow tissues. RESULTS: NQO1 was induced in the liver by felbamate (p=0.004) and phenytoin (p=0.007) at day 1. Levels of NQO1 at day 3 and 5 remained elevated in both felbamate (p=0.001) and phenytoin (p=0.005) groups. Slight induction in hepatic NQO1 levels for mice treated with clozapine, carbamazepine or lamotrigine was also observed within five days. Hepatic GST levels increased at day 1 in phenytoin-treated animals (p=0.02), and at day 3 in the felbamate group (p=0.004) and in both groups remained elevated at day 5. GST levels in the carbamazepine group were elevated at day 1 (p=0.02) and they decreased by day 3 to control levels. In response to clozapine and lamotrigine, there was also a slight increase in GST levels. No NQO1 induction was observed in the bone marrows of any of the five different treatment groups. DISCUSSION: It appears that some drugs that cause IDRs do cause cell stress *in vivo*, but many further experiments will be required to determine if this is a characteristic feature of these drugs, and if so, what biomarkers best predict the ability of a drug to cause IDRs.

1191 ESTABLISHMENT OF BIOASSAY SYSTEM FOR ENVIRONMENTAL SAMPLES USING pERE-MCF-7 CELLS AND p1A1-HEPA I CELLS.

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In order to establish the rapid and easy-to-perform methods using ERE-MCF-7 cells by luciferase assay. MCF-7 stable cells which are stably transfected with phERE-Luc were treated with many chemicals and then luciferase activity were determined. Estradiol (E2) and synthetic estrogen, diethylstylbesterol (DES) were induced luciferase activity in dose dependent manner ranging 20-30 folds over that of control, and their activities were blocked by Tam treatment. 29 Flavonoids and 5 curcumin derivatives were tested in this system. Their E2 equivalent concentrations (EEQs) were calculated as a concentration of E2 that resulted in the same luciferase reading of test compound from the dose response curve. These data show that these methods are valuable tools for screening estrogenic activity of chemical. We have also examined the dioxin like activites using Hepa I cells were transfected with pCYP1A1-Luc. Environmental samples were extracted using combined solid-phase extraction in static adsorption mode with soxhlet extraction. The EEQ was determined based on the luciferase activity of 17 beta-estradiol. Kumho River of Korea showed 0.77 pM EEQ in upstrean and 7.7pM EEQ in downstream. Kum River of Korea showed 3.5pM and 1.7pM EEQ in upstream and downstream respectively. Mankyung River of Korea showed 61fM and 0.41 pM EEQ in upstream and downstream respectively. Miho Stream of Korea showed 0.2pM EEQ only in the upstream. All these water samples were tested with pCYP1A1-Luc activity using Hepa I cells containing pCYP1A1-Luc as described in pERE-Luc and results showed there were more dioxin like activities in sediments than water from the river. Domestic and industrial effluents have been discharged to that they presumed to be Kumho River, Kum River, Mankyung River and Miho Stream of Korea, so contaminated with various organic compounds. [Supported by grant from the Ministry of Environment of Korea]

1192 AN ENZYMATIC TISSUE DIGESTION METHOD FOR RECOVERY OF NYLON RFP FROM THE LUNGS OF EXPOSED RATS: VALIDATION STUDIES.

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The determination of lung burden in animal biopersistence studies requires that RFP (respirable-sized, fiber-shaped particulates) be recovered without loss or damage. Preparations from digested lung tissue must also contain a minimum of lung residue, so that accurate fiber counts can be made. Numerous reagents have been used to recover fibers from lung tissue, and these include, sodium hypochlorite (bleach, hydrochloric acid, hydrogen peroxide, and potassium or sodium hydroxide). Many of these reagents are useful for inorganic fibers but are not useful for organic fiber-types such as p-aramid, which are reactive in these oxidizing agents. The current studies were conducted to provide and validate a method for recovery of Nylon RFP from the lungs of rats following a 4-week inhalation study. Using *in vitro* pilot studies, we subjected Nylon RFP to varying concentrations of Clorox hypochlorite solution at several digestion time courses. A known quantity of Nylon RFPs incubated in saline was utilized as a control sample. The numbers and dimensions of Nylon RFPs were then evaluated both by phase contrast optical microscopy (PCOM) and scanning electron microscopy (SEM). The results from these studies demonstrated that the final digestion conditions for the most effective digestions were determined to be 25% filtered Clorox hypochlorite concentration = 5.25% normal Clorox/4 = 1.31% final hypochlorite solution), shaken vigorously for 30 seconds and incubated at 60 oC for 9 minutes. The digestate was then quickly deposited on filters for analysis. Subsequently, we conducted *in vivo* studies with known quantities of instilled Nylon RFP into the lungs of rats and implemented clearance (digestion) studies 4 hrs later to validate this methodology.

1193 DETERMINANTS OF URINARY PORPHYRINS AND MERCURY AMONG DENTAL PERSONNEL.

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Assessments of mercury (Hg)exposure require the use of a biomarker that demonstrates unique specificity and sensitivity to Hg as well as identification of determinants of Hg exposure that are prevalent in human populations. We investigated potential associations between urinary Hg, porphyrin excretion patterns, medical conditions and use of medications in 89 male dentists (DDS) and 109 female dental assistants (DAs).Subjects provided a urine sample and completed a questionnaire covering demographic data, symptoms (Neuroquest), mood (Profile on Mood States), personal habits, and medical and work histories. Crude correlations were used to identify a set of potential determinants ($p<.05$) that were later tested in multiple regression models. Differences between sexes were sufficient to support separate analyses. Pooled copro-, precopro-, and penta-carboxyl porphyrin levels were strongly associated (Beta =.42 or 0.49) with urinary Hg between 0-10 ug/l. Among DDS, only urinary Hg significantly correlated with urinary porphyrins, whereas among DAs, pack-years (Beta=.29), ever having had anesthesia (Beta= -.18), and having an immune (Beta= -.20)or endocrine (Beta= -.18) disorder were also correlated. Methyl mercury exposure (#fish/week) was not associated with porphyrin excretion. The determinants of urinary mercury also differed by sex with respect to work- related factors, personal factors. There was an inverse association with alcohol consumption. These observations support the utility of urinary porphyrins as a biomarker of Hg exposure in humans and suggests their utility as a measure of altered immune and endocrine and immune function in women. Supported by ES04696 and ES07033.

1194 AN INTERVENTION ANALYSIS FOR THE REDUCTION OF EXPOSURE TO METHYLMERCURY FROM THE CONSUMPTION OF SEAFOOD.

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Previously, an exposure model was developed (Risk Analysis 22:689-699, 2002) to predict levels of methylmercury (MeHg) in blood and hair in the US population based on the frequency of seafood consumption, the amount consumed per serving, and the types of seafood consumed. Steady-state relationships that employed descriptive statistics to account for pharmacokinetic variation were used to predict levels of mercury in blood and hair. The model incorporates an uncertainty dimension that is intended to represent the range of plausible interpretations of the data. In the present analysis, the model was used to predict the impact of limitations in the amount or types of seafood consumed on predicted blood and hair MeHg levels. Specifically, simulations for three scenarios were developed: 1) limitation of the consumption of seafood to 12 oz per week, 2) elimination of the consumption of shark, swordfish and other seafood with MeHg levels exceeding 0.5 ppm., and 3) limiting the amount of seafood consumed and eliminate consumption of high MeHg species (> 0.5 ppm). In the baseline model, the median (uncertainty) estimates for the 50th, 95th, 99th, and 99.9th per capita population percentiles were 0.6, 5.4, 9.9, and 15.9 ppb MeHg in blood, and 0.2, 1.8, 3.7, and 6.4 ppm MeHg in hair. After restriction of seafood consumption to no more than 12 oz per week, the median (uncertainty) estimates for the 50th, 95th, 99th, and 99.9th per capita population percentiles were 0.6, 5.0, 8.1, and 11.5 ppb MeHg in blood, and 0.2, 1.7, 3.0, and 4.6 ppm MeHg in hair. Elimination of high MeHg species, with average concentrations above 0.5 ppm, resulted in very modest decrements in MeHg blood and hair levels, in comparison to either the baseline or the reduced consumption scenario. These results suggest that strategies to reduce MeHg exposure by reducing the amount of fish consumed are more effective at trimming the high end of the US exposure distribution than are strategies intended to change the types of fish consumed.

1195 QUANTITATIVE DISEASE PREVENTION AND COST UTILITY CONSIDERATIONS FOR A SUITE OF BIOMARKERS FOR CHRONIC BERYLLIUM DISEASE.

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The value of the information provided by biomarkers for exposure-associated diseases is difficult to estimate. Chronic beryllium disease (CBD) provides an exceptional example to study this problem since there are well-developed biomarkers of susceptibility and exposure/early disease. CBD continues to occur despite major reductions in occupational exposure levels. We have previously developed a model for optimization of use of the beryllium lymphocyte proliferation test (BeLPT), an indicator of beryllium sensitization, which may be detected prior to development of CBD. Our model includes assumptions based on recent studies indicating that the development of a positive BeLPT is not dose related but the development of CBD is dose related. Our model also includes per worker cost considerations such as test cost, worker retraining, and cost of illness. Our results concurred with other recommendations of annual testing. CBD is strongly associated with a genetic polymorphism in the Human Leukocyte Antigen gene variant, HLA-DPβ1*201, characterized by glutamate substitution at position 69 (Glu69+). Here we expand our original model to include consideration of genetic testing prior to Be exposure and compare the cost per worker for individuals who are Glu 69+ to those who are Glu 69-. We used estimates of Glu 69 frequency in the general population (33%) and estimates of Glu 69 frequency in diseased persons (~90%) from the literature. The cost per worker calculated from our model for Glu 69+ and Glu69- workers was $150, 000 and $20, 000 respectively for workers exposed at 0.2 μg/m3 (ACGIH and USDOE recommendations) and given the BeLPT annually. This indicates significant financial and disease reduction value for encouraging alternative occupations for susceptible individuals and the importance of careful consideration of ethical ramifications of this information. This research was supported by the CRESP II(US DOE DE-FG26-00NT40938), CEEH(NIEHS 5 P30 ES07033) and CSIR.

1196 ATSDR'S INTERMEDIATE-DURATION ORAL MINIMAL RISK LEVEL FOR COBALT.

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The Agency for Toxic Substances and Disease Registry (ATSDR) recently released the Toxicological Profile for Cobalt as a draft for public comment. Developed under an interagency agreement between ATSDR and the Department of Energy, the cobalt profile updates the previous version released in 1992. The risk assessment for cobalt is interesting in that cobalt (specifically, cobalamin) is an essential component of vitamin B_{12}. Moreover, cobalt has been used in treating anemia because it stimulates the production of red blood cells in humans. Nonetheless, cardiomyopathy, gastrointestinal effects, visual disturbances, and thyroid effects following ingestion of cobalt (as cobalt sulfate in beer or as cobalt chloride as a treatment for anemia) are documented in the literature. Although no oral minimal risks levels (MRLs) were derived for cobalt in the 1992 document, a reevaluation of the toxicological database for cobalt resulted in the derivation of an intermediate-duration oral MRL of 0.01 mg/kg/day based on the study by Davis and Fields (1958). An intermediate-duration MRL is an estimate of daily exposure to a substance that is likely to be without an appreciable risk of adverse effects over a period of 15-364 days. In the Davis and Fields study, six male volunteers were exposed to 120 or 150 mg/kg/day of cobalt chloride (~1 mg Co/kg/day) for up to 22 days, resulting in polycythemia in all six subjects (16%-20% increase in pre-treatment red blood cell numbers). The implications of polycythemia observed from exposure to cobalt are unclear since the increase in red blood cells produced in this study likely produced no clinical signs. However, secondary polycythemia is clearly a biomarker of effect for cobalt exposure and a reasonable end point for the derivation of a health-based guidance value.

1197 BIOAVAILABILITY OF SILVER COMPOUNDS IN RATS.

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Studies were undertaken to estimate the relative bioavailability in rats of several silver compounds of photo processing and environmental significance. Several indices of bioavailability were evaluated, including tissue glutathione concentrations, glutathione peroxidase activity in tissues, and serum ceruloplasmin (CP) activity. CP activity was found to be the most sensitive and reproducible biomarker of silver exposure over relatively short exposure times. CP (EC 1.16.3.1) is a plasma glycoprotein, which, in addition to its ferroxidase and radical scavenger activity, is the primary copper transport and homeostasis protein in mammals, normally carrying 6-7 copper atoms per CP molecule. Silver(I) appears to deactivate CP when it is incorporated into the protein in place of copper during *de novo* synthesis in the liver. To estimate silver bioavailability, groups of 4 female SD rats were given 14-day repeat oral doses of the reference silver compound, silver acetate, dissolved or suspended in 1% aqueous methyl cellulose. Tissues were collected on day 15 and serum CP was assayed by measurement of its diamine oxidase activity. CP activity was depleted in a dose-dependent pattern, with >98% inhibition following a 960 μmol/kg dose of silver acetate. Male SD rats showed a similar dose response, but had only about ½ the native CP oxidase activity measured in females. Subsequent experiments indicated that similar responses could be obtained with as few as 4 daily repeated doses of 960 μmol/kg silver acetate. Four-day repeated dose studies in female rats were conducted with several other silver compounds, which resulted in variable levels of CP inhibition and revealed the following relative bioreactivity of silver from these compounds; **Silver Sulfide 〈 Silver Chloride 〈 Silver Thiosulfate ≅ Silver Acetate ≅ Silver Nitrate.** Silver sulfide, the ultimate environmental form of silver from photo processing discharges, had no effect on CP oxidase activity at 960 μmol equiv. silver/kg, indicating that it is the least bioavailable and bioreactive silver species among the common photo industry/environmental forms of silver.

1198 TERATOGENICITY OF CYANOBACTERIAL EXTRACTS TO FETAX EMBRYOS.

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Several species of cyanobacteria are known to produce potent toxins which have detrimental effects in wildlife, livestock and in humans. Several toxins (microcystin, saxitoxins, anatoxins etc.) may be produced simultaneously by one cyanobacterial species. The highest concentrations of these toxins, which are released upon cyanobacterial cell/filament rupture, are to be found in shallow water, which represents the primary location for aquatic early life-stage development. The effects of pure microcystin-LR (MLR) and RR (MRR) toxins on amphibian development, has been investigated. However, the effects of extracts from cultured cyanobacteria, which may contain several different toxins and hence may more accurately represent the situation in lakes and rivers, have not been assessed. Using the FETAX system, we have investigated the effects of two Planktothrix rubescens and two Microcystis aerogenosa extracts as well as pure microcystin-YR (MYR), on the development of Xenopus laevis embryos. The endpoints evaluated were mortality, malformation and growth demonstrated that neither P. rubescens extract nor MYR had any effect on embryonal survival. In contrast, M. aerogenosa extract caused a dose-dependent increase in mortality. All four extracts caused narrowing of the face in 96hr embryos. This was not observed in embryos exposed to MYR. Similarly, reduced embryonal growth, which was evident in embryos exposed to cyanobacterial extracts (M. Aerogenosa > P. rubescens), was not apparent in MYR-exposed embryos. This implies that the observed toxicity is not due to the presence of pure microcystin but rather other toxins produced by cyanobacteria.

1199 A RELIABLE SCORING SYSTEM TO ASSESS DEVELOPMENTAL TOXICITY OF ENVIRONMENTAL CONTAMINANTS USING ZEBRAFISH EMBRYOS.

C. Willett, T. Fremgen, P. McGrath and C. Zhang. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: P. Mayeux.

Biological testing has become an increasingly major component of ecological risk assessment and monitoring. A rapid, reproducible and inexpensive predictive vertebrate model for hazard identification would greatly contribute to ecological risk assessment. We are developing such a model using the zebrafish embryo, which has several inherent advantages: the free-living zebrafish embryo is completely transparent, facilitating experimentation and analysis, its entire body plan is established by 24 hours post-fertilization (hpf), and by 5 days post-fertilization (dpf), virtually all organs are functioning, including the liver. We have developed a simple, rapid and reliable scoring system to detect and quantify developmental toxicity. Endpoints include mortality, hatching frequency, heart rate, circulatory abnormalities and other morphological parameters. Embryos were treated by semi-static immersion in compounds diluted in fish culture medium from 24 hours post-fertilization (hpf) to 120 hpf; fresh compound was added daily. Endpoints were assessed every 12 hours until 72 hpf, and then every 24 hr until 120 hpf. Using this scoring system, we have analyzed five compounds representing different classes of environmental toxins: 2, 3, 7, 8-tetracholorodibnezo-p-dioxin (TCDD), benzene, hexachlorobutadiene (HCBD), ethanol and 2, 4-dinitrotoluene (DNT). Our results indicate that the scoring system is predictive of toxicity in other vertebrates, and that the zebrafish is a promising model for assessing environmental toxicity.

1200 DEVELOPMENTAL TOXICITY OF MIXTURES OF DI- AND TETRACHLOROETHANE AND DICHLOROPROPANE IN EMBRYO CULTURE.

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Drinking water chlorination results in numerous chlorinated byproducts. We evaluated the developmetal and embryo toxicity of 1, 3-dichloropropane (1, 3DP), 2, 2-dichloropropane (2, 2DP), 1, 1-dichloroethane (1, 1DE) and 1, 1, 2, 2-tetrachloroethane (TCE) in rat whole embryo culture (WEC) due to their presence in chlorinated drinking water, their structural similarities, and a lack of developmental toxicity data for these compounds. Humans could be exposed to these four chlorinated propanes and ethanes (CPEs) simultaneously in drinking water and hence we evaluated them alone and in combination. Toxicity profiles were generated by exposing gestational day (GD) 9.5 rat embryos in WEC to the CPEs for 48 hours. The individual CPEs were all dysmorphogenic in WEC and embryonic exposure resulted primarily in rotation and heart defects. The embryonic effects from exposure were compared based on developmental score (DEVSC), death and dysmorphology as the parameters of comparison. Concentrations of individual CPEs chosen for the mixture studies were predicted to produce DEVSCs 25% below control values. These equipotent mM concentrations (14.5 1, 1DE, 1.5 TCE, 16 2, 2DP, 5.5 1, 3DP) were then used to determine the toxicity of all possible combinations, based on a dose-additivity model, of the four CPEs. Eight of mixture combinations gave experimental DEVSCs which were not significantly different from the predicted scores while three of the mixtures (1, 3DP/2, 2DP;TCE/1, 3DP/2, 2DP;TCE/1, 3DP/2, 2DP/1, 1DE) gave scores which were significantly lower than predicted. Embryo mortality was additive in ten of the eleven treatment groups, with one mixture significantly more embryo toxic (27% mortality) than predicted. Dysmorphology was significantly elevated in all treatment groups compared to controls and was neither significantly different between the groups nor different from dysmorphology seen in embryos following exposure to the individual compounds. These data suggest that the developmental toxicity of these halogenated propanes and ethanes is additive. This abstract does not necessarily reflect EPA Policy.

1201 ZEBRAFISH BIOASSAYS FOR ASSESSING SUBSTANCE ABUSE.

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Substance abuse often leads to clinical impairment or distress resulting profound social, occupational and medical impact. New approaches to reduce the harm caused by the use of alcohol, tobacco, prescriptive and illicit drugs are urgently needed. Due to inherent attributes including, optical clarity, rapid development, a well-defined nervous system and predictable behavior, we used the zebrafish embryo as an animal model to study substance abuse. We observed growth retardation, abnormal morphology in the heart, brain, trunk, craniofacial structure and nerve system, as well as, induced apoptosis in response to continuous administration of alcohol. These results suggest ethanol-treated zebrafish embryos exhibit clinical manifestations similar to those of human fetal alcohol syndrome and it is therefore a model for studying the impact of alcohol and other substance abuse on fetus. In addition, using subtractive hybridization, we showed that gene expression is regulated in response to alcohol treatment.

1202 TEST RESULTS WITH EIGHT CHEMICALS IN A DROSOPHILA-BASED DEVELOPMENTAL TOXICITY PRESCREEN.

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To further characterize the Drosophila-based prescreen to detect developmental toxicants, the following 8 chemicals were evaluated - methyl mercury chloride (MMC), methotrexate (MTX), L-phenylalanine (LPA), sodium arsenate heptahydrate (SAH), cadmium chloride (CC), vinblastine sulfate (VBS), aminopterin (APN) and mitomycin C (MC). All of the test agents are mammalian developmental toxicants and/or teratogens. One-to-three experiments, each employing multiple concentrations and including a concurrent control, were conducted with each chemical using our published protocol (Teratogenesis, Carcinogenesis, and Mutagenesis 11:147-173, 1991). Drosophila were exposed throughout development (egg through third instar larva) in culture vials to medium containing the test chemical. A mated, untreated, Oregon-R wild-type female (Mid-American Drosophila Stock Center, BGSU, 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. In cases where replicate data were available at a given concentration, incidence data were also pooled and compared to the pooled controls. The incidence of bent humeral bristles was statistically increased ($p<0.05$) in flies exposed to MMC, LPA, SAH, CC, VBS, and MC. VBS also statistically increased ($p<0.05$) the incidence of eye defects. The incidence of wing blade defects was statistically increased ($p<0.05$) in flies exposed to MTX and APN. These results with 8 diverse chemicals provide additional support for increased utilization of this assay as a prescreen for the detection of developmental toxicants.

1203 ZEBRAFISH AS A PREDICTIVE MODEL FOR ASSESSING TOXICITY OF CHEMOTHERAPEUTICS.

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The efficacy of many chemotherapeutics is mitigated by high toxicity. A reproducible and inexpensive predictive method for screening novel therapeutics for general toxicity would be extremely valuable. We previously demonstrated the feasibility of using zebrafish embryos to study toxicity by assessing the LC50 of 20 commercially available compounds and found good correlation between zebrafish and other mammalian models. We have further tested the model by assessing the general toxicity, including the LC50, MTD, and target organ morphology, of 15 chemotherapeutic compounds, including paclitaxel, tamoxifen, dexamethasone and actinomycin D. Embryos were treated by semi-static immersion in compounds diluted in fish culture medium from 24 hours post-fertilization (hpf) to 120 hpf. Mortality was assessed and fresh compound was added daily. MTD and organ morphology were assessed at 120 hpf. Our data correlates well with mammalian data and supports zebrafish as an alternative animal model for general toxicity testing of pharmaceutical compounds.

1204 THE TERATOGENIC EFFECTS OF ENVIRONMENTAL ETHANOL EXPOSURE.

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The long-range goal of this project is to elucidate the molecular mechanism by which ethanol perturbs embryonic and fetal development, and to identify genes that plays a role in the sensitivity to ethanol-induced teratogenesis. Maternal exposure to ethanol from alcoholic beverages and many consumer products has been linked to developmental abnormalities in human and laboratory animals. Fetal exposure to alcohol is primarily dictated by voluntary maternal behavior and societal influences. Generally, consumption of alcohol induces alterations in facial features, major organs, and bone structures and will trigger damaging effects on the brain, which will lead to learning disabilities and behavioral problems. This can have a tremendous toll on the affected individuals, their families, and society as a whole. Data from twin studies and animal models argue strongly for a robust genetic component to ethanol-induced teratogenesis. In order to take advantage of the powerful genetic capabilities of the zebrafish to identify genes that influence sensitivity,

we must first identify the effects of ethanol exposure on zebrafish development. We have identified dose-dependent changes in zebrafish embryo/larval mortality, neurocranial and craniofacial skeletal development, CNS apoptosis, expression of selected developmentally regulated genes, and cardiac development and function. This data will be discussed in relation to our mutagenic screen for genes that influence sensitivity to ethanol-induced teratogenesis.

1205 CARBONYL PROTEIN ADDUCTS AS A BIOMARKER OF CIGARETTE SMOKE EXPOSURE IN MAMMALIAN CELLS AND HUMANS.

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Carbonyl protein adducts have been used as a biomarker of exposure for compounds or mixtures that can cause oxidative damage. Cigarette smoke can cause oxidative stress and contains many compounds that can cause oxidative damage. Specific examples include aldehydes (e.g., acrolein, acetaldehyde, formaldehyde) and some phenolic compounds (e.g., catechol, hydroquinone). Initial experiments to characterize the assay used a purified protein (bovine serum albumin) and a single compound (acrolein). Carbonyl protein adduct formation was linear with addition of 0-100 ug/mL of acrolein to the bovine serum albumin. A one-hour exposure of acrolein to the protein resulted in the maximum concentration of carbonyl protein adducts. The objective of subsequent experiments was to quantify the formation of carbonyl protein adducts *in vitro* and in humans after exposure to cigarette smoke. Carbonyl protein adducts were measured in Chinese hamster ovary (CHO) cells after exposure to single compounds found in cigarette smoke; cigarette smoke condensate from a reference cigarette; or the whole smoke from the same reference cigarette. Aldehydes incurred a greater amount of carbonyl protein adducts in CHO cells than phenolic compounds. CHO cells exposed to reference cigarette smoke condensate or whole reference cigarette smoke had higher levels of carbonyl protein adducts than the respective controls. The carbonyl protein adducts in a buccal wash from smokers and non-smokers indicated an approximate 4-fold increase of adducts in smokers compared to non-smokers. The measurement of carbonyl protein adducts serves as a sensitive biomarker for cigarette smoke exposure and may serve as an assay to guide the development of potential reduced risk cigarettes.

1206 HEMOGLOBIN ADDUCTS FROM *N*-METHYLOLACRYLAMIDE IN RATS: COMPARISON WITH THOSE FORMED BY ACRYLAMIDE.

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Acrylamide (AM) and *N*-methylolacrylamide (NMA) are used in the formulation of grouting materials. AM is metabolized to a reactive epoxide, glycidamide (GA). Both AM and GA react with hemoglobin to form adducts which can be related to exposure to AM. To evaluate whether NMA could also cause the same hemoglobin adducts as AM, *N*-(2-carbamoylethyl)valine (AAVal derived from AM) and *N*-(2-carbamoyl-2-hydroxyethyl)valine (GAVal derived from GA) were measured following a single oral dose of AM (50 mg/kg) or NMA (71 mg/kg) in male F344 rats. Blood was collected by cardiac puncture at 24 h following dosing, globin was isolated, and AAVal and GAVal were derivatized by a modified Edman degration to produce phenylthiohydantoin derivatives. The adduct derivatives were analyzed by liquid chromatography/mass spectrometry. In the AM-treated rats, AAVal was 21 ± 1.7 pmol/mg globin (mean ± SD, $n = 4$), and GAVal was 7.9 ± 0.8 pmol/mg. In the NMA-treated rats, AAVal was 41 ± 4.9 pmol/mg, and GAVal was 1.4 ± 0.1 pmol/mg. This study indicated that AAVal and GAVal can be detected following administration of NMA. Whether AAVal was derived from reaction of NMA with globin followed by loss of the hydroxymethyl group from the adduct, or loss of the hydroxymethyl group to form AM with subsequent reaction with globin is not known. However, the much higher ratio of AAVal:GAVal in the NMA-treated rats (29 vs. 2.6 in AM-treated rats) suggests that reaction of NMA with globin is the predominant route to these adducts in NMA-treated rats, rather than conversion to AM. The detection of GAVal in NMA-treated rats indicates oxidation of NMA, either directly, or following conversion to AM. The lower levels of GAVal on NMA administration suggests a much lower level of epoxide formed in these animals compared with AM treatment.

1207 CYCLIC N-TERMINAL HEMOGLOBIN ADDUCT AS A BUTADIENE DIEPOXIDE BIOMARKER.

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1, 3-Butadiene (BD), a widely used industrial chemical that is carcinogenic in animals and probably humans, is oxidized by cytochrome P450 to diepoxybutane (DEB), the most mutagenic of BD metabolites. DEB can react with the N-terminal valines of hemoglobin (Hb), forming the cyclic adduct (2, 3-dihydroxypyrrolidin-1-yl)-3-methylbutanoic acid (PYRV). All three DEB metabolites (two enantiomers and one meso form) are expected to form PYRV adducts, resulting in three diastereomers. These adducts are potentially highly specific biomarkers for DEB. The aim of this study was to develop a sensitive isotope dilution mass spectrometric assay for measurement of PYRV in globin from animals and humans exposed to BD. Key to this procedure were the synthesis of alkylated terminal peptide (PYR-VLSPADKTNVK for the human α-chain) as standard and its stable isotopically labeled analog ($^2H_3$) as internal standard (IST), as well as developing a reliable procedure for concentration of the modified heptapeptide (PYR-VLSPADK) after trypsin hydrolysis. The syntheses of the standard and IST were accomplished by incorporation of PYRV (both OH groups protected by t-butylation) to the synthesized peptide sequence LSPADKTNVK. The peptides were characterized by ESI-MS/MS sequencing and exact mass measurement. The presence of all three diastereomeric peptides in the synthesized standard was established by 2D COSY ^{1}H NMR. The standard PYR-VLSPADKTNVK provided a hapten to raise polyclonal antibodies (AB) for immunoaffinity (IA) enrichment. Trypsin digested samples of standard and IST were applied on IA columns, washed extensively and eluted in 10% formic acid. Quantitation was performed by LC-ESI-MS in SIM mode, using the masses of both singly and doubly charged ions. The results showed 60-80% recovery of PYR-VLSPADK peptide after IA separation. The present limit of detection for standards was about 150 fmol (injected). PYRV is a promising biomarker for humans and animals to better understand mechanisms of carcinogenesis and more accurately predict risk.

1208 LC-ESI-MS/MS QUANTITATION OF HEXENAL-DERIVED 1, N^2-PROPANODEOXYGUANOSINE ADDUCTS.

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E-2-Hexenal (hexenal) is an α, β-unsaturated aldehyde that is naturally formed in plants and is present in various flavorings. Hexenal has been shown to be genotoxic in a variety of *in vitro* assays and forms a pair of diastereomeric exocyclic 1, N^2-propanodeoxyguanosine adducts (H-dGuo 1 and 2) upon reaction with DNA. A sensitive and specific adduct quantitation method employing mass spectrometry is needed for measurements of adducts at low doses. To this end, we are developing and optimizing a method to quantitate H-dGuo in DNA. Analyte standards have been synthesized and characterized by UV, MS, and NMR. ESI-MS and ESI-MS/MS analysis revealed ions at the anticipated $[M + H]^+$ and [M + H − deoxyribose + H]$^+$ *m/z* for H-dGuo (*m/z* 366 and *m/z* 250, respectively). Synthesis and characterization of stable isotope internal standards ($[^{13}C_4\,^{15}N_2]$H-dGuo 1 and 2) are underway. Studies with H-dGuo spiked into DNA have shown that solid phase extraction (SPE) is an effective means of separating H-dGuo from unmodified nucleosides and other sample contaminants. LC-ESI-MS/MS was used to analyze H-dGuo spiked into DNA at varying concentrations and purified from the enzymatic DNA hydrolysate by SPE. Diversion of LC flow to waste prior to mass spectrometric analysis of H-dGuo was used to further enrich samples, and the selected reaction monitoring mode (SRM; *m/z 366→m/z 250*) was used to analyze H-dGuo. From this study, we estimated an approximate limit of quantitation of 4.2 H-dGuo 1 or 2 per 10^7 dGuo. We anticipate that utilization of an internal standard and further method optimization will allow accurate quantitation at lower concentrations of H-dGuo, allowing quantitation of H-dGuo in a variety of DNA samples. This work was supported in part by grants from the NIEHS (T32ES07126, ES11746, P30ES10126), the NCI (P30CA16086) and the Flavor and Extract Manufacturers Association (FEMA).

1209 IDENTIFICATION OF ADDUCTS FORMED BY THE REACTION OF ISOPRENE MONOEPOXIDES WITH 2'-DEOXYADENOSINE.

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Isoprene (IP, 2-methylbuta-1, 3-diene) is ubiquitous, since it is emitted by plants, produced by combustion processes, and is the major exhaled hydrocarbon by humans. It is also an important industrial chemical. Since IP exhibits differential tumorigenic activity in rats and mice, it is important to establish biomarkers for IP metabolites so that differences in endogenous and exogenous metabolism can be understood. These data should lead to a more accurate assessment of the risk of cancer for humans. In the present study 2'-deoxyadenosine (dAdo) and 2'-deoxyinosine (dIno) were incubated with IP monoepoxides isoprene-1, 2-oxide (IP-1, 2-O, 2-ethenyl-2-methyloxirane) and isoprene-3, 4-oxide (IP-3, 4-O, propen-2-

yloxirane). Racemic mixtures of IP-1, 2-O, as well as of IP-3, 4-O can form four adducts on each position of the nucleoside. These arise from attack of the enantiomeric epoxides with either their internal or external oxirane carbon. The adducts were separated by HPLC and identified by diode array UV detection and electrospray-mass spectrometry. NMR characterization, as well as studies with the individual epoxide stereoisomers are ongoing. HPLC-separation of the reaction mixtures of dAdo with IP-1, 2-O or IP-3, 4-O resulted in four or three N^6-dAdo adduct peaks, respectively. These were the main adducts. In addition, unstable adducts at the N1-position and their deamination products (N1-dIno adducts) were identified and characterized. Characterization of the nucleoside adducts of IP is the first step toward establishing useful DNA adducts as biomarkers of IP metabolism and exposure. N^6-adenine adducts have been used as biomarkers of exposure to butadiene in humans, however, these adducts have been shown to be non-promutagenic. Recent data suggest that N1-dIno adducts might provide a good biomarker since they are stable and potentially mutagenic.

1210 CHARACTERIZATION OF 1, N^2-PROPANODEOXYGUANOSINE ADDUCTS FORMED BY HYDROXYMETHYLVINYL KETONE.

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1, 3-Butadiene (BD) is carcinogenic in rodents and may also be a human carcinogen. While several epoxides formed during BD metabolism are mutagenic and may contribute to BD carcinogenicity, another proposed metabolite, hydroxymethylvinyl ketone (HMVK), could also play a role. By analogy with other α, β-unsaturated carbonyls, HMVK might be mutagenic *via* formation of promutagenic DNA adducts, specifically 1, N^2-propanodeoxyguanosine adducts. A significant quantity of HMVK is likely to be formed since it is a proposed intermediate in the metabolism of 3-butene-1, 2-diol (BD-diol) to 1, 2-dihydroxy-4-(*N*-acetylcysteinyl)-butane (MI), the major mercapturic acid metabolite of BD in humans. In addition, BD-diol is a major BD metabolite in rodents. Understanding potential contributions to BD carcinogenicity made by metabolites in this pathway is critical for accurate risk assessment. Since HMVK may be a significant metabolite in both humans and rodents it is necessary to determine if it is indeed able to form promutagenic DNA adducts. The hypothesis underlying this study is that HMVK forms 1, N^2-propanodeoxyguanosine adducts *in vitro*. To test this, HMVK was synthesized and incubated with 2'-deoxyguanosine at 37°C for ~24 hours under basic conditions. Adducts were separated from the unreacted nucleoside by HPLC. The resulting chromatograph showed two product peaks. Rechromatography of each collected peak yielded a chromatograph with the two original product peaks, suggesting equilibration of diastereomeric products. Full scan MS confirmed the expected molecular ion at m/z = 354 ($M + H^+$), and MS/MS gave a fragmentation pattern consistent with 1, N^2-propanodeoxyguanosine adducts. The chemical structure was definitively established by ^{1}H NMR. Proton assignments, verified by 2D NOESY and COSY experiments, confirmed the presence of the equilibrating diastereomers suggested by HPLC. The results show that HMVK is capable of forming promutagenic DNA adducts and could therefore potentially contribute to BD carcinogenicity.

1211 DEVELOPMENT OF NOVEL INTERNAL STANDARD DNA FOR DNA ADDUCT ASSAYS.

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Application of stable isotope labeled internal standards (IS) dramatically enhances the accuracy of mass spectrometry analyses for DNA adducts. IS bases or nucleosides, however, cannot reflect artifacts arising during DNA hydrolysis, such as additional oxidation of unmodified nucleotides, insufficient hydrolysis of DNA to nucleosides, and reactions with other molecules, all of which hinder accurate measurement. To overcome this, we prepared high purity ^{15}N-labeled DNA from *E. coli* after culture in minimum salt medium containing $(^{15}NH_4)_2SO_4$ as a single nitrogen source for bacterial growth. The presence of ^{14}N-purines was less than 10 ppb in labeled DNA. We utilized the ^{15}N-labeled DNA to measure pyrimido[1, 2-α] purin-10(3H)-one (M_1G), the major DNA adduct induced by malondialdehyde (MDA). Under physiological conditions, M_1G is hydrolyzed to a ring-opened aldehyde form by base pairing with dC, which does not occur with M_1G base or its nucleoside under same conditions. This difference limits the use of IS M_1G or IS M_1G-dR not only due to different recoveries, but also due to competitive reaction of the aldehyde moiety toward amines, including Tris, a common buffer. The ^{15}N-DNA was treated with MDA to induce a high number of M_1G adducts in the DNA. 23ng of IS was added to sample DNA followed by mild acid hydrolysis for depurination. Depurinated M_1G and $^{15}N_5$-M_1G were selectively labeled with aldehyde reactive probe (ARP). M_1G-ARP and $^{15}N_5$-M_1G-ARP were measured using LC/MS/MS with selected reaction monitoring (*m/z* 519.3 → *m/z* 188.2 for M_1G-ARP and *m/z* 524.3 → *m/z* 193.2 for $^{15}N_5$-M_1G-ARP). Using this IS DNA, 1~3 M_1G per 10^7 nucleotides were detectable in less than 10 μg of mouse liver DNA. ^{15}N-IS DNA preparation from labeled *E. coli* is a very time and cost efficient way to produce high quality ^{15}N-IS using a simple 2~3 day bacterial culture and routine DNA isolation without any chemical or enzymatic synthesis. Such ^{15}N-labeled DNA should be suitable for most of DNA adduct assays, including multiple DNA adduct arrays. Furthermore, it is useful for the study of any artifact induced during the DNA isolation/hydrolysis process.

1212 A NOVEL ASSAY TO QUANTIFY OXIDATIVE DAMAGE USING BASE EXCISION REPAIR ENZYME 8-OXOGUANINE N-GLYCOSYLASE.

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7, 8-dihydro-8-oxoguanine (8oxoG) is one of the major DNA base modifications resulting from oxidative stress. This base modification is repaired during the BER pathway by the excision of the damaged base by 8-oxoguanine-DNA glycosylase (Ogg1), which is a bi-functional enzyme with both glycosylase and AP lyase activity. Ogg1 excises oxidized purines by cleaving the N-glycosidic bond with an activated nucleophilic amino acid, which reacts with C-1′ of deoxyribose. This cleavage of the N-glycosidic bond creates a lesion known as the apurinic/apyrimidinic (AP) site, which may become mutagenic if not repaired by an AP lyase. The accurate quantitation of DNA adducts can provide us with an indication of exposure to mutagens or carcinogens and is an important tool in cancer risk assessment. Currently, there is no convenient method to measure oxidized purines in genetic material when only small amount of DNA are available. To address this limitation a new assay for oxidative DNA damage was developed. In this Ogg1-coupled Slot Blot assay, DNA (8-16$_{\mu g}$) is treated with sodium borohydride to reduce aldehydic sites. This DNA is then incubated with Ogg1 to excise oxidized purines and generate an aldehydic site. Finally, an aldehyde reactive probe (ARP) is added to the C-1′ aldehyde on the ring opened sugar resulting from the excision of the oxidized purine base from DNA, followed by application to a nitrocellulose membrane, chemiluminescence and densitometric quantitation. DNA from HeLa cells treated with submillimolar to millimolar concentrations of hydrogen peroxide was used to determine the assay's limit of detection at ~1 per 10^6 nucleotides. Further modification of this assay should lower its current limit of detection. This assay has the ability to measure 8-oxoG, its secondary products and other oxidized purines. The Ogg1-coupled slot blot assay cannot differentiate between these products, however this measurement may give a better idea of oxidative damage than the current methods for 8-oxoG.

1213 DETERMINATION OF DNA-ADDUCTS IN LIVER SAMPLES OF B6C3F1 MICE EXPOSED TO ETHYLENE OXIDE.

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Ethylene oxide (EO) is an important chemical intermediate in the polymer industry and can also be formed in humans by metabolism of endogenous ethylene. EO is known to be mutagenic and carcinogenic in rodents and reacts directly with DNA to form N-7-hydroxyethylguanine (7-HEG), O^6-hydroxyethylguanine (6-HEG), and N-3-hydroxyethyladenine (3-HEA). The purpose of this investigation was to refine a method we previously developed for analysis of these adducts by HPLC and tandem mass spectrometry (HPLC-MS/MS). The sensitivity of the method was improved, and the method was validated in detail. Within-series and between-day imprecision for 3-HEA and 6- and 7-HEG was between 3.9% and 14.6% depending on the analyte and concentration, while recovery was determined to be between 84.7 and 112.6%. The limit of quantitation (LOQ, calculated, S/N 10:1) is 0.01, 0.03, and 0.02 pmol/mg DNA for 3-HEA, 6-HEG, and 7-HEG, respectively. The method was used to analyze 3-HEA and 6- and 7-HEG in liver samples from B6C3F1 mice (n = 4) exposed to 100 ppm EO by inhalation for 6 h/d and 5d/wk for 6wk. Following neutral thermal hydrolysis, 7-HEG was determined to be between 21.9 and 27.3 pmol/mg DNA, while 3-HEA was between < LOQ and 0.02 pmol/mg DNA. No 6-HEG could be analyzed. To release 6-HEG from DNA, a second clean-up, including an acidic hydrolysis of DNA, was carried out. Nevertheless, no 6-HEG could be found in liver samples of exposed mice. DNA samples from liver tissue of control mice (n = 4), which were not exposed to EO, were also analyzed for their adduct levels. Only 7-HEG could be detected in the range between 0.21 and 0.45 pmol/mg DNA. No 3-HEA and 6-HEG could be found. The results presented here are part of an on-going study of DNA adduct dosimetry in various target and nontarget tissues of B6C3F1 mice, which were exposed to EO for 0, 10, 25, 100 and 200 ppm by inhalation for up to 48 wk.

1214 FORMATION OF DHP-DERIVED DNA ADDUCTS FROM METABOLIC ACTIVATION OF CLIVORINE, A REPRESENTATIVE OTONECINE-TYPE PYRROLIZIDINE ALKALOID, AND *LIGULARIA HODGSONNII* HOOK PLANT EXTRACT.

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Plants that contain pyrrolizidine alkaloids (PAs) are widely distributed in the world. PA-containing plant extracts and pure PAs have been shown to be genotoxic and tumorigenic in experimental animals. Our recent mechanistic studies indicated that riddelliine, a tumorigenic retronecine-type PA, induced liver tumors *via* a genotoxic mechanism mediated by the formation of eight 6, 7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP)-derived DNA adducts. To date it is not known whether this mechanism is general to PAs of other types, such as otonecine-type PAs. The *Ligularia hodgsonnii* Hook, an herbal plant used as antitussive Chinese medicine, was found to contain several otonecine-type PAs and clivorine is the predominant PA present in this plant species. Clivorine has been shown to induce tumors in rats. In this study, we report that metabolism of the PA-containing extract of this plant by F344 rat liver microsomes results in the formation of DHP. When incubation of this plant extract in the presence of calf thymus DNA, eight DHP-derived DNA adducts were formed. Similar results were obtained from metabolism of the isolated pure clivorine under similar experimental conditions. These results indicate eight DHP-derived DNA adducts were also formed from metabolic activation of otonecine-type PAs in the presence of DNA. This metabolic activation pathway involves (i) formation of the corresponding dehydropyrrolizidine (pyrrolic) derivatives through oxidative N-demethylation of the necine base followed by ring closure and dehydration; and (ii) binding of the pyrrolic metabolites to DNA leading to the DNA adduct formation and tumor initiation. The results also suggest that these eight DHP-derived DNA adducts are potential biomarkers of PA exposure and tumorigenicity.

1215 ASSESSMENT OF DNA STRAND BREAKS IN LEUKOCYTES OF WORKERS OCCUPATIONALLY EXPOSED TO 1-BROMOPROPANE.

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As part of two NIOSH Health Hazard Evaluations, the comet assay was performed on peripheral leukocytes from workers to determine if occupational exposure to 1-bromprompane (1-BP) induced DNA strand breaks. Start-of- and end-of-workweek blood and urine samples were collected from 41 and 22 workers at 2 facilities where 1-BP was used as a solvent for spray adhesives in furniture manufacturing. Exposure to 1-BP was assessed from personal breathing zone samples collected for 1-3 days up to 8 hrs per day for calculation of 8 hr time waited average (TWA) 1-BP concentrations. Bromide was measured in blood and urine as an internal biomarker of exposure. Overall, 1-BP TWA concentrations ranged from 0.2 - 271 ppm at site 1 and from 4 - 42.7 ppm at site 2. The highest exposures were in workers classified as sprayers. 1-BP TWA concentrations were statistically significantly correlated with blood and urine bromide concentrations. For estimation of DNA damage, comets were produced by alkaline microgel-electrophoresis and analyzed using VisComet image analysis software. One hundred leukocytes from each blood sample (stored at -80 oC until analysis) were evaluated for comet extent, tail extent, tail moment, and tail integrated intensity among other endpoints as a indexes of DNA single strand breaks/alkali labile sites. Start-of- and end-of-workweek comet endpoints were compared with environmental and internal exposure indices using linear regression analysis. Comet results were also stratified based on job classification. Preliminary analyses indicate that occupational exposures to 1-BP at these two facilities were not associated with the level of DNA strand breaks in leukocytes of exposed workers.

1216 THE OCCURRENCE OF SEVERE ALLERGIC REACTIONS TO FOOD IN NORWAY, BASED ON DOCTORS REPORTS TO THE NORWEGIAN REGISTER OF SEVERE ALLERGIC REACTIONS TO FOOD.

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Background: It is important to know the incidens of severe allergic reactions to Food. We have limited information about the problem in Norway, and the current register has been undertaken to obtain information about risk groups, offending foods, the circumstances under which the reactions take place, treatment and diagnostic follow-up. Methods: The reporting system became active July 1. 2000. Information material, reporting and informed consent forms were mailed to all identified first line care providers. Serum samples were analysed for specific IgE antibodies to common food allergens. Results: The first 18 months 100 cases were reported. There were 47% males and 53% females. There were marked peaks for young adults and small children. About 2/3 of cases were reported to have known allergies, and 55% had known food allergies. Asthma was reported in 26%. The location of food intake was restaurant (8%), party/visit (7%), institution (3%) home (1%) and unknown (81%). In about half of the cases some assumption could be made about the causative food, and most common were nuts, peanuts and shellfish. Of 12 food allergens used as a standard battery, seropositivity (UniCap*) was most common against peanut, hazelnut; shellfish; and celery (falling order). First symptoms were edema/swelling, urticaria, pruritus and gastrointestinal symptoms. About 50% had their reaction within 30 min after food intake. A strong correlation between seropositivity and early symptom onset was observed. The combination adrenalin/steroids/antihistamine was given in 30% of cases, 9% got adrenalin only, 34% were given various combinations without adrenalin. Bronchodilators was the main treatment in 3% of cases. No deaths were reported.

1217 REDUCTION OF FUMONISIN MYCOTOXINS IN BT. CORN.

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Biotechnology has made it possible to develop corn hybrids that are protected against a major corn pest, the European corn borer (Ostrinia nubilalis). The coding sequence for Cry1Ab protein derived from Bacillus thuringiensis (Bt.) has been introduced into corn plants (event MON 810). Cry proteins are the active insecticidal ingredients of Bt. microbial insecticides that have been safely used on agricultural crops around the world for 40 years. The Cry1Ab protein is produced throughout corn plant tissues providing season-long protection against corn borers. Decreased insect damage to corn kernals reduces ports of entry for fungi that produce fumonisin mycotoxins. Fumonisins are toxic to farm animals, cause cancer in rodents and may contribute to high cancer rates in farmers in Africa and China that consume large amounts of fumonisin contaminated corn. Field trials with event MON 810 hybrids conducted in the US, France, Argentina and Turkey found generally reduced fumonisin levels in event MON 810 hybrids. In US field trials, fumonisin levels in Bt hybrids were on average 57% of levels in non transgenic controls. Many sites had a 3 fold or greater reduction in fumonisin levels. In Argentina, fumonisin levels in a Bt hybrid were on average, 39% of controls. In France, fumonisin levels were decreased several fold in Bt. hybrids. In Turkey, fumonisin levels were decreased 7 fold in a Bt. hybrid. Implications of reduction in fumonisin levels on human an animal health will be discussed. In countries where fumonisin mycotoxin contamination is high and corn is a major dietary staple, fumonisin exposures can considerably exceed the TDI of 2 ug/kg body weight/day established by Codex. Biotechnology can help improve the food security of corn grain for human and animal consumption.

1218 SAFETY EVALUATION OF AN α-AMYLASE ENZYME PREPARATION DERIVED FROM THE ARCHAEAL GENUS *THERMOCOCCALES* EXPRESSED IN *PSEUDOMONAS FLUORESCENS* BIOVAR I.

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The engineered α-amylase, BD5088, derived from the domain *Archaea* and the genera *Thermococcus* and *Pyrococcus*, was selected based on its characteristics of low-pH optimum and high thermotolerance, properties that are suited to corn wet milling applications. The host organisms of the three α-amylases from which BD5088 was derived were isolated from marine hydrothermal systems. The recipient strain of the host that is used to produce amylase BD5088, *Pseudomonas fluorescens* DC88, was avirulent after oral administration to mice. An extensive battery of studies designed to meet FDA requirements for Generally Recognized As Safe (GRAS) substances was performed on the enzyme preparation. Analytical characterization confirmed the identity and composition. Repeat-dose oral gavage studies of the BD5088 enzyme preparation in rats, for up to 13-weeks duration, showed no systemic toxicity. Inflammation of the nasal mucosa and the lung was seen in some

rats and was attributed to regurgitation and / or aspiration of the test material that contained lipopolysaccharide from the *Pseudomonas* production strain. This interpretation was validated by administering the enzyme preparation to rats in-feed following pre-mixing in a corn oil slurry. The NOAEL was 890 mg/kg/day as Total Organic Solids. There was no genotoxic activity based on Ames (treat and plate), mouse lymphoma, mouse micronucleus and rat lymphocyte chromosome aberration tests. There was no evidence of allergic potential based on comparison with sequences contained in a database of known allergens and on a digestibility study in simulated gastric fluid. The margin of exposure (safety factor) is $>10^6$ for producing dextrose and fructose syrups by corn wet milling.

1219 CONTAMINATION RESPONSE SYSTEM AS FOOD SAFETY TOOL.

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The Food Safety and Inspection Service (FSIS), is a public health regulatory agency of the United States. Department of Agriculture (USDA). FSIS enforces the tolerances and action levels set by FDA and EPA. FDA has statutory authority for setting tolerances and/or action levels for veterinary drugs under the FFDCA. EPA has statutory authority for setting tolerances and/or action levels for pesticides under the FIFRA and FFDCA, as modified by FQPA. The Contamination Response System (CRS) is a part of the FSIS Residue Program that is designed to monitor, detect and identify, and reduce and control residues of animal drugs, pesticides, and other chemical contaminants in meat, poultry and egg products. The CRS is activated in the event of a chemical contamination and provides rapid response to suspicion or discovery of residues or other contaminants with the potential to cause widespread adulteration of meat, poultry and egg products. The CRS includes procedures for expediting communication within and between Federal agencies, collection of information, evaluation, and decision making. In a four year period (1998-2001), we identified 42 CRS cases. Highest number of cases reported in year 1999 (20 cases) followed by year 1998 (10), 2000 (6) and 2001 (6). Bovines had highest number (17 cases) of contaminants as compared to other species of the cases such as 7 incidents in horses, 2 in sheep, 8 in goats, 7 in pigs and 1 in turkey. Polychlorinated biphenyls (45%) are the most predominantly identified environmental pollutants. Other pollutants such as benzene hexachloride (1) diphenyl dichlorotrichloroethane (4), chlordane (4), carbodax (1), endosulfan (2), famphur (3), heptachlor (1), lindane (2), methoxychlor (1), pentachloroaniline (1), poly brominated biphenyls (1), phenylbutazone (1) and toxaphen (1) constitute the remainder. USDA/FSIS CRS program in conjunction with EPA and FDA plays a pivotal role in protecting consumers from meat and poultry products that contain chemical contaminants.

1220 ANTIMICROBIAL RESIDUES IN THE UNITED STATES HORSE, SHEEP AND GOAT MEAT.

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The National Residue Program of the Food Safety and Inspection Service (FSIS) of the USDA includes a comprehensive testing program for residues of pesticides, drugs and other chemical contaminants in meat and poultry. In the current investigation, nine years of data from the National Residue Monitoring Program (1992-2000) were examined. FSIS conducted 2112 analyses for antibiotics and 1369 analyses for sulfonamides in horses between 1992 and 2000. During this period only 154 and 5 violative animals for antibiotic and sulfonamides were found, respectively. Penicillin and streptomycin were the most commonly detected antibiotics in horse during the examined period (30 and 135 detection respectively). There was steady increase in the number of detections of penicillin (2 vs. 9) and streptomycin (10 vs. 58) during 1993-2000. Because there is no established tolerance for most of the examined antibiotics in horsemeat, any detected amount is considered a violation. Gentamicin and oxytetracycline residue were detected only once each during 1999 and 2000. During the 1992-2000 period, 2377, 3518, and 2586 mature sheep, lambs, and goats were tested for antibiotics respectively. During the same period, 1422, 2581, and 2361 mature sheep, lambs, and goats were tested for sulfonamides, respectively. Chlortetracycline and tetracycline were the most commonly detected antibiotics in lambs, with 8 and 6 detections respectively. Chlortetracycline was also the most commonly detected antibiotics in mature sheep. With five detections, sulfamethazine was the most commonly detected sulfonamide in goats. Only one mature sheep, eight lambs, and one goat carcass contained violative levels of antibiotics. Three lambs and seven goat carcasses contained violative levels of sulfonamides.

1221 URINARY METABOLITES OF AFLATOXIN IN DOGS AND DIETARY PROTECTION BY CLAY AGAINST CANINE AFLATOXICOSIS.

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Six dogs were given a single low level non-toxic dose of aflatoxin B_1. Aflatoxin M_1 detected in the urine regularly cleared the dog's system in 48 h. On average, 71.5% of this metabolite cleared in the first 6 h after dosing, increasing to 90.4% after 12 h. Aflatoxin metabolite P_1 was not found in the urine compared to large amounts of M_1 and trace levels of Q_1. In 1998, 55 dogs died in Texas from eating dog food contaminated with aflatoxin at levels ranging from 150 - 300 ppb. Corn used in the pet foods fed was contaminated with aflatoxin. NovaSil Plus (NSP) is a naturally occurring sorbent used as an anticaking agent in animal feeds. Previous research has shown that NSP is capable of tightly and selectively adsorbing aflatoxin *in vitro* and *in vivo*. Studies were conducted to investigate the ability of NSP to protect dogs from aflatoxin in the diet. In a crossover design, six dogs were randomly fed a complete and balanced diet containing no clay (control) or coated with NSP (0.5%) for a period of 7 days. On day 7, the dogs were given a low level dose of aflatoxin B_1. During the next 48 h, urine samples were collected and analyzed using HPLC. After a 5-day washout period, the diets were switched and the process repeated. Urinary metabolites were measured and compared between the controls and experimental dogs to determine the efficacy of NSP. The NSP diet provided an average decrease in urinary aflatoxin metabolites of 48.7% $\pm$ 16.6 S.D. versus the control diet. Studies incorporating NSP in the extrusion process are underway. Control and NSP-inclusion poultry and rice dog foods were extruded. Preliminary results of an eight dog crossover study indicate similar findings. In conclusion, NSP is effective in protecting dogs fed pet foods that contain aflatoxin. Despite regular screening of ingredients for aflatoxin, low levels may reach the final product undetected. NSP may provide the pet food industry further assurance of canine diet safety. (Supported by Engelhard Corp, Mark L. Morris Prof in Clin Nutr, and NIEHS P42-ESO4917)

1222 MATERNAL AND DEVELOPMENTAL ASSESSMENT OF MONTMORILLONITE CLAYS COMMONLY ADDED TO ANIMAL FEEDS: TOXICITY EVALUATION AND METAL BIOAVAILABILITY IN THE PREGNANT RAT.

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A variety of common clay minerals are frequently added to animal feeds to bind and reduce the bioavailability of mycotoxins in the gastrointestinal tract. However, the safety of many of these products has not been thoroughly investigated. Those that act nonselectively may interact with nutrients, minerals, and other feedborne chemicals to pose significant hidden risks. In this study, a calcium montmorillonite clay (NovaSil Plus), that is commonly used to bind aflatoxins, and a sodium montmorillonite clay (Source Clay Minerals Repository, Columbia, MO) were examined. Clay minerals were supplemented in the diet of pregnant Sprague-Dawley rats throughout pregnancy at a level of 2%(w/w). Evaluations of toxicity were performed on day 16 and included maternal body weights, maternal feed intakes, litter weights, and embryonic resorptions. Liver, kidneys, tibia, brain, uterus, pooled placenta, and pooled embryonic mass were collected and weighed. Tissues were lyophilized and neutron activation analysis (NAA) was performed. Elements considered by NAA include: Al, Ba, Br, Ca, Ce, Co., Cr, Cs, Cu, Dy, Eu, Fe, Hf, K, La, Lu, Mg, Mn, Na, Nd, Ni, Rb, S, Sb, Sc, Se, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, University, V, Yb, Zn, and Zr. Animals supplemented with either NovaSil Plus or sodium montmorillonite clay were comparable to controls with respect to toxicity evaluations and metal analysis suggesting that these clays do not interact with mineral utilization in the pregnant rat. Although aluminum is a major component in these clays (and of particular interest), the metal was not detected above background limits in any of the tissues evaluated. Thus, this study indicates that even at high concentrations in the diet, neither clay mineral results in maternal or fetal toxicity (Supported by NIEHS P42-ES04917, TAES H6215 and USAID TAM50).

1223 ENHANCED CLAY-BASED ENTEROSORBENT FOR THE PREVENTION OF AFLATOXICOSIS: *IN VITRO* AND *IN VIVO* CHARACTERIZATION.

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Appropriate interventions that can diminish foodborne exposure to mycotoxins are high priorities. A practical approach to the aflatoxin problem has been the dietary inclusion of a calcium montmorillonite clay (NovaSil) that preferentially binds aflatoxins in the GI tract of animals. In this study our objectives were to characterize

the sorption of aflatoxin onto the surfaces of an enhanced NovaSil clay, i.e. NovaSil Plus (NSP). Equilibrium adsorption of AfB_1 to NSP was investigated using isothermal analysis. Toxin was mixed with clay at different temperatures for 24 hr; the concentration adsorbed (mol/kg) versus the equilibrium concentration in the supernatant (mol/L) was compared to standard isotherms. Data were fitted to multiple isotherm equations including: Langmuir, Freundlich, Toth, and various transforms in order to gain an insight into the molecular mechanism(s) and site(s) of AfB_1 adsorption. NSP was shown to possess a higher capacity for AfB_1 (i.e., 459 nmol/mg) versus 325 nmol/mg for NS. The enthalpy of binding was centered at -49.2 kJ/mol, suggesting chemisorption of ligand. Distribution coefficients of 2.3 $x10^5$ and 7.5 x 10^5 (for NS and NSP, respectively) confirmed high affinity binding for both clays. The sorption of AfB_1 to heat-collapsed NSP was reduced by more than 90%, suggesting that the interlayer (and calcium ions) play a key role in the mechanism of binding. The composition of matter for both clays was determined using neutron activation analysis. Oxidative metabolites in the urine of AfB_1 exposed rats were decreased in the presence of both clays. Molecular models were generated for the NSP-AfB_1 complex and support the conclusion that AfB_1 may react at multiple sites, but especially within the gallery of NSP. In summary, NSP binds aflatoxin with enhanced affinity and capacity versus NS. The mechanism appears to be similar for both clays (Supported by USAID TAM50 and NIEHS P42-ES04917).

1224 CLINICOPATHOLOGIC CHARACTERIZATION OF FUMONISIN B_1 (FB1) INDUCED HEPATO-, NEPHRO- AND NEURO-TOXICITY IN HORSES.

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Fumonisins induce hepatotoxicity in all species. In horses, FB1 induces cardiotoxicity and death due to neurotoxicity (leukoencephalomalacia) or liver failure. To investigate the pathogenesis of fumonisin toxicosis in horses and attempt to determine a NOEL, we characterized the time and dose-relationship of FB1 induced serum and urinary biochemical changes and correlated them with morphologic alterations. FB1 was administered iv at 0.20, 0.10, 0.05, 0.01 or 0 mg FB1/kg BW/day (n=3 or 4). Horses were euthanized on day 28 or when neurologic signs became severe (≥7 days, ≥0.05 mg FB1/kg). FB1 induced dose- and time-dependent increases in serum total bilirubin, bile acids and total protein concentrations (at all doses); SDH, AST, ALP, GGT activities and cholesterol (≥0.05 mg FB1/kg); and creatinine concentration (≤0.10 mg FB1/kg). In urine, GGT activity and GGT:creatinine ratio were increased at all doses. In the nervous system, a dose-dependent vasculopathy with severe proteinaceous perivascular edema, leukocytosis and hemorrhage was present in brain and spinal cord at all doses. Icterus was present at ≥0.05 mg FB1/kg with hepatocellular apoptosis (± necrosis) at all doses but especially at 0.05 mg FB1/kg. Renal lesions were not observed. Therefore, FB1-induced serum biochemical changes occurred at all doses and correlated with morphologic alterations in liver but not in kidney. A NOEL was not determined. Supported by FDA and USDA-CSREES grant # 928-39453.

1225 DETERMINATION OF ZEARALENONE IN SOME CEREALS, NUTS AND DRIED FRUITS COLLECTED FROM ALEXANDRIA MARKETS.

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Wheat, corn and rice, of two production locations in El-beheira Governorate, and several nuts and dried fruits samples were collected from Alexandria markets and analyzed for the presence of zearalenone (ZEA). Samples were subjected for acid/base extraction and for cleanup with liquid-liquid separation. High performance liquid chromatography (HPLC) with UV detection was used for ZEA identification and quantitation. Results indicated that wheat samples collected directly after harvesting were ZEA-free, while 2 out of 3 wheat samples collected late post harvesting were positive for ZEA with concentrations of 877 and 921 ug/kg. Seven out of eleven corn and 2 out of 3 rice samples were positive for ZEA. The level of ZEA in six of the positive corn samples were in the range of 236 to 1970 ug/kg, while the level in the seventh sample sharply exceeded this range reaching unexpected value of 15703 ug/kg. This huge level could be attributed to the unacceptable storage conditions. Two out of the three tested rice samples were found to contain ZEA at levels of 254 and 476 ug/kg. Hazelnut; walnut; pistachio nut and coconut samples were positive for ZEA with ranges of 188-3838; 95-148; 84-808 and 35-768 ug/kg, respectively.Almond samples were ZEA-free and so were the tested dried fruit samples. Farmers and distributers of cereals and nuts should mentain good storage conditions for these products in order to reduce the risk to concumers by the generated mycotoxins.

1226 EFFECTS OF COOKING ON THE BIOLOGICAL ACTIVITY OF FUMONISINS.

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Fumonisin (FB) mycotoxins are found in corn and corn-based foods. Cooking decreases FB concentrations under some conditions, but little is known about how cooking effects its biological activity. Baked cornbread (BCB), pan-fried corn cakes (PFC), and deep fried corn fritters (DFF) were made from corn meal spiked with *Fusarium verticillioides* culture material (FCM) and fed to male rats (n=5) for 2 weeks at high (1% w/w FCM equivalents) or low (0.1 % FCM equivalents) doses. Controls were fed 1% w/w sound corn. Toxic response to BCB, PFC, DFF, and FCM included decreased body weight gain (1% diets only), decreased kidney weight, and microscopic kidney and liver lesions of the type caused by FB. FB concentration (FB_1 + FB_2) in the 1% w/w PFC diet (92.2 ppm) was slightly, but not significantly ($p<0.05$), lower than those of the 1% w/w BCB (132.2 ppm), DFF (120.3 ppm) and FCM (130.5 ppm) diets. In a second experiment, 10 g of corn containing 26 ppm FB_1, masa made from the corn, and baked and fried tortilla chips made from the masa were extracted twice with 50 ml acetonitrile/water (1:1). Extracts were combined, dried, and redissolved in 1.0 ml DMSO. Vero cells were dosed with 4 µl of the redissolved extracts for 48 hr. The corn extract inhibited ceramide synthase as indicated by significantly increased cell sphinganine (Sa) concentrations (mean=132 pmole/well), whereas Sa was unaffected by the masa, baked chip and fried chip extracts (mean Sa 14-24 pmole/well). FB_1 concentrations of the masa and chips (2.3-4.6 ppm) were likewise reduced more than 80% compared to the corn. In summary, baking and frying had no significant effect on the biological activity or concentration of FB in corn-based foods while, in contrast, nixtamalization reduced both biological activity and FB concentration in masa. Furthermore, these studies provided no evidence for the formation of unknown, biologically active FB products during cooking.

1227 FATE OF FUMONISIN IN MAIZE DURING NIXTAMALIZATION AND TORTILLA PRODUCTION BY MAYAN COMMUNITIES IN GUATEMALA.

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Fumonisin B1 (FB1) is a carcinogenic mycotoxin found in maize. In the preparation of tortillas, maize is boiled in a lime solution, which hydrolyzes at least a portion of the FB1 to the aminopentol backbone (HFB1). The alkali processing of maize (nixtamalization), when conducted in a laboratory or commmercial setting, reduces the total fumonisin content. The purpose of the present study was to 1) determine if the traditional method of nixtamalization as practiced by rural Kaqchikel-speaking Mayan communities in Guatemala reduces the level of fumonisins in tortillas produced from fumonisin-contaminated maize, and 2) determine the steps in the traditional process where reduction in fumonisin levels is most likely to occur. Analysis of cooked tortillas prepared by the Mayan traditional process revealed detectable amounts of FB1, FB2 and FB3 and their hydrolyzed counterparts. There was approximately equal molar amounts of FB1 and HFB1 in the cooked tortillas but the total amount of FB1 plus HFB1 was reduced approximately 60% compared to that originally in the uncooked maize. The total FB1 plus HFB1 in the lime-water used to cook the maize and water washes of the nixtamalized maize accounted for approximately 50% of the total FB1 in the uncooked maize. The amount of HFB1 in lime-water after steeping was much greater than FB1. A total of 11% of the FB1 in the uncooked maize was accounted for in the combined water washes (3). The three fractions (tortillas, lime-water and washes) together accounted for 94% of the FB1 originally in the uncooked maize. These results show that the traditional Mayan method of nixtamalization reduces the total fumonisins in the final product and that the majority of the lost fumonisins are found in the alkali steep water; findings that are similar to that of studies conducted using commercial or non-traditional methods of nixtamalization.

1228 FUMONISIN B_1 TOXICITY IN THE BRAIN DURING COEXISTING LIPOPOLYSACCHARIDE-RELATED ENDOTOXEMIA IN BALB/C MICE.

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Fumonisin B_1 (FB_1) is a mycotoxin synthesized by *Fusarium verticillioides*, a widespread contaminant of corn. FB_1 is a causative agent of animal diseases including equine leukoencephalomalacia. This experiment studied the modulation of FB_1

toxicity in the brain regions after administration of endotoxin known to disrupt the blood-brain barrier (BBB). The BBB integrity after lipopolysaccharide (LPS) treatment was evaluated in female BALB/c mice (4/group) after a single intraperitoneal (i.p.) dose of LPS (3 mg/kg). After 24 and 48 hours 200 ml of 0.5% sodium fluoresceine (NaFl) was injected intravenously. Animals were sacrificed 1 hour later, brains dissected and fluorescence determined. An increase in NaFl brain levels was observed at both time points. Next, female BALB/c mice (5/group) were injected i.p. with 3 mg/kg of LPS on the first day of treatment and/or with 2.25 mg/kg of FB_1 daily subcutaneously for 3 days. Cortex, cerebellum and medulla oblongata but not midbrain indicated increased free sphinganine in the FB1 group compared to control, as determined by high performance liquid chromatography. All regions showed a greater increase in free sphinganine in LPS/FB_1 group. Reverse transcriptase polymerase chain reaction analysis assessed the expression of proinflammatory cytokines in the brain. Treatment with LPS alone induced expression of interferon γ (IFNγ), interleukin (IL)-1β, IL-6 and IL-12. Treatment with FB_1/LPS further increased expression of tumor necrosis factor a, IL-6 and IL-12, while a decrease was observed for IFNγ and IL-1β compared to LPS treatment alone. Results indicated the endotoxin-related BBB disruption was followed by inhibition of de novo synthesis of sphingolipids by FB_1 in the brain. The changes in IFNγ, IL-1β, IL-6 and IL-12 expression between LPS and LPS/FB_1 dose groups suggest interactions between endotoxin and FB_1. The exposure to FB_1 coexisting with bacterial infection may have adverse effect on the brain. (Partially supported by NIH ES09403).

1229 SUBCHRONIC TOXICITY IN RATS FED CULTURE MATERIALS OF FUMONISIN-PRODUCING AND MONILIFORMIN-PRODUCING FUNGAL ISOLATES.

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Fumonisin (FB) and moniliformin (MN) are *Fusarium* mycotoxins that co-occur in corn and foods. Their effect on human health is unclear, but both have been implicated as a factor in one or more diseases. FB is hepato- and nephrotoxic in various species and causes liver and kidney cancer in rodents. The heart is not considered a target in rodents, but FB does affect cardiac function in swine *in vivo* and in frog heart atria *in vitro*. The heart, kidney and liver are target organs of MN in poultry and rats. Additive toxicity has been found in poultry co-exposed to FB and MN. To study the effect of co-exposure in a mammalian species, male rats (2 replicate experiments; n=3/group per replicate) were fed diets spiked with FB-producing (fumonisin B_1 + B_2 + B_3) *Fusarium verticillioides* and MN-producing *F. fujikuroi* culture materials for two weeks *ad libitum*. Mycotoxin concentrations of the diets were: 0 ppm FB/0 ppm MN (control group), 400 ppm FB/0 ppm MN, 400 ppm FB/200 ppm MN, or 400 ppm FB/500 ppm MN. The replicate experiments yielded similar results. All FB-fed groups exhibited decreased body weight, decreased relative (% body weight) liver and kidney weights, and increased tissue sphinganine concentrations indicative of ceramide synthase inhibition. Apoptosis and other microscopic liver and kidney lesions were also consistent with FB toxicity and, like the other effects, were not modified by MN. Relative heart weight (% body weight) of the 400 ppm FB/500 ppm MN group was slightly increased, however, gross or microscopic heart lesions were not found in any group. In summary, MN did not significantly modify the subchronic toxicity to rats of FB-containing diets. Further investigations are needed to study the effects of chronic co-exposure to FB and MN.

1230 KINETICS OF DEOXYNIVALENOL (VOMITOXIN) DISTRIBUTION AND CLEARANCE FOLLOWING ORAL EXPOSURE IN THE MOUSE.

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Deoxynivalenol (DON or vomitoxin) is a trichothecene mycotoxin commonly found in cereal grains that adversely affects the gastrointestinal and immune function. Here we developed approaches to analyze for DON in tissue by immunochemical assay and used these to follow the kinetics of distribution and clearance of this mycotoxin in the mouse. Spiking studies revealed that DON in plasma could be analyzed directly using a competitive direct enzyme-linked immunosorbent assay (ELISA) providing that standard curves were prepared in human plasma as diluent. For analysis of organs, tissues were ground with a mortar and pestle in phosphate buffered saline (1:5 ratio). Resultant extracts were then heated in boiling water for 5 min, centrifuged, and supernatants analyzed by ELISA. Tissue disposition and clearance of DON were measured in B6C3F1 male mice (8 wk-old) that were orally administered 25 mg/kg BW of the toxin. Blood was collected from retroorbital plexus and organs removed after 30 min to 24 hr intervals. Maximal DON was detected at 30 min in all tissues tested with a rapid clearance following over a 24 hr period. At 30 min, DON concentrations in ng/g were 5680±1480 in kidney, 4530±1140 in heart, 4430±440 in plasma, 3900±206 in liver, 3640 ± 105 in thymus, 2990±110 in spleen and 763±61 in the brain. DON concentrations were significantly higher in all the organs tested from 30 min to 8 hr compared to untreated mice. At 24 hr, DON concentrations dropped to levels which were not significantly higher except in kidney. Taken together, the results showed that, in the mouse, DON rapidly distributed in all organs within a short time after exposure according to the rank order kidney> heart> plasma> liver> thymus> spleen> brain. (supported by NIH Grants ES 03358, ES-09521 and DK 58833).

1231 HUMAN CYTOKINE mRNA RESPONSE TO DEOXYNIVALENOL (VOMITOXIN) USING WHOLE BLOOD CULTURES.

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Deoxynivalenol (DON or vomitoxin), a tricothecene mycotoxin, is a naturally occurring contaminant frequently found in grain-based foods. This toxin has adverse effects on human and animal health, with the GI tract and immune system being primary targets. A critical step in DON toxicity is action on leukocytes by activation of cytokines. Previous studies have shown an upregulation of several cytokines, in U937 cells, a cloned human macrophage model. To study the acute effects of DON on human cytokine production in peripheral mononuclear blood leukocytes, we have developed a culture approach using a 20 percent dilution of whole blood in RPMI-1640 media and a 6 hour exposure. Cultures were exposed to DON at concentrations of 0, 10, 50, 100, 250, and 500 ng/ml. RNA was then isolated and assayed using real-time PCR primer/probes, for both cytokines (IL-6, IL-8, and TNF-alpha) and 18S rRNA. Cytokine levels were normalized using 18S rRNA levels and relative expression levels determined. IL-6 was significantly induced by DON at 250 ng/ml (~9.5 fold) and 500 ng/ml (~14 fold). IL-8 was significantly induced by DON at 250 ng/ml (~8.5 fold) and 500 ng/ml (~3 fold). TNF-alpha was significantly induced by DON at 10 ng/ml (~1 fold), 250 ng/ml (~1 fold), and 500 ng/ml (~4 fold). Taken together, the capacity of DON to induce IL-8, IL-6, and TNF-alpha gene expression and the threshold doses to achieve these effects were consistent with previous findings in cloned human and mouse macrophage cultures. Interestingly, a high degree of variability was observed in blood cultures from different donors, thus raising the possibility that some individuals may have greater sensitivity to DON than others. Further research is being undertaken to clarify this possibility.

1232 INTERACTIONS BETWEEN MACROPHAGES AND NONPARENCHYMATOUS LIVER CELLS IN RESPONSE TO FUMONISIN TREAMENT *IN VITRO*

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Fumonisin B_1 (FB_1), a common mycotoxin produced by *Fusarium verticillioides* found in corn, causes animal diseases such as equine leukoencephalomalacia and porcine pulmonary edema. It induces tumors in male rat kidney and female mouse liver, and has been associated with human esophageal cancer in areas highly contaminated with fumonisins. Liver and kidney are the target organs of fumonisin in laboratory animals, but cells from liver are resistant to fumonisin toxicity *in vitro*. We have shown that FB_1 induces localized activation of cytokine network in mouse liver. Expression of tumor necrosis factor (TNF)α, interferon (IFN) γ, and interleukin (IL) 12 in mouse liver was increased in response to FB_1. A positive amplification loop involving TNFα, IFNγ and IL-12 has been implied and involves liver macrophage (Kupffer) cells and the hepatic lymphocytes. The resistance of individual cells to the cytotoxic effects of FB_1 *in vitro* may be due to the absence of interactions between the cytokines that are produced by different types of cells. We investigated the cellular interactions in FB_1-induced toxicity using mixed cultures of macrophages (J774A.1) and nonparanchymatous liver cells (NmuLi). The results indicated that treatment of the mixed cells with 10 μM FB_1 for 48 h or 72 h produced a greater increase in lactate dehydrogenase release than either J774A.1 or NmuLi cells alone. Expression of *TNFα* and *IL-12* in mixed cultures was increased following 10 μM FB_1 treatment for 24 h, while individual types of cells were unresponsive to FB_1 treatment. Transfer of conditioned medium from J774A.1 cells treated with 10μ M FB_1 for 24 h to NmuLi cultures produced an increase in *IFNγ* expression in NmuLi after 6 h incubation. These results indicate that interactions between different types of cells are involved in hepatotoxicity and can be a contributory factor in FB_1 toxicity. (Supported in part by NIH grant ES 09403).

1233 CHRONIC DIETARY TOXICITY STUDY OF DAG (DIACYLGLYCEROL) IN BEAGLE DOGS.

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This study evaluated the possible chronic toxicologic effects of DAG, when given in the diet for 12 months to dogs. DAG is a cooking oil which contains > 80% diglycerides, < 20% triglycerides and <5 % monoglycerides. For this study, a special diet

was prepared with no dietary fat so that all of the dietary fat could be provided by DAG, at various concentrations together with a control oil. The control oil, TG (triacylglycerol), was prepared to contain > 85% triglycerides, < 10% diglycerides and <5 % monoglycerides. The fatty acid composition for DAG and TG was closely matched. Dietary concentrations of 0% DAG/9.5% TG (TG control), 1.5% DAG/8.0% TG, 5.5% DAG/4.0% TG, and 9.5% DAG/0% TG were presented daily, seven days per week, for 52 weeks. A second concurrent control group received the standard basal diet (Certified Canine LabDiet 5007, which has a fat content of 9.5%). The basal diet, control article-treated and DAG-treated groups each consisted of four males and four female dogs. Treatment was initiated in prejuvenile (2.5 months old) dogs. Statistical evaluations compared the DAG-treated groups both to the basal diet and 9.5% TG control groups. The clinical condition of the animals, body weights, body weight gains and food consumption were unaffected by DAG. Hematology and urinalysis parameters were unaffected. No serum chemistry changes indicative of a toxic effect were observed. There were no effects noted on ECG data. No test article-related gross or histopathologic findings or changes in organ weights were observed. While there were no identifiable differences between the effects of TG and DAG, both caused some changes relative to the basal diet (decreased food consumption, higher alkaline phosphatase, cholesterol and triglycerides). These changes were not toxicologically significant and were attributed to the differences in the diet. Thus, DAG at dietary concentrations up to 9.5% for one year were not toxic, and had no effect on normal canine growth and development.

1234 BISPHENOL A INTERFERES WITH MICROTUBULES IN CULTURED HUMAN FIBROBLASTS.

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Bisphenol A (BPA) is widely used for the manufacturing of plastics, coatings and resins. In addition to being estrogenic, BPA exhibits genotoxic potential in cultured cells, e.g. by inducing micronuclei with whole chromosomes in Chinese hamster V79 cells and causing near-diploid aneuploidy and morphological transformation in Syrian hamster embryo cells. Since the induction of aneuploidy is often associated with the disruption of microtubules (MT), the ability of BPA to interfere with MT in cultured human AG01522C fibroblasts was assessed in the present study. We determined the mitotic index, the structure of the mitotic spindle and of the cytoplasmic microtubule complex (CMTC), and the number of freshly divided cells. The mitotic spindle and the CMTC were visualized by immunochemical staining of α-tubulin. Cytotoxicity or growth inhibition were assessed by electronic counting of the number of living cells. After exposure of the fibroblasts to BPA concentrations of 200 to 450 μM for half a population doubling (12 h), both the number of living and of freshly divided cells and the mitotic index declined in a concentration-dependent manner. The CMTC of BPA-treated cells contained abnormal structures resembling rings and loops (R&L), which to our knowledge have not been observed before. When fibroblasts were treated with 300-400 μM BPA for 12 h and the number of R&L determined at various time points after treatment, abnormal cells reached a maximum (30-50% of cells containing 1-3 R&L) after 1-4 h and declined steadily thereafter; control levels were reached 36 h after incubation with 300 or 350 μM BPA, whereas abnormal cells were still elevated 48 h after incubation with 400 μM BPA. The decrease of R&L did not correlate with cell growth. When cells were kept at 4°C for MT depolymerization, only about two thirds of the abnormal MT structures disappeared within a few minutes, whereas one third persisted. These data suggest that BPA interferes with MT polymerization in cultured human fibroblasts. The underlying mechanism is presently unknown and requires further studies.

1235 FUMONISIN B_1 (FB1) ALTERS SPHINGANINE (SA) AND SPHINGOSINE (SO) CONCENTRATIONS IN SERUM, TISSUE, URINE AND CEREBROSPINAL FLUID (CSF) OF HORSES.

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Fumonisins alter sphingolipid biosynthesis and induce hepatotoxicity in all species. In horses, FB1 induces cardiotoxicity and death due to neurotoxicity (leukoencephalomalacia) or liver failure. To investigate the pathogenesis of fumonisin toxicosis in horses, we evaluated the time and dose response of sphingolipids to FB1 in serum, tissue, urine and CSF and correlated with pathophysiologic findings (Haschek et al, SOT 2003 abstract; Smith, GW, et al, Am J Vet. Res 63:538-545, 2002). FB1 was administered iv at 0.20, 0.10, 0.05, 0.01 or 0 mg FB1/kg BW/day (n=3 or 4). Horses were euthanized on day 28 or when neurologic signs became severe (≥7 days, ≥0.05 mg FB1/kg). Blood, urine, CSF and selected tissues were analysed for So and Sa concentrations by HPLC. FB1 induced 1) dose- and time-dependent increases in serum Sa and So at all doses; 2) dose-dependent increases in liver and kidney Sa at ≥0.05 mg FB1/kg and So at all doses; 3) dose-dependent increases in lung Sa and So at all doses; 4) dose-dependent increases in heart and skeletal muscle Sa at all doses and So at ≥0.05 mg FB1/kg; 5) an increase in brain So at 0.10 mg FB1/kg and a decrease in spinal cord Sa at 0.01 mg FB1/kg; 6) a dose-dependent increase in urine Sa ≥0.05 mg FB1/kg and Sa:So at all doses; and 7) an increase in CSF Sa at 0.01 mg FB1/kg. Based on this and other studies, we conclude that increases in serum sphingolipid concentrations are the most sensitive and specific indicators of FB1 exposure in horses. Altered sphingolipid metabolism in liver, kidney and heart correlate with organ specific injury as determined by physiologic and pathologic evaluation, while alterations in lung and central nervous system sphingolipid metabolism do not appear directly correlated with organ specific injury. Supported by FDA and USDA-CSREES grant # 928-39453.

1236 TUMOR NECROSIS FACTOR-α INDUCTION IN FUMONISIN B_1-TREATED PORCINE RENAL EPITHELIAL CELLS IS MEDIATED VIA ACTIVATION OF PROTEIN KINASE C-α AND NUCLEAR FACTOR-κB.

N. V. Gopee and R. P. Sharma. *University of Georgia, Athens, GA.*

Fumonsin B_1 (FB_1) is a toxic metabolite produced by *Fuasrium moniliforme* (*F. verticillioides*), a fungus predominantly present in corn. FB_1 is known to be responsible for a variety of species-specific diseases including pulmonary edema in pigs and leukoencephalomalacia in horses. This mycotoxin has been reported to be a renal carcinogen in male rats, hepatocarcinogen in female mice and has been implicated as a contributing factor in human esophageal cancer. It inhibits ceramide synthase, enhances tumor necrosis factor-α (TNFα) production, and causes apoptosis. We previously showed that 1 μM FB_1 caused transient activation of protein kinase C-α (PKCα) in porcine renal epithelial cells (LLC-PK_1). It has been established that PKC activates nuclear factor-κB (NF-κB), an important transcription factor for the proinflammatory cytokine, TNFα. Therefore, this study investigated the implications of PKCα activation by 1 μM FB_1 on NF-κB activation and subsequently on TNFα gene expression in LLC-PK_1 cells. FB_1 (1 μM) induced a rapid and transient activation of PKCα and nuclear translocation of NF-κB at 5 min, followed by their down-regulation. This FB_1-induced translocation was inhibited by preincubating LLC-PK_1 cells with the PKC inhibitor, calphostin C. TNFα mRNA expression was significantly increased following 15 min exposure to FB_1. Co-exposure of cells with FB_1 with phorbol 12-myristate 13-accetate (PMA) resulted in an amplified expression of TNFα, as compared to FB_1 alone. Calphostin C abrogated the FB_1-induced increase in TNFα gene expression. This study puts forward compelling evidence that short-term activation of PKCα by FB_1 results in the activation of NF-κB, which transcribes TNFα in LLC-PK_1 cells. The PKCα-dependent induction of TNFα is one of the mechanisms involved in FB_1-induced toxicity in LLC-PK_1 cells. (Supported in part by NIH grant ES09403)

1237 90-DAY CHRONIC TOXICITY STUDY OF A NOVEL (-)-HYDROXYCITRIC ACID EXTRACT OF *GARCINIA CAMBOGIA.*

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(-)-Hydroxycitric acid (HCA), a widely used dietary supplement for weight management, is a natural extract from the dried fruit rind of *Garcinia cambogia* . This fruit exhibits a distinctive sour taste and has been used for centuries in Southern Asia for making culinary dishes. The addition of this fruit makes meals more filling and satisfying. Previous studies demonstrated the novel beneficial effect of a novel HCA extract (HCA-SX, commercially known as Super CitriMax) in weight management, as well as its safety in acute toxicity studies. In this study, we have conducted a 90-day chronic toxicity evaluation in male and female Sprague-Dawley rats. The animals were administered 0.2%, 2.0% and 5.0% HCA-SX in their daily feed. 0.2% HCA-SX intake in feed equals 4.62 gm as a 60% HCA-SX extract (2, 770 mg HCA), which is the recommended dosage for human weight loss consumption. The animals were given Purina Lab. Chow and tap water, and sacrificed upon 30, 60 and 90 days of treatment. Changes in body weight, liver and testis weight, and serum chemistry analysis were monitored. Hepatic and testicular lipid peroxidation, and DNA fragmentation were also conducted. Histopathological examination was conducted on the major organs obtained at the time of autopsy. A time-dependent increase in animal body weight was observed in control animals, while HCA-SX administration induced a dose- and time-dependent reduction in body weight gain in both male and female rats. No significant differences in liver and testis weight were observed between the groups at 30, 60 and 90 days of treatment. No changes in hematological and biochemical variables were noted. No significant differences were observed in hepatic and testicular lipid peroxidation and

DNA fragmentation. These results demonstrate that chronic administration of HCA-SX for 90-days does not adversely affect the variables tested in these animals and is safe under the conditions studied.

1238 INDUCTION OF THE PROCARCINOGEN-ACTIVATING CYP1A2 BY A HERBAL DIETARY SUPPLEMENT IN RATS AND HUMANS.

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Herbal dietary supplements, which are popular worldwide because they are believed to promote health, may in fact be double edge-swords. A herbal dietary supplement which contains extracts of kola nut, grape, green tea and ginkgo biloba, and is used as an agent for weight management, was administered to rats to test whether it induced CYP1A2, a procarcinogen-activating enzyme. Western blot analysis indicated that treatments with 0.5, 1 and 2 g/kg of the supplement for 3 days increased CYP1A2 expression in rat liver microsomes in a dose-dependent manner. The 0.5, 1 and 2 g/kg treatments increased rat liver microsomal CYP1A2 activity *via* the conversion of caffeine to paraxanthine to 212, 331 and 473% of normal, respectively. In humans, the intake of 2 and 4 capsules of the supplement for 3 days increased CYP1A2 activity to 194 and 203%, respectively, as assessed by the change in the urinary ratio of 1, 7-dimethylxanthine plus paraxanthine to unmetabolized caffeine. Intake of the herbal supplement increased CYP1A2 activity to levels higher than that observed from smoking (179%). This study suggests that the long-term intake of the dietary supplement inducing CYP1A2 may increase the incidence of liver, breast, bladder, prostate and colon cancers caused by procarcinogens activated by CYP1A2.

1239 SAFETY OF A NOVEL BOTANICAL EXTRACT FORMULATION FOR AMELIORATING *ALLERGIC RHINITIS.*

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Allergic rhinitis (known as hay fever) is found to be the most frequently occurring immunological disorder affecting 40 million people in the US. Symptomatically, this is an inflammation and irritation of the nasal mucosal membrane. Allergy is defined as a state of hypersensitivity caused by exposure to a particular antigen (allergen) resulting in marked increases in reactivity to that antigen upon subsequent exposure. A novel botanical formulation (Aller-7) was developed for the treatment of *Allergic rhinitis* using a combination of 7 standardized medicinal plant extracts from *P. emblica, T. chebula, T. bellerica, A. lebbeck, P. nigrum, Z. officinale* and *P. longum.* This formulation demonstrated potent antihistaminic, antiinflammatory, antispasmodic, antioxidant and mast cell stabilization activity. This study focused on the safety and antimutagenic potential of the formula, which was tested in Swiss Albino mice at the doses of 125, 250, 500, 1000 and 1500 mg/kg. Following 15 days of feeding, the animals were sacrificed. No histopathological changes were observed in vital organs. A separate study in rats demonstrated no toxicity up to 2 gm/kg bw. Sub-acute toxicity was conducted in Albino Wistar rats at a dose of 90 mg/kg bw for 3 days, which was graduated to 180 mg/kg for the next 3 days then to 270 mg/kg for 3 weeks. After 28 days, the animals were sacrificed and tested, upon which no toxicity was observed. In a subchronic toxicity study, there was no observed effect level (NOEL) at 1 gm/kg bw in rats. In teratologic assay, at the dose of 1.8 g actives (20-times more than the recommended dose), there were no visceral or skeletal anomalies observed in the fetuses. No maternal changes were observed when Aller-7 was administered during gestation and lactation. No evidence of mutagenicity was observed at doses up to 5000 mg/plate of Aller-7 in *S. typhimurium* cells. In conclusion, these studies demonstrate the broad spectrum safety of this novel formulation for human consumption.

1240 TRANS-10, CIS-12 CONJUGATED LINOLEIC ACID INHIBITS DIFFERENTIATION IN 3T3-L1 ADIPOCYTES AND DECREASES PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ EXPRESSION IN MICE.

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The trans10, cis12 (t10c12) isomer of conjugated linoleic acid (CLA) has been shown to reduce body fat gain when fed to mice and inhibit lipoprotein lipase activity in cultured 3T3-L1 adipocytes. We report herein that t10c12 CLA and conjugated nonadecadienoic acid (CNA, a 19-carbon CLA cognate) block preadipocyte differentiation and nullify the differentiation enhancing effect of thiazolidinedione (TZD, a PPARγ activator). Treatment of differentiating 3T3-L1 preadipocytes with t10c12 CLA or CNA resulted in decreased intracellular triglyceride accumulation and decreased mRNA expression of the adipogenic genes fatty acid synthase, aP2, and PPARγ. T10c12 CLA also decreased PPARγ, C/EBPα protein expression with no change of C/EBPβ. Mice fed diet supplemented with 0.5% CLA mixture for 4 weeks significantly reduced body fat gain without affecting body weight or serum parameters. In accordance with cell culture study, dietary CLA treatment decreased PPARγ and its downstream lipoprotein lipase (LPL) mRNA expression. The data indicate that CLA, especially t10c12 isomer, can inhibit adipogenesis in part by its influence on the expression/activation of PPARγ.

1241 TOXICOLOGY STUDIES ON CONJUGATED LINOLEIC ACID (CLA).

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Conjugated linoleic acid (CLA) is the term given to a group of positional and geometric isomers of the essential fatty acid linoleic acid. CLA is found naturally in foods such as dairy and meat products where the c9, t11 isomer predominates. Commercial preparations of CLA contain a mixture of isomers, with c9, t11 and t10, c12 often occurring in equal proportions. Many beneficial effects of CLA have been reported including anticarcinogenic activity. However, very little safety data are available on this material. A programme of toxicology studies was conducted to confirm the safety of a preparation containing a mixture of CLA isomers (Clarinol). Clarinol was tested in two *in vitro* mutagenicity assays, an Ames Test and an *in vitro* cytogenetics assay, and a 90-day repeat dose oral toxicity rat study. Clarinol was non-mutagenic in both *in vitro* assays. In the 90-day study, Clarinol was administered to Wistar (Crl: (WI) WU BR) rats at dose levels of 1%, 5% and 15% in a synthetic diet (AIN-93G). A high fat control diet containing 15% safflower oil was also tested in the study. A 4-week recovery period was included at the end of the study. At a dose level of 15%, Clarinol produced hepatocellular hypertrophy in female rats. This effect was reversible upon withdrawal of test material. An increase in plasma insulin levels was also observed in high dose females on study but there was no effect on plasma glucose levels. Insulin levels returned to control values at the end of the recovery period. A No Observed Adverse Effect Level of 5% was identified in the study.

1242 LACK OF ESTROGENIC OR ANTI-ESTROGENIC ACTIVITY OF THE ANTIOXIDANT NAO FOUND IN SPINACH.

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The developing fetus is uniquely sensitive to perturbation by chemicals with estrogenic and/or endocrine-disrupting activity. The carcinogenic effect of developmental exposure to estrogens has been well documented in humans and experimental animals. Since some natural antioxidants and flavonoids, used increasingly in nutritional and pharmaceutical applications for infants and children, as well as adults, exhibit estrogenic activity, we investigated the estrogenic potential of the natural antioxidant NAO isolated from spinach leaves using an *in vivo* rodent uterotropic bioassay and an *in vitro* transcriptional activation assay. Outbred 17-day-old female CD-1 mice were treated with 17b-estradiol (500 mg/kg/day) as a positive control or NAO at doses ranging from 1000 to 1, 000, 000 mg/kg/day for 3 consecutive days and sacrificed on the fourth day. Body weights and uterine wet weights were determined. Mice treated with17b-estradiol showed a statistically significant increase in uterine wet weight, but mice treated with NAO did not show any increase over untreated controls at any dose tested. The estrogenic activity of NAO was also determined *in vitro* using a transcriptional activation assay in which BG1Luc4E2 cells, which express estrogen receptor, were stably transfected with a luciferase reporter gene responsive to estrogens. The positive control, 17b-estradiol, showed a dose-dependent increase in luciferase activity; however, NAO did not demonstrate any significant increase over control. NAO was also tested for anti-estrogenic activity by adding it to the cells in combination with17b-estradiol; NAO did not inhibit the effect of17b-estradiol. Together, these data suggest that NAO does not have estrogenic or anti-estrogenic activity. Additional studies using the neonatal mouse are underway to determine any potential long-term effects of NAO if exposure occurs during critical periods of early development.

1243 *IN VIVO* EXPOSURE TO AN EPHEDRA CONTAINING METABOLIC NUTRITION SYSTEM DOES NOT ALTER SERUM BIOCHEMISTRY AND HISTOPATHOLOGY OF SEVEN VITAL TARGET ORGANS OF B6C3F1 MICE.

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Although conventional biomedical research has largely focused on mechanisms of weight loss and genetic aspects of obesity, most medical solutions are plagued by side-effects and fraught with complex questions. As a consequence, consumers are

seriously considering herbal products, nutraceuticals and functional foods rather than conventional medications. This is evidently driven by a growing consumer understanding of diet/disease links, aging populations, rising health care costs, and advances in food technology and nutrition. This study investigated the effects of subchronic exposure to a metabolic nutrition system (MNS Orange-AdvoCare®) on serum biochemistry and target organ histopathology (kidney, liver, heart, brain, lung, spleen and duodenum) in mice. MNSO is a unique combination of vitamins, minerals, omega-3 fatty acids and herbal extracts designed to provide a strong foundation of nutritional support, enhance thermogenesis and boost energy levels. MNSO contains citrus, ephedra, gingko, green tea and Ocimum extracts. In this study, ♀B6C3F1 mice were fed control (-MNSO) or MNSO (1X-10X; 1X=daily human dose) diets. Animals were sacrificed after 4 mo., at which time blood was collected for serum chemistry analysis and tissue sections for H&E-staining. Food consumption and body-weight changes were also monitored throughout. MNSO exposure did not adversely affect the serum chemistry profiles(electrolyte, lipid, carbohydrate, protein), nor did it disturb normal tissue architecture. Although animals on MNSO diet consumed more food, they were relatively more lean and active compared to controls. These results suggest that long-term MNSO exposure produces normal serum chemistry and histology in mice fed up to ten times the normal human dose [Supported by AdvoCare, Carrolton, TX].

1244 A SUBCHRONIC FEEDING STUDY OF ANNATTO EXTRACT (NORBIXIN), A NATURAL FOOD COLOR EXTRACTED FROM THE SEED COAT OF ANNATTO (*BIXA ORELLANA* L.), IN SPRAGUE-DAWLEY RATS.

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A subchronic oral toxicity study of annatto extract (91.6 % as norbixin), a natural carotenoid, was conducted. Groups of 10 male and female Sprague-Dawley rats were fed annatto extract at dietary levels of 0, 0.1, 0.3 and 0.9 % for 13 weeks. There were no treatment-related adverse effects with reference to body weight, food and water consumption, and ophthalmology and hematology data. Serum biochemistry revealed changes confined to rats of the 0.9 and 0.3 % groups in both sexes, including increased alkaline phosphatase, phospholipid, total protein, albumin and the albumin/globulin ratio. Marked elevation in absolute and relative liver weights was also found in both sexes receiving 0.9 or 0.3 %, but not 0.1 %. Hepatocyte hypertrophy was evident and an additional electron microscopic examination demonstrated this to be linked to abundant mitochondria after exposure to a dietary level of 0.9 % annatto extract for 2 weeks. Thus, the No-Observed-Adverse-Effect-Level (NOAEL) was judged to be a dietary level of 0.1 % (69 mg/kg body weight/day for males, 76 mg/kg body weight/day for females) of annatto extract (norbixin) under the present experimental conditions.

1245 TRANSCRIPTIONAL RESPONSES OF AAG KNOCKOUT MOUSE EMBRYONIC STEM CELLS UPON EXPOSURE TO ME-LEX.

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We have previously generated the 3-Methyladenine DNA glycosylase Aag null cells by double-targeting the Aag gene in mouse ES cells. This knockout cell line is sensitive to alkylating agent-induced p53 induction, S phase arrest, sister chromatid exchange, and apoptosis. Expression profiling of Aag null and wild type mouse ES cells treated with methyl-lexitropsin (Me-Lex), an alkylating agent that almost exclusively induces 3MeA DNA lesions, was performed using oligonucleotide microarrays (GeneChip, Affymetrix). Aag +/+ and Aag -/- cells were treated at both equitoxic and equimolar Me-Lex dose. Total RNA was isolated 0, 2, and 24 hour after treatment and used for microarray analysis. Hundreds of genes were up or down regulated at each time point for both the wild type and Aag null cells. Data analysis was focused to gene groups related to apoptosis, cell cycle, and DNA repair. Some wild type- and Aag null cell specific expression patterns identify candidate genes involved to MeLex-induced toxicity. We are now in the process of carrying out the computational analysis of the data.

1246 ROLE OF NITRIC OXIDE IN ROTENONE-INDUCED NIGRO-STRIATAL INJURY.

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Selective degeneration of the nigro-striatal system is the main pathology of Parkinsons' disease (PD). A deficit of mitochondrial complex I activity and increased reactive oxygen and nitrogen species have been suggested in the pathogenesis of PD. Rotenone, a widely used pesticide causes a syndrome in rats that mimics, both behaviorally and pathologically, the symptoms of PD. The present study evaluated the role of nitric oxide (NO) in the nigro-striatal injury resulting from a chronic administration of rotenone. After a 40 day administration of rotenone to adult male Sprague-Dawley rats, a relatively stable rotenone concentration was observed in the cortex and striatum. Furthermore, a significant (45%) increase in the number of nicotinamide adenine dinucleotide phosphate diaphorase(NADPH-d)-positive cells in the striatum was observed, accompanied by a 70% increase in the production of 3-nitrotyrosine, a marker for peroxynitrite generation. We also observed a highly significant increase in striatal neuronal nitric oxide synthase expression after rotenone administration. In addition, there was a 53% decrease in striatal dopamine (DA) levels and a 28% loss of substantia nigra tyrosine hydroxylase (TH) immuno-positive neurons. Moreover, the subcutaneous injection of 7-Nitroindazole (7-NI), a selective neuronal nitric oxide synthase inhibitor, provided significant protection against rotenone-induced nigro-striatal damage and attenuated the increased production of peroxynitrite. Therefore, our data suggest that chronic rotenone administration can lead to a significant damage to the nigro-striatal dopaminergic system and this damage can be mediated by the increased generation of nitric oxide and peroxynitrite.

1247 CHROMIUM EXPOSURE DISRUPTS TRANSCRIPTION FROM PAH-INDUCIBLE PROMOTERS.

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Environmental exposures to toxic or carcinogenic compounds rarely result from the presence of a single, isolated toxicant. More frequently, the toxic agent is a complex mixture of chemical entities, often including a combination of metals, such as chromium, and PAHs, such as benzo[a]pyrene (B[a]P). Work from our laboratory has shown that chromate disrupts the coordinate induction of phase I and phase II detoxification genes by aryl hydrocarbon receptor ligands. To test the hypothesis that combined exposure to mixtures of B[a]P and chromate disrupts the regulatory mechanisms that control transcription from B[a]P-inducible gene promoters, we have used Inductively Coupled Plasma Mass Spectrometry and exposure to $^{51}CrO_4K_2$to measure the uptake, distribution and biological effects of chromium in mouse hepatoma Hepa-1 cells. In cells exposed to hexavalent chromium, conversion of Cr(VI) to Cr(III) is very rapid, with less than 5% of the input remaining as Cr(VI) after 1 hour. Twenty five percent of the total chromium is present in the nucleus and 8 - 10% of nuclear Cr(III) is found in association with chromatin. B[a]P-induced expression of a luciferase reporter driven by the mouse Cyp1a1 gene promoter stably integrated in Hepa-1 cells is almost completely repressed by chromium exposure. Overexpression of the transcriptional co-regulators SRC-1, SRC-2, p300/CBP, and RIP-140 did not reverse the chromium-mediated repression of B[a]P-dependent gene induction; however, treatment with Na butyrate, a histone deacetylase inhibitor, fully reversed the effect of chromium. These results suggest that chromium interacts with transcriptionally poised chromatin to block some feature of transcription initiation or progression from inducible promoters. *(Supported by NIH 1R01-ES10807, and NIH P42-ES04908).*

1248 ARYL HYDROCARBON RECEPTOR REGULATION AND TRANSACTIVATION DURING OSTEOBLAST DIFFERENTIATION.

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Many toxic compounds released into the environment by fossil fuels, waste incineration and cigarette smoking exert adverse effects on the skeleton. Several metabolic bone diseases arise as a result of an imbalance in bone remodeling and these toxicants may be contributing factors in the progression of these pathological conditions. One candidate molecule that has been implicated to control the toxic effects of these exogenous compounds on bone cells is the aryl hydrocarbon receptor (AHR). The AHR is a ligand activated transcription factor that binds poly-aromatic hydrocarbons and congeners of dioxin/furan compounds. We have documented the presence of the AHR in osteoblasts and suggest that it might not only be responsible for participating in toxicity reactions but also be involved in skeletal development. We utilized cultures of osteoblasts derived from neo-natal rat calvaria as well as tissue specimens from both wild type and AHR -/- mice. AHR is expressed in primary cultures of osteoblasts that are induced to differentiate. Both the mRNA and protein levels of the receptor increase through day 15 at which point they begin to decline. This pattern of expression parallels the induction of a number of early bone-specific genes involved in the maturation of osteoblasts. We also show by immunocytochemistry that the receptor is present in calvarial osteoblasts. The AHR functions as a transactivating receptor in osteoblasts as evidenced by its ligand-de-

pendent migration to the nucleus and its association with known dioxin response elements. Dioxin exposure for 4 hours to osteoblasts in culture induces the expression of CYP1A1 and Cyclooxygenase-2 protein levels. Together these data imply that the AHR may not only mediate the effect of aromatic toxicants on bone cells but, with an as yet unidentified ligand, be involved in the differentiation pathways of the osteoblast.

1249 TEMPORAL GENE EXPRESSION CHANGES FOLLOWING TREATMENT WITH ANTHRACYCLINES IN MALE RATS.

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Temporal gene expression changes associated with the treatment of anthracyclines in male rats were investigated. Doxorubicin was administered to rats by intravenous injection for up to 2 weeks at a dosage of 1 mg/kg/day. Daunorubicin, another anthracycline was used as a comparator drug. Evaluated parameters included clinical observation, clinical pathology, light microscopy, and ultrastructural pathology. Changes in cardiac tissue gene expression of treated rats were found to occur in the absence of downstream lesions. There were minimal changes in clinical and anatomical pathology of anthracycline treated groups when compared to control. Early changes in gene expression in the absence of any downstream lesions may be suggestive of a possible initial response (compensatory event) to the exposure to doxorubicin. The genes of interest that were up-regulated included superoxide dismutase, plasma GSH peroxidase precursor, and antioxidant protein 2. The functional relevance of the modulation observed was presumably thought to be a reflection of the roles of these genes in the antagonism of Reactive Oxygen Species. Collectively the findings suggest that additional studies will be required to better determine if the gene expression changes observed represent a reliable indicator of the early onset of toxicity or simply a compensatory event stemming from anthracycline exposure.

1250 EXPRESSION AND REGULATION OF THE PLANT STEROL HALF TRANSPORTER GENES ABCG5 AND ABCG8 IN RATS.

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The Abcg5 and Abcg8 genes encode half-transporter proteins that heterodimerize to form a transporter of plant sterols and cholesterol. Mutations in these genes result in the human condition sitosterolemia, characterized by the accumulation of plant sterols in the blood, leading to premature arteriosclerosis and coronary artery disease. While the expression and regulation of these genes has been studied in mice and to a lesser extent in humans, it has been largely ignored in rats. Therefore, the purpose of this study was to examine the expression and regulation of Abcg5 and Abcg8 at the mRNA level in the Sprague-Dawley rat. Both Abcg5 and Abcg8 mRNA were expressed primarily in rat small intestine, with the highest levels in jejunum and lower levels in the duodenum and ileum. Both genes were also highly expressed in rat liver, but were minimal in all other tissues examined (brain, kidney, large intestine, lung, and stomach). There were no significant gender differences in the expression of these genes. The effects of a 1% cholesterol diet on the regulation of Abcg5 and Abcg8 were also examined, and compared with those seen in C57BL/6 mice. In mice, a 1% cholesterol diet caused a 3.5-fold increase in the expression of liver Abcg5 mRNA, and a 5-fold increase in liver Abcg8 mRNA, compared to liver mRNA levels in mice fed a standard diet. However, in rats, the 1% cholesterol diet suppressed liver Abcg5 mRNA levels by 75%, and suppressed liver Abcg8 mRNA levels by 88%, in comparison to levels seen in rats fed a standard diet. In mouse small intestine, Abcg5 and Abcg8 mRNA levels were only modestly induced by the 1% cholesterol diet, with similar results observed in rat small intestine. However, compared to mouse, a more robust increase was seen in rat ileum, where both genes were induced 2.5-fold. These results suggest variation between rats and mice in regulatory mechanisms controlling Abcg5 and Abcg8 expression, and may explain some differences in lipid metabolism observed between these two species. (Supported by NIH grants ES-09716 and ES-07079)

1251 TISSUE AND GENDER-SPECIFIC EXPRESSION OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEINS 1-6 IN MICE.

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The family of Multidrug resistance-associated proteins (Mrps) are a class of ATP-dependent transporters that are important in the export of various conjugated and unconjugated organic anions out of cells. The tissue distribution of these transporters has been thoroughly conducted in rats, but similar characterization of the Mrp family in mice is incomplete. Therefore, the purpose of this study was to quantitatively determine relative mRNA levels of the Mrp family in nine mouse tissues (liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, and whole brain) and to determine whether there are gender variations in expression of Mrps in the C57BL/6 mouse. Mrp1 was present in all tissues examined, with highest expression in stomach and large intestine. Mrp2 mRNA levels were highest in small intestine, followed by liver and kidney. Mrp3 was ubiquitous, with highest levels in large intestine. Mrp4 message levels were predominant in kidney, with relatively minimal expression elsewhere. Mrp5 had a unique pattern of expression with high mRNA levels in brain, lung and stomach. Finally, Mrp6 message was primarily observed in liver and small intestine. Female-predominant expression was observed in kidney (Mrp3 and Mrp4) as well as liver (Mrp1, Mrp4 and Mrp6), suggesting possible endocrine regulation of these transporters. In general, the tissue expression correlates well with that in rat, with only minor differences observed. However, some gender differences in Mrps seem to be species-specific (Mrp3, Mrp4, and Mrp6), and differ from results seen in rat. (Supported by NIH grants ES-09716 and ES-07079)

1252 TISSUE DISTRIBUTION OF MOUSE ORGANIC ANION TRANSPORTING POLYPEPTIDES.

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Organic anion transporting polypeptides (Oatps), which belong to the Slc21a family of solute carrier transporters, mediate Na+-independent transport of xenobiotics and endogenous compounds. The purpose of this study was to determine the tissue-specific distribution of eight known members of the Oatp family, namely Oatp1-5, 9, 12 and prostaglandin transporter (PGT) in mice, as well as to determine potential gender differences in their expression. The mRNA levels of each Oatp in liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, and whole brain, were quantified with the branched DNA signal amplification assay. Oatp1 mRNA was mostly detected in liver and kidney, but with substantially higher levels in male than female mice in both tissues. Oatp2 mRNA levels were highest in liver and brain. Furthermore, female mice had 2.5-fold more Oatp2 gene transcripts in liver than males. Oatp3 mRNA levels were low in all tissues examined, with the highest of these levels in lung. Oatp4, also named liver-specific transporter (lst), was almost exclusively detected in liver, whereas Oatp5 mRNA was only found in kidney. Oatp9 mRNA was ubiquitous, with the highest concentration in liver and all three parts of the small intestine. Oatp12 mRNA was found at similar concentration in all tissues examined, with the lowest levels in liver. PGT mRNA levels had the highest abundance in lung and stomach. Additionally, there was no gender difference found in Oatp3-5, 9, 12 or PGT mRNA. In conclusion, these data indicate that the tissue distribution of each mouse Oatp is unique. However, the tissue distribution of these mouse Oatps correlates well with corresponding rat Oatps, suggesting the functions in both species may be similar. Gender differences only in Oatp1 and Oatp2 mRNA suggest that endocrine hormones may influence the regulation of these two genes, but not other Oatps. (Supported by NIH grant ES-09649)

1253 *TERT*-BUTYL HYDROQUINONE (TBHQ) PROTECTS AGAINST ARSENITE-INDUCED CYTOTOXICITY.

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The carcinogenic metaloid arsenic and benzo(a)pyrene (B[a]P) are among the top 20 hazardous substances in the environment and they are often found as co-contaminants, including in a full 50% of the 1, 200 Superfund sites in the National Priority List. The long-range goal of this research is to develop an understanding of the mechanisms underlying the adverse health effects of complex mixtures of B[a]P and arsenic. We are exploring the hypothesis that combined exposure to a mixture of B[a]P and arsenite will disrupt the regulatory mechanisms that control transcription from B[a]P-inducible gene promoters, and will cause an uncoupling of phase I and phase II gene expression with a concomitant imbalance in B[a]P metabolism. In embryo fibroblasts (MEF) from C57BL/6J mice, low doses of arsenite (1 - 2 μM) synergize with B[a]P, but not with tBHQ, in the induction of the phase II quinone oxidoreductase gene, NQO1. Global profiling experiments indicate that tBHQ inhibits the induction by arsenite of a large number of genes, suggesting that tBHQ could protect against oxidative damage induced by arsenite. To test this hypothesis, be used MEF's from GCLM knock-out mice, lacking the modifier subunit of glutamylcysteinyl ligase, which have been shown to be extremely susceptible to killing by oxidants. In GCLM-KO MEFs, tBHQ was fully protective of the cytotoxicity induced by arsenite. These data suggest that a major component in the

cytotoxic effects of arsenite is the inability to adapt quickly to an oxidative challenge and synthesize glutathione *de novo* and that tBHQ protects against this challenge. *(Supported by NIH 1R01-ES10807, and NIH P42-ES04908).*

1254 INDUCTION OF UDP-GLUCURONOSYLTRANSFERASE 1A AND 2B mRNA LEVELS IN RAT LIVER AND DUODENUM BY CLASSES OF MICROSOMAL ENZYME INDUCERS THAT ACTIVATE VARIOUS TRANSCRIPTIONAL PATHWAYS.

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Microsomal enzyme inducers (MEIs) upregulate Phase I biotransformation enzymes, most notably the cytochrome P450s (CYPs). Transcriptional upregulation by MEIs occurs by at least four different nuclear receptor mechanisms: aryl hydrocarbon receptor (AhR; CYP1A inducers), constitutive androstane receptor (CAR; CYP2B inducers), pregnane-X receptor (PXR; CYP3A inducers), and peroxisomal proliferator activating receptor-α (PPARα; CYP4A inducers). Other mechanisms include the antioxidant/electrophile response element (ARE/EpRE; quinone reductase inducers) and CYP2E inducers which upregulate enzymes through post-transcriptional mechanisms including mRNA stabilization. UDP-glucuronosyltransferases (UGTs) are Phase II biotransformation enzymes, most of which are expressed in liver, but some are primarily expressed in intestine. MEIs are known to increase UGT activity; however, information concerning transcriptional regulation of individual UGT isoforms is incomplete. Therefore, the purpose of this study was to examine effects of MEIs on UGT1A and 2B isoform mRNA levels in rat liver and duodenum. All three AhR ligands induced UGT1A6 and 1A7 mRNA levels in liver, but not duodenum. Two AhR ligands induced UGT1A3 mRNA in liver only. All four CAR activators induced UGT2B1 mRNA in liver but not duodenum. Similarly, three CAR activators induced UGT1A1 and 1A6 mRNA levels in liver only. Two of the three PXR ligands induced UGT1A2 mRNA in duodenum, but not liver. Two of the three PPARα ligands induced UGT1A1 and UGT1A3 mRNA levels in liver only. Two EpRE ligands induced UGT2B12 mRNA in liver and duodenum, and UGT1A6, 1A7, 2B1, and 2B3 mRNA in liver only. All three CYP2E inducers upregulated UGT1A3 mRNA levels in liver only. In summary, some UGTs, like CYPs, are upregulated by MEIs, though MEIs had minimal effects on UGT expression in duodenum. Additionally, several UGT isoforms are induced by the various mechanisms responsible for CYP regulation. (Supported by NIH grants ES-03192 and ES-07079)

1255 ROLE OF TRANSCRIPTION FACTORS IN LPS-INDUCED DECREASE OF MOUSE ORGANIC ANION TRANSPORTING POLYPEPTIDE (OATP) 4 EXPRESSION.

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Previous studies indicate that lipopolysaccharide (LPS) causes a time- and dose-dependent decrease in mouse oatp4 mRNA levels. Oatp4 mRNA levels were lowest at 12 hr after LPS treatment. Because LPS is reported to transcriptionally down-regulate Ntcp and Mrp2, the purpose of the present study was to determine the effect of LPS on the binding activities of seven transcription factors predicted to bind to the regulatory sequence of oatp4, namely HNF-1α, C/EBP, HNF-3β, CHOP-C, HFH-1 and HFH-2, and RARα:RXRα, using electrophoretic mobility shift assays (EMSAs). Nuclear extracts were prepared from livers of C3H/OuJ mice excised at 0, 1.5, 3, 6, 12, 16, 24, and 48 hr after treatment with LPS (5 mg/kg). CHOP-C, HFH-1 and HFH-2 showed no indication of binding to the oatp4 regulatory sequence in EMSAs. However, the binding to the putative binding sequences of HNF-1α, C/EBP, HNF3β, and RARα:RXRα was detected in mouse liver by EMSAs. Their lowest levels were observed at 1.5, 1.5 to 3, 1.5, and 1.5 to 3 hr after LPS treatment, respectively, then subsequently increased to control values. Specific unlabeled oligonucleotides (100- to 500-fold molar excess) abolished the complex recognized by ^{32}P-labeled oligonucleotides specific to HNF-1α, C/EBP, HNF3β, and RARα:RXRα. Anti-HNF-1α antibody caused a supershift with HNF-1α putative binding sequence, but anti-HNF-1β antibody did not. To further address the function of HNF-1α in the regulation of mouse oatp4 expression, oatp4 mRNA levels were determined with the branched DNA signal amplification assay in livers of HNF-1α-null (*Tcf*$^{-/-}$) and wild-type mice. Mouse oatp4 mRNA levels in HNF-1α-null mice were markedly lower than that in wild-type mice. Therefore, these data suggest that HNF-1α, C/EBP, HNF3β and RARα:RXRα might be involved in the down-regulation of mouse oatp4 by LPS, but CHOP-C, HFH-1 and HFH-2 do not seem to be involved. Moreover, these data also suggest that HNF-1α plays a critical role in the constitutive expression of mouse oatp4. (Supported by NIH grant ES-09649)

1256 ROLE OF THE TGF-BETA PATHWAY IN THE PATHOGENESIS OF STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY.

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Diabetic nephropathy, a major complication of diabetes, is characterized histologically by thickening of the glomerular basement membrane, glomerular hypertrophy, mesangial matrix accumulation, and interstitial fibrosis. These changes correlate well with the loss of renal function. The molecular pathogenesis of diabetic nephropathy, however, has not been fully elucidated. To determine the molecular mechanisms involved in this condition, we have studied changes in gene expression in the kidneys of streptozotocin (STZ)-treated mice, a well-characterized model of diabetes. Male CD-1 mice (3 per group and per time point) were treated with a single intraperitoneal injection of STZ (200 mg/kg) or vehicle, sacrificed 2 and 6 months after treatment, and the kidneys were collected for histologic and molecular evaluation. GEArray, real-time quantitative RT-PCR and Western blot analysis revealed many changes in gene expression, in particular in the TGF-beta pathway. We also localized these changes in gene expression in the different compartments of the kidney by using laser capture microdissection (LCM) to isolate glomeruli and cortical tubules, respectively. By real-time quantitative RT-PCR, TGF-beta and SREBP-1 gene expression were increased in both glomeruli and cortical tubules, while cdkn1a (p21/Waf1/Cip1) expression was mainly increased in cortical tubules. In addition, although not detected in the RNA extracted from whole STZ-diabetic kidneys, nephrin expression was dramatically increased in isolated glomeruli. Collectively, these data confirmed that genes in the TGF-beta pathway are involved in the pathogenesis of diabetic nephropathy and indicated the potential value of LCM in transcriptomics studies.

1257 CONSTITUTIVE EXPRESSION OF VARIOUS XENOBIOTIC AND ENDOBIOTIC TRANSPORTER mRNAS IN THE CHOROID PLEXUS OF ADULT SPRAGUE-DAWLEY RATS.

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A major function of xenobiotic and endobiotic transporters is to move a wide range of organic substances and metals across cell membranes. The choroid plexus plays an important role in regulating the exchange of materials between blood and cerebrospinal fluid, thereby protecting the brain from various toxic insults. Therefore, choroid plexus is one of the most toxicologically important tissues of the central nervous system. The aim of this study was to quantitatively determine the constitutive expression of various transporters in the choroid plexus of adult Sprague-Dawley rats. The mRNA levels of 39 genes (Mrp1, 2, 3, 4, 5, and 6; Mdr1a, 1b and 2; Oatp1, 2, 3, 4, 5, 9 and 12; Oat1, 2, 3, and K; Oct1, 2, 3, N1 and N2; Ntcp, Bsep, Ibat; Dmt1, Menke's and Wilson's; Ent1 and 2, Cnt1 and 2; Pept1 and 2; as well as Abcg5 and 8) were measured in choroid plexus by the branched DNA signal amplification method. The mRNA levels of these genes were also determined in liver, kidney and ileum in order to compare relative expression levels. The expression of 8 genes (Mrp1, 4, and 5, Oatp3, Menke's, Dmt1, Ent1, and Pept2) was higher in choroid plexus than in liver, kidney and ileum. An additional 7 genes (OctN1 and N2, Oatp2, Oat2 and 3, and Cnt1 and 2) were all significantly expressed in choroid plexus, but choroid plexus was not the tissue with the highest expression. The remaining 24 transporters were expressed at low levels in choroid plexus as compared to other tissues. The constitutive expression levels of different transporters in choroid plexus may provide insight into the range of xenobiotics that can potentially be transported by the choroid plexus, thereby providing a means to protect the brain from toxicity. (Supported by NIH grants ES-09716, ES-09649, and ES-07079)

1258 INDUCTION OF MULTIDRUG RESISTANCE PROTEIN 3 (Mrp3) *IN VIVO* IS INDEPENDENT OF CONSTITUTIVE ANDROSTANE RECEPTOR.

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Previous studies demonstrated that the organic anion transporter Mrp3 is induced in rat liver by phenobarbital (PB) and several other microsomal enzyme inducers that induce cytochrome P450 2B (CYP2B). CYP2B is known to be induced by constitutive androstane receptor (CAR)-retinoid X receptor (RXR) heterodimer binding to a PB-responsive promoter element. Hepatic mRNA levels of CYP2B

and Mrp3 were measured in three models of altered CAR activity (Wistar Kyoto rats, RXRα$^{-/-}$ and CAR$^{-/-}$ mice) in order to determine the involvement of CAR in the induction of Mrp3. In Wistar Kyoto rats, a strain in which females express very little CAR protein, the induction of CYP2B1/2 mRNA by PB, diallyl sulfide, and trans-stilbene oxide, but not by oltipraz, was significantly higher in males than in females. In contrast, Mrp3 mRNA was induced by each of these treatments to an equal level in males and females. In hepatocyte-specific RXRα$^{-/-}$ mice, CYP2B10 mRNA was not induced by diallyl sulfide or oltipraz, and the induction by PB and trans-stilbene oxide was diminished as compared to wild-type mice. Mrp3 however, was induced by PB, diallyl sulfide, trans-stilbene oxide and oltipraz in both wild-type and RXRα$^{-/-}$ mice. Additionally, the constitutive expression of Mrp3 was significantly reduced in the RXRα$^{-/-}$ mice. In CAR$^{-/-}$ mice, the robust induction of CYP2B10 by PB seen in wild-type mice was completely absent. However, Mrp3 mRNA was equally induced by PB in both wild-type and CAR$^{-/-}$ mice. These data clearly demonstrate that induction of hepatic Mrp3 is CAR-independent and implies a role for RXRα in the constitutive expression of Mrp3. (Supported by NIH grants ES-09716, ES-09649, and ES-07079)

1259 SPECIES AND GENDER DIFFERENCES IN ORGANIC ANION TRANSPORTER mRNA.

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Significant gender differences in organic anion transporter (Oat) mRNA levels are known in rat kidney and liver. Oats are uptake transporters that facilitate the initial steps of excretion. Mechanisms by which Oat gender differences occur could be revealed using mouse knockout (KO) models, but similar expression patterns between species must first be determined. Thus, the aim of this study was to determine whether oat1, oat2 and oat3 mRNA levels in mouse tissues exhibit similar gender-specific patterns observed in rat. Tissues were collected from male and female 129J and C57BL/6 mice, two strains used in KO development. Oat mRNA levels were determined by branched DNA and compared with rat Oat mRNA levels. Rat Oat1 mRNA was prevalent in kidney, where male levels exceeded female levels. Mouse oat1 in both strains was analogous to rat levels. Rat Oat2 mRNA was highest in female kidney, and markedly higher than in male kidney. Although oat2 was highest in kidney of both mouse strains, 129J female levels were only slightly higher than male; no gender difference was evident in C57 kidneys. Additionally, rat Oat2 mRNA in males was highest in liver at only 1/3 of female kidney levels. Conversely, highest oat2 levels in male mice were in kidney. Oat3 mRNA was highest in kidney for both species. However, female predominance was only observed in 129J kidney. Oat3 mRNA levels in rat liver were low compared with kidney and were male-specific. Similarly, oat3 mRNA was low in liver of both mouse strains, with male levels higher than female levels. However, oat3 mRNA in mouse liver was notably lower relative to kidney than was observed in rat. Overall, species differences regarding gender-specific mRNA levels were evident for oat2 and oat3, but oat1 mRNA levels were similar between species. Therefore, the use of mouse KO models may not be appropriate to study the mechanisms of Oat2 and Oat3 gender differences. Moreover, characterizing Oat mRNA species differences may aid interpretation of anion toxicity or clearance discrepancies if mRNA and protein trends coincide. (Supported by NIH grants ES-09716 and ES-07079)

1260 COACTIVATION OF ESTROGEN RECEPTOR α BY DRIP 205 IN HUMAN BREAST CANCER CELLS.

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Vitamin D interacting protein 205 (DRIP 205) has been characterized as a component of the mediator complex of transcription factors which interact with multiple transcription factors including nuclear receptors. Previous studies in this laboratory have demonstrated that DRIP 205 coactivates estrogen receptor α (ERα)-mediated transactivation in cells cotransfected with constructs containing three tandem estrogen response elements (pERE$_3$) or GC-rich motifs (pSp1$_3$) that bind ERα and ERα/Sp1, respectively. DRIP 205 mutants containing C-terminal (aa 714-1566) and N-terminal (aa 1-516) deletions also coactivate ERα- or ERα/Sp1-mediated transactivation in ZR-75 breast cancer cells, and both of these mutants contain LXXLL box motifs that interact with activation function 2 (AF2) of ERα. Therefore, a DRIP 205 mutant (DRIP 205 Δ588-635, DRIP 205m3) that did not contain the LXXLL boxes was generated to investigate contributions of these motifs on coactivation. In cells transfected with pERE$_3$ or pSp1$_3$, DRIP 205m3 significantly coactivated both ERα- and ERα/Sp1-mediated transactivation, respectively. Coactivation of ERα by DRIP 205m3 was further investigated in ZR-75 cells transfected with pERE$_3$ and increasing concentrations of expression plasmids encoding the AF1 domain of ERα (ERα-AF1) and the LXXLL motifs in steroid receptor coactivators (GRIP1-NR box). Expression of both ERα-AF1 and GRIP1-NR box peptides decreased transactivation coactivated by DRIP 205m3. These results suggest that DRIP 205 can coactivate ERα- and ERα/Sp1-mediated transactivation in breast cancer cells, and this response is LXXLL box-independent. Based on results of competitive peptide inhibition studies, coactivation of ERα by DRIP is complex and may involve both AF1 and AF2 domains of ERα. Current studies are focused on identifying functional interacting domains of ERα and DRIP 205. (Supported by ES09106 and CA76636)

1261 CELL CONTEXT-DEPENDENT DIFFERENCES IN THE MECHANISM OF HORMONAL ACTIVATION OF E2F1 IN BREAST CANCER CELLS.

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17β-Estradiol (E2) induces E2F1 gene expression in MCF-7 breast cancer cells, and deletion analysis of the E2F1 gene promoter showed that the -146 to -54 region was the minimal estrogen-responsive sequence. Subsequent analysis of this promoter in MCF-7 cells indicated that E2-induced transactivation was dependent on both upstream GC-rich and downstream CCAAT motifs that bound estrogen receptor α (ERα)/Sp1 and NFYA, respectively. Moreover, the resulting ERα/Sp1/NFYA complex was required for enhanced gene expression. E2 also induces E2F1 gene expression in ER-positive ZR-75 breast cancer cells, and deletion analysis of the E2F1 gene promoter also demonstrated that the -146 to -54 region was the minimal E2-responsive sequence. Subsequent mutation/deletion analysis of the E2F1 promoter showed that both the upstream GC-rich and downstream CCAAT sites were independently E2-responsive, and this was in direct contrast to results obtained in MCF-7 cells where both motifs were required for transactivation. Further analysis showed that the upstream GC-rich sites (-169 to -111) were hormonally-activated by genomic ERα/Sp1 in which ER induced transactivation through protein-protein (ERα/Sp1) and not protein-DNA (promoter) interactions. In contrast, the downstream (-122 to -54) CCAAT motif binds NFY proteins, and hormone-induced transactivation is associated with activation of cAMP/PKA which in turns activates NFYA in ZR-75 cells. These results demonstrate the importance of cell context in the molecular mechanisms of E2F1 activation and illustrate significant differences even among ER-positive breast cancer cells. (Supported by NIH ES09106 and ES09253).

1262 ESTROGEN RECEPTOR/Sp1 COMPLEXES ARE REQUIRED FOR INDUCTION OF *CAD* GENE EXPRESSION BY 17β-ESTRADIOL IN BREAST CANCER CELLS.

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The *cad* gene is trifunctional and expresses carbamoylphosphate synthetase/aspartate carbamyltransferase/dihydroorotase which are required for pyrimidine biosynthesis. *Cad* gene activities are induced in MCF-7 human breast cancer cells, and treatment of MCF-7 or ZR-75 cells with 10 nM 17β-estradiol (E2) resulted in a 3- to 5-fold increase in cad mRNA levels in both cell lines. The mechanism of hormone-induced *cad* gene expression was further investigated using constructs containing the growth-responsive -90 to +115 (pCAD1) region of the *cad* gene promoter. E2 induced reporter gene (luciferase) activity in MCF-7 and ZR-75 cells transfected with pCAD1 which contains three upstream GC-rich and two downstream E-box motifs. Deletion and mutation analysis of the *cad* gene promoter demonstrated that only the GC-boxes which bind Sp1 protein were required for E2-responsiveness. In many cell lines, interaction of c-*myc* with the E-box motifs is required for *cad* expression. Although E2 induces c-*myc* in breast cancer cells, high endogenous expression of USF1/2 which is bound to the E-box sequences in gel mobility shift assays precludes *myc* binding. Results of electrophoretic mobility shift and chromatin immunoprecipitation assays show that both Sp1 and estrogen receptor α (ERα) interact with the GC-rich region of the *cad* gene promoter. Moreover, in transactivation assays with pCAD1, hormone-induced transactivation was inhibited by cotransfection with dominant negative Sp1 expression plasmid and small inhibitory RNA for Sp1 which silences Sp1 expression in the cells. These results demonstrate that, in common with many other genes involved in E2-induced cell proliferation, the *cad* gene is also regulated by a non-classical ERα/Sp1-mediated pathway. (Supported by NIH ES09106 and CA76636)

1263 TRANSCRIPTIONAL REGULATION OF HUMAN *CYP2F1* LUNG SPECIFIC EXPRESSION.

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CYP2F1 is expressed preferentially in human lung and is believed to play important roles in bioactivating pulmonary toxicants. DNAse I footprinting analysis with nuclear extracts from human lung revealed a single protected region (−152 to −182) in

the proximal promoter of the *CYP2F1* gene. This region contains two E–box regulatory motifs that normally bind deltaEF1, E12, E47 and AP4 transcription factors. The entire promoter region from +106 to −1685 was scanned, but no additional protected regions were identified. In EMSA assays, performed with a 31bp probe corresponding to the region from −152 to −182, prominent protein DNA complexes were detected with nuclear extracts from human lung tissue but not from liver and heart tissue. Binding of nuclear proteins was abolished by addition of non–radiolabeled specific probe but not by adding nonspecific probe. Supershift EMSA analysis did not identify the protein involved in binding this 31bp probe, all antibodies (deltaEF1, AP4, E2A, HEB, TAL1) used in this study failed to yield a supershift. In addition, supershift EMSA assays identified three Sp1/Sp3 binding sites with BEAS–2B and A549 lung cell nuclear extracts; one partially overlapped the DNAse protected region (−164 to −193), and another two were located at −49 to −72 and −103 to −125. Luciferase reporter assay shows that the later two Sp1/Sp3 binding sites drive the transcription activity of *CYP2F1* in human lung A594 and BEAS–2B cells. (Supported by PHS Grant #HL60143).

1264 MECHANISMS OF ARYL HYDROCARBON RECEPTOR-MEDIATED DISRUPTION OF ANDROGEN RECEPTOR FUNCTION IN LNCAP CELLS.

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2, 3, 7, 8, Tetrachlorodibenzo-p-dioxin (TCDD) blocks androgen-induced responses in male rats exposed *in utero* and lactationally, and to a lesser extent in adult rats. In experimental animals, reduced fertility following TCDD exposure has been attributed to decreased growth of the testis, epididymis, and accessory sex organs, and decreased daily and ejaculated sperm numbers. Several studies have shown the prostate to be most sensitive to TCDD treatment. Proposed mechanisms of the anti-androgenic effects of TCDD and other aryl hydrocarbon receptor (AHR) ligands include: (1) enhanced ligand metabolism; (2) altered hormone synthesis; (3) down-regulation of receptor levels; and (4) interference with hormone-induced gene transcription and cell proliferation in hormone-responsive tissues. In the present study we utilized androgen-dependent human Lymph Node Cancer of the Prostate (LNCaP) cells to determine the effect of TCDD on androgen receptor-(AR) mediated gene expression and DNA binding. Luciferase reporter assays indicate that TCDD-activated AHR inhibits induction by dihydrotestosterone (DHT) of AR-mediated gene transactivation. Electrophoretic mobility shifts assays reveal that TCDD blocks binding of the DHT-activated AR to its cognate sequence, but does not interfere with nuclear translocation of the activated AR. Global gene profiling using microarray analysis shows that TCDD blocks expression of several androgen responsive genes, including several members of the kallikrein gene family, which includes PSA, the androgen-inducible marker of prostate cancer. These results point to a role of the AHR in disruption of androgen-mediated responses through interference with AR promoter binding. *(Supported by NIH Grants RO1 ES06273 and P30 ES06096).*

1265 REGULATION OF CYCLOOXYGENASE 2 BY 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN.

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TCDD (2, 3, 7, 8 tetrachlorodibenzo-p-dioxin) is a ubiquitous environmental contaminant that has been shown to cause carcinogenic, reproductive, developmental, immunological, hepatic, and neurotoxic responses in animals. Many actions of TCDD are mediated by binding to the cytosolic arylhydrocarbon receptor (AhR) and induction of the classical dioxin-inducible gene battery. However, little is known about the complex molecular actions of TCDD beyond those associated with the AhR and it is likely that specific toxic effects of TCDD are mediated independent of the AhR. TCDD has been shown to induce *COX2* (cyclooxygenase 2) in rat liver and repress *COX2* in rat ovary; these responses may be AhR dependent. Physiologically, *COX2* is induced by a host of stimuli associated with inflammation, cytokines, and tumor necrosis factor-α. TCDD may regulate the *COX2* gene *via* CYP1A1 (cyctochrome P450 1A1) mediated arachidonic acid oxidation and activation of the NF-kB (nuclear factor kappa beta) pathway. Using large scale gene arrays we have previously shown that 50 nM TCDD x 8 h upregulates *COX2* 2.5 fold in C6 rat glioma cells. In order to further study this effect we have treated MO59K human glioma and H42E rat hepatoma cells with 50 nM TCDD for 4, 8, 24 h. Using quantitative real-time RT-PCR our data shows a cell line and time-specific regulation of *COX2* and *CYP1A1*, which is measured as a positive indicator of AhR activity. In conclusion, TCDD mediated dysregulation of *COX2* and *CYP1A1* may contribute to the toxicity of TCDD.

1266 STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR PROTEIN) ACTIVITY IN FISH.

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The steroidogenic pathway is targeted by some environmental contaminants, resulting in reduced levels of steroid hormone synthesis. Alterations in steroid production can lead to severe reproductive abnormalities and other physiological dysfunction. The Steroidogenic Acute Regulatory (StAR) Protein transports cholesterol across the mitochondrial membrane, which is a rate-limiting step in steroidogenesis. Using largemouth bass (LMB) as a model, we have begun to examine the molecular mechanism(s) involved in the regulation of StAR. We have cloned the entire coding region of LMB StAR and have used this sequence to develop a real-time PCR assay to measure changes in StAR mRNA levels in ovarian tissue cultures. Preliminary data shows significant induction of StAR mRNA levels in LMB ovarian tissue cultures by 1mM dbcAMP after 4 hours, which suggests that mammalian and fish StAR may be similarly regulated. A 3 kb portion of the LMB StAR promoter was also cloned to further understand the transcriptional regulation of StAR in LMB. The transcriptional start site for the LMB promoter was located using RACE and the sequence upstream of the start site has been analyzed for consensus sites. A putative consensus binding site for SF-1 (sterol factor 1) has been identified close to the transcriptional start site. This suggests that SF-1, like in mammalian systems, may be involved in the regulation of LMB StAR. Elucidating the molecular pathway and determining sites for steroidogenic regulation provides crucial information to the understanding of normal cellular functioning.

1267 EFFECT OF SYNTHETIC ANTIOXIDANTS ON ALPHA CLASS GLUTATHIONE S-TRANSFERASE GENE EXPRESSION AND GLUTATHIONE BIOSYNTHESIS IN HUMAN LIVER SLICES.

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The human glutathione S-transferases (hGSTs) comprise a multigenic family involved in a variety of detoxification reactions. The alpha classes GSTs (hGSTA1, hGSTA2, and hGSTA4), in particular, exhibit catalytic activities toward reactive aldehydes as well as peroxidase activities toward hydroperoxides. In the current study, we examined the effect of exposure to known rodent GST inducers on alpha class GST expression and glutathione biosynthesis in cultured human liver slices. Precision human liver slices were incubated up to 24 hours in the presence of *tert*-butyl-hydroquinone (TBHQ), ethoxyquin (EQ), and butylated hydroxyanisole (BHA). Cultured liver slices generally remained viable throughout the exposures, with the exception of high does of EQ (640 uM) and TBHQ (200 uM) which triggered losses in slice potassium concentrations at 24 h of exposure. In slices prepared from one donor, 40 uM EQ elicited a 40% increase in hepatic GSH, whereas exposure to 640 uM EQ elicited a modest (1.8-fold) increase in hGSTA4 mRNA expression but decreased the levels of hGSTA1 and hGSTA2 mRNAs. In a second experminent, the treatments were generally unaffective with regards to alpha GST gene expression, although some transient increases (<25%) in hGSTA1 mRNA expression were observed on exposure to 10 uM BHA. In summary, our results suggest that the expression of mRNAs encoding the alpha GST isozymes hGSTA1, hGSTA2, and hGSTA4 are not strongly modulated by certain synthetic antioxidants in cultured human liver slices. These results may be reflection of 1) an overall poor induction response of human alpha GST by antioxidants relative to rodents, 2) a limited responsiveness of these genes to inducing agents in cultured human liver slices, or 3) variability in inducibility among liver donors. Supported by NIH ES09427 and USEPA STAR 827441.

1268 EXAMINATION OF THE ROLE OF P53-ASSOCIATED CELL CYCLE GENE EXPRESSION INDUCED BY METHYLMERCURY IN MOUSE EMBRYONAL FIBROBLASTS.

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Exposure to methylmercury (MeHg) in the environment poses a risk of impaired function and death to human neurons, with the developing brain being more susceptible. Our lab has hypothesized that MeHg damage can be characterized by changes in cell cycle progression—namely G_2 M arrest. P53, acting as a transcription factor in response to genotoxic stress, serves as a cell cycle checkpoint and thus may play a role in mercury toxicity. We have employed cultures of p53 transgenic mouse embryonal fibroblasts to examine differential sensitivity to MeHg, and specifically the pathways induced by p53. Of particular interest for their promotion and inhibition of cyclin dependent kinases were cyclin B1 and p21, respectively.

Fibroblasts were exposed to 0.75, 2, and 4μM MeHg, and total RNA harvested from cells at 8, 24, and 48 hours post-exposure. Since we speculated that MeHg induced alteration of various genes related to the inhibition of the proteasomal pathway, lactacystin, a specific proteasomal inhibitor, was used as an additional control. By preventing ubiquitination and degradation of proteins, lactacystin would thereby increase endogenous p53. Experiments which assayed for changes in mRNA expression by semi-quantitative RT-PCR demonstrated that cyclin B1 was downregulated by treatments for up to 24 hours in the $p53^{+/+}$ cells, while in $p53^{-/-}$ cultures cyclin B1 expression was only initially depressed by lactacystin. Basal expression of p21 was higher in the wildtype compared to the null, but all samples expressed p21 suggesting p53-independent regulation. GADD153, another transcription factor capable of causing cell cycle arrest, increased with MeHg dose in $p53^{+/+}$ cells but not in the $p53^{-/-}$ and not as a result of lactacystin exposure. Given the responses of 3 representative p53-inducible genes, MeHg toxicity is associated with changes in expression of cell cycle genes determined in part by p53 status and proteasomal activity. Supported by NIH Grants ES10613-01, ES11387-01, ES09601-02, and ES07033.

1269 INTERACTION BETWEEN THE ARYL HYDROCARBON RECEPTOR AND TRANSCRIPTION FACTOR E2F LEADS TO GENE REPRESSION.

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The molecular basis of the biological effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is largely unknown. TCDD mediates the transactivation of the CYP1 family of cytochrome P450 monooxygenases, but activation of the CYP1A1, CYP1A2 and CYP1B1 genes does not adequately explain the diversity of TCDD effects. Complexes formed between the activated AHR, the retinoblastoma protein RB and the transcription factor E2F at E2F binding sites of natural promoters, are important in repression of S-phase-specific genes and cell cycle arrest. Gene expression profiling has shown that more genes are repressed by TCDD treatment than they are induced, even when secondary effects of exposure are blocked by protein synthesis inhibitors. Transactivation by the activated AHR can explain gene induction, but not gene repression, which constitutes a major effect of dioxin exposure that has not been characterized at the molecular level. Based on preliminary evidence, we hypothesized that AHR can also cause gene repression by forming complexes with the transcription factor E2F independently of RB and interfering with E2F-dependent transcription. To test this hypothesis, we analyzed protein interactions between AHR and E2F in pull-down and gel retardation assays. In pull-down assays using GST-E2F fusions as bait we found that AHR binds directly to GST-E2F, forming stable protein complexes. Using gel retardation assays, we show that AHR can specifically displace RB from its interaction with E2F at E2F binding sites occupied by RB(p105)/E2F/DP1 complexes, generating a novel complex containing E2F and AHR. We also used transient expression of a luciferase reporter driven by the promoter of the DHFR gene in RB-negative mouse hepatoma Hepa-1 cells to show that direct AHR-E2F interactions lead to gene repression. These data suggest that AHR not only establishes active repression interactions with RB, but that it can also bind to E2F and down-regulate gene expression possibly through a mechanism of E2F sequestration. *(Supported by NIH R01 ES06273).*

1270 TRAPPING GENES ASSOCIATED WITH TOBACCO USE.

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Data from epidemiological studies have strongly linked the development of squamous cell carcinomas of the upper aerodigestive tract to consumption of tobacco, particularly when combined with alcohol. The exact mechanisms leading from tobacco use to disease are not well understood, but for tumorigenesis those mechanisms are surely genetic. Understanding how carcinogens induce tumor formation ultimately relies on identifying and characterizing the genes that are targeted for mutation and/or dysregulation by those carcinogens. To this end, many mutations have been found in cancer cells from tobacco users, but not all mutations have been associated with specific genes. Thus, the goal of this project is to identify, mutate and characterize those genes. This goal is part of the long-term objective of understanding the genetic mechanisms underlying the development of cancer. To address this goal, we have deployed a gene-trapping strategy to identify tobacco-sensitive genes. Briefly, mouse embryonic stem (ES) cells are transfected with a promoterless reporter gene construct. Successful transfection of an ES cell will knockout an endogenous gene, but the reporter gene will be active and under the control of the endogenous promoter. Large numbers of unique gene-trapped ES cell clones are then screened in the presence or absence of cigarette smoke condensate (CSC), and altered reporter gene expression is identified and recorded. The reporter gene itself is then used as the starting point for sequencing of the endogenous genes. Sequences are then compared to those in the GenBank database to identify novel genes as well as previously known genes that were unknown to be altered/dysregulated by tobacco. In addition, mutant ES cells created as described above can be used to generate knockout mice. Those mice in turn will then be studied to understand the role of each gene relative to the development of cancer and disease.

1271 HEPATOBILIARY TRANSPORTER INDUCTION IN ALTERED THYROID HORMONE HOMEOSTASIS: A MICROARRAY ANALYSIS.

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The mechanism by which UDPGT inducers alter hepatic clearance of thyroid hormones in rodents has been well established. DPC 904 (a pyrazolopyrimidine) reduced serum T4 and T3 and increased TSH, and caused thyroid follicular cell hypertrophy and hyperplasia, increased liver weights, and hepatocellular hypertrophy with increased mitoses in rats given ≥200 mg/kg/day for 5 days. The biliary clearance of both T4-glucuronide and unconjugated T4 was increased with treatment; however, the majority of excreted T4 was unconjugated. We hypothesized that the reduction in serum T4 was related to increased hepatobiliary transport of both conjugated and unconjugated T4. The aim of this study was to determine whether expression of genes involved in thyroid hormone homeostasis (e.g. hepatic enzymes and transporters) is altered following treatment with DPC 904. Mature male Sprague-Dawley rats were given a single 200 mg/kg dosage of DPC 904, and serum and livers were collected at 3, 6, 10, 24, or 48 hours. Serum T4 and T3 levels were decreased (0.6× and 0.7×, respectively) at 10 hours, and TSH was increased (1.9×) by 24 hours and remained elevated. Hepatic gene expression profiles were generated using Affymetrix Rat Genome U34A arrays and Rosetta Resolver software. Induction of oatp2 (Slc21a5) mRNA was evident at 3 hours, coinciding with a strong and sustained increase in CYP3A expression. There was also a dramatic increase in CYP2B expression at 3 hours. Mrp2 was induced at 6 hours, and UGT1A1, 1A2, 1A5, 1A6, and 2B1 expression was increased by 10 hours. Generally, peak gene expression changes were observed at 24 hours. In support of the microarray results, oatp2 protein levels were markedly increased in treated rats. Based on these results, hepatic uptake of T4 is likely increased by induction of oatp2, whereas increased biliary excretion correlates with increased UGT and mrp2 expression. Cluster analysis supports the conclusion that DPC 904 regulates hepatic gene expression through both PXR and CAR activation.

1272 GENE EXPRESSION IN RAT SKIN FOLLOWING DERMAL EXPOSURE TO JP-8 JET FUEL.

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Dermal irritation resulting from occupational exposure of Air Force personnel to jet fuels continues to be an important occupational health issue. Irritant contact dermatitis, the most common occupational skin disease, costs the government millions of dollars each year. Gene array technology can serve as a tool for assessing chemical irritancy potential. To determine the transcriptional response to jet fuel, male Fisher 344 rat skins were exposed to JP-8 for 1 hour *in vivo*. Total skin RNA was then isolated at 0 h (control), 1 and 4 hrs following the beginning of the exposure to monitor transcriptomic profiles using the Affymetrix RatTox U34 array. Our results indicate dermal exposure to JP-8 causes significant change in gene expression: 187 transcripts changed at 1 hour and 216 genes changed at 4 hours when compared to controls. Of the 187 genes showing differential expression at 1 hour, 67 were expressed sequence tags (EST). Of the remaining 120 transcripts, 33 showed increased expression and 67 had decreased expression with respect to controls. At 4 hours there were 73 ESTs, 64 increases and 55 decreases. Genes were grouped according to the following functional categories: metabolism, cell structure, extracellular matrix, differentiation and cell division, signal transduction, transporters and ligands, cellular and oxidative stress, cytokines/growth factors/receptors, and miscellaneous. A strong temporal change of gene expression was observed in the skin following exposure to JP-8. Characterization and analysis of the transcriptomic response of skin to jet fuel may enhance the risk assessment of JP-8-induced dermal irritation.

1273 UNRAVELLING THE MOLECULAR MECHANISMS UNDERLYING HYDROXYUREA GENOTOXICITY IN MOUSE LYMPHOMA L5178Y CELLS USING TOXICOGENOMICS.

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The application of gene expression profiling technology to genetic toxicology is expected to allow a significant advance in understanding toxic mechanisms of DNA reactive genotoxins versus non-DNA reactive genotoxins such as Hydroxyurea

(HU), and should ultimately aid in a better risk assessment. The main objectives of the study were to (1) examine the relative sensitivity of DNA microarray technologies using HU as a model compound, (2) investigate the relationship between gene expression changes and traditional genetic toxicology endpoints and (3) better characterize the molecular mechanisms of HU genotoxicity. Mouse lymphoma L5178Y cells were treated for 4 hrs with increasing doses of HU (from 0.1mM to 1mM) and harvested immediately or left for a 20hr-recovery period (n = 3 independent biological experiments). Cytotoxicity was evaluated by cell count and genotoxicity by *in vitro* micronucleus assay at 24hrs. Gene expression profiling of HU-treated L5178Y cells was performed using Affymetrix mouse MG-U74A GeneChips (Affymetrix, USA). Data analysis showed a dose-dependent increase in the number of genes modulated, 75% of which was significantly down-regulated, whatever the doses or the time-points. Hierarchical clustering analysis of the gene expression profiles allowed a better discrimination of the genotoxicity data at 4 hrs as compared to 24 hrs, suggesting a putative link between gene expression modulation at 4hrs and the observed genotoxicity. The modulated genes at 4 hrs were involved in cell cycle, chromosome assembly, nucleotide metabolism and DNA repair pathway (less clear gene expression patterns at 24hrs). Therefore, the gene expression modulation observed at 4hrs could represent HU molecular signature, most probably linked to its non-DNA reactive genotoxic properties. Additional experiments with other non-DNA reactive genotoxins should help determine whether the observed HU gene expression fingerprint is compound- or chemical class-specific.

1274 LIVER-TARGETING PRODRUG OF PMEA INDUCES A MUCH MORE FAVORABLE KIDNEY AND LIVER TOXICOLOGICAL GENE EXPRESSION IN RATS COMPARED TO BISPOM-PMEA.

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Background: PMEA is an acyclic nucleotide phosphonate analog with potent anti-hepatitis B virus (HBV) activity. However it has low oral bioavailability and accumulated at high levels in kidney. Two prodrugs of PMEA are being developed. BisPOM-PMEA is an orally bioavailable esterase-activated prodrug, whereas hepavir B is a liver-targeting cytochrome P450 (CYP) 3A4-activated prodrug. **Aim:** To profile and compare the renal and hepatic toxicological gene expression in rats in response to prolonged treatment of BisPOM-PMEA and hepavir B. **Methods:** Five male rats per group were dosed orally for 8 days with 40 and 300 mg/kg/day of BisPOM-PMEA and hepavir B, respectively. Affymetrix Rat Toxicology Arrays were employed. 3-fold difference of gene expression compared to controls is taken as cutoff. Changes in expression of selected genes were confirmed by competitive RT-PCR analyses. **Results:** *Gene Expression Profile in Kidney:* Both BisPOM-PMEA and hepavir B treatments markedly increased the mRNA levels of multidrug resistance gene (mdr1b, >20 folds) as well as cell cycle genes (cyclin B and G, and GADD153, 3-5 folds). In addition, hepavir B at 300 mg/kg/day also induced other genes including stearyl-CoA desaturase (>25 folds) and fatty acid synthetase (25 folds). In contrast, BisPOM-PMEA at 40 mg/kg/day down-regulated more than 50 genes including canalicular multispecific organic anion transporter (cMOAT, 3.2 folds, specific for the adefovir excretion), and a large number of drug metabolizing enzymes (CYP1A, 2A, 2B, 2C, 3A, GST and UDPGT, 5-40 folds). *In Liver:* Only minor changes in gene expression were observed, except that hepavir B at 300 mg/kg/day down-regulated stearyl-CoA desaturase (9.6 folds) but up-regulated mdr1b (>40 folds). **Conclusion:** BisPOM-PMEA (at 40 mg/kg/day) dramatically down-regulated many more toxicological genes in kidney compared to hepavir B (at 300 mg/kg/day). The data suggested that hepavir B may have less nephrotoxicity potential compared to BisPOM-PMEA.

1275 THE EFFECT OF GLUTATHIONE REGULATION ON GENE EXPRESSION IN RAT PRIMARY HEPATOCYTES.

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Evaluation of the cellular response to chemical or environmental changes has led to utilization of microarray technology. Toxicologists are investigating this technology as a way to monitor changes in thousands of genes in order to find more sensitive predictive methods for drug and chemical safety analyses. Genomic modeling research shows the potential of using genomic data for predictive models. Glutathione (GSH) plays an important role in modulating the toxicity of chemicals and is often measured as an indicator of xenobiotic metabolism. The rate-limiting step of GSH synthesis is controlled by the enzyme γ-glutamylcysteine synthetase (GCS) that is feedback inhibited by GSH. L-Buthionine sulfoximine (BSO) is a potent inhibitor of GCS. This research investigated the effect of GSH depletion on the transcriptome of primary rat hepatocytes. To determine the gene expression profiles for normal (0 μM BSO) and those GSH-depleted hepatocytes, total RNA from these cultures were harvested at several time points (0h, 2h, 4h, 6h, 12h, and 24h) during treatment with 0 μM, 50 μM, and 200μM BSO. These samples were analyzed with the Affymetrix rat genome U34A arrays. These studies create a baseline of gene expression in naïve and GSH-depleted primary rat hepatocytes in the presence and absence of a GSH synthesis inhibitor. Both the metabolism and function of GSH involves many interconnected biochemical pathways, such as protein synthesis, regulation of enzyme activity, amino acid transport, catabolism of reactive oxygen species, and coenzyme activity for synthesis of endogenous compounds. This research establishes the groundwork for modeling the GSH pathway in primary rat hepatocytes.

1276 DEVELOPMENT OF A GENE-EXPRESSION ARRAY FOCUSING ON THE HYPOTHALAMUS-PITUITARY-THYROID AXIS IN XENOPUS LAEVIS.

J. Korte, S. J. Degitz and J. E. Tietge. *MED, NHEERL, ORD, USEPA, Duluth, MN.* Sponsor: J. Nichols.

As recommended by the Endocrine Disrupter Screening and Testing Program Advisory Committee (EDSTAC), the USEPA has been developing a screening test capable of detecting effects of Endocrine Disrupting Chemicals (EDCs) on the hypothalamus-pituitary-thyroid (HPT) axis in *Xenopus laevis*. As part of this, we developed an oligonucleotide array to assess changes in expression of important thyroid-axis genes, allowing comparison of the results with morphometric and biochemical information. The method consists of hybridizing amplified, biotinylated RNA from test organisms with membrane-bound synthetic DNA oligomers of 65 to 70 bases. The biotin label is detected by chemiluminescence and quantified. Currently, there are two different oligos for each of the 70 different genes represented on the array, and each oligo is spotted twice. When tested on samples from stage 53 to 56 tadpoles, the system shows robust responses from housekeeping genes (actin, elongation factor 1-α, ribosomal protein L8, etc.), as well as strong responses from genes important to the HPT axis (thyroid stimulating hormone, thyroid hormone receptor, corticotropin releasing factor, proopiomelanocortin, prohormone convertase, growth hormones A and B, etc.) The method has numerous attractive features: 1) very sensitive, surpassing radioactive methods 2) system does not require complete sequence information, useful in species such as *X. laevis* where complete genomic information is not available 3) high flexibility, allowing genes to be added as new sequences become available. 4) does not require robotics or other highly specialized/expensive equipment 5) potential to detect hundreds of genes at once 6) fast, the results are available a few hours after the hybridization step. The array has been run successfully with RNA from whole tadpoles, tail sections, and even on individual brains weighing only a few milligrams. *This abstract does not necessarily reflect EPA policy.*

1277 IDENTIFYING NOVEL DIOXIN-INDUCIBLE GENE TARGETS IN NEURONAL CELL LINES.

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A great deal of concern exists that even low levels of dioxins transferred to a developing fetus across the placenta can induce neurological injury. A first step to understanding mechanisms of dioxin injury is to identify modifications in neuronal gene expression resulting from dioxin exposure. Using human glioma (MO59K) and neuroblastoma (SH-SY5Y) cells we identified dioxin-inducible genes by suppression subtractive hybridization (SSH) and serial analysis of gene expression (SAGE). Of particular interest is the aryl hydrocarbon receptor (AhR) nuclear translocator II (ARNT2) protein which is a basic helix-loop-helix (bHLH) transcription factor that is highly expressed in neurons and is thought to play an integral role in neuronal AhR-mediated responses to dioxin. In cDNA libraries enriched for dioxin-induced genes from human glioma and neuroblastoma cells we have identified individual dioxin-induced genes in subtracted libraries and compared expression in the two cell types. Identification of dioxin responsive genes, that include a combination of Ahr, Arnt, Arnt2 and hypoxia inducible factors (Hif), among others, provides a foundation from which to examine cross-talk and signaling between the identified dioxin responsive genes and their gene products.

1278 TRANSCRIPTIONAL CHANGES IN NORMAL HUMAN LIVER CELLS EXPOSED TO TETRACHLOROETHYLENE METABOLITE USING MICROARRAY ANALYSIS.

N. Keshava and T. Ong. *Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV.* Sponsor: P. Joseph.

Trichloroacetic acid (TCA) is a major metabolite of tetrachloroethylene (TCE) in both humans and experimental animals. Occupational exposure to TCE can occur through inhalation, skin contact or ingestion during its use in dry cleaning and de-

greasing. TCE is converted to TCA mediated through cytochrome P450 pathway. Although TCE is classified as a probable carcinogen to humans by International Agency for Research on Cancer, inadequate evidence limits classifying TCA as a probable carcinogen. Studies have been conducted in our laboratory to determine whether exposure to TCA causes alterations in expression of cancer related genes in cultured human cells. Exponentially growing normal human liver cells were exposed to 200 and 400 uM concentration of TCA for 12h. Total RNA was used for the preparation of double stranded cDNA. Biotin labeled cRNA transcript was synthesized using cDNA, fragmented and hybridized to HuGeneFL GeneChip probe arrays representing more than 6800 human genes and expressed sequence tags. The arrays were stained with streptavidin-phycoerythrin and biotinylated anti-streptavidin antibodies. The differential gene expression data analysis was performed using GeneChip 4.0 software. The 3'/5' cRNA transcript ratios for both GAPDH and b-actin were found to be consistent between control and treatment groups over a period of 12h exposure. Altered gene expression patterns were observed in 133 RNA transcripts with at least a 2 fold change. Alterations in expression include metabolic genes (CYP11B1, MGMT), cell cycle control and DNA repair genes (GADD45, Cyclin G2, Cyclin D3, Cyclin A) and transcription factors (IF2, IF3). Whether alterations in the expression pattern of these genes, particularly transcriptional factors, is associated with carcinogenic potential of TCA needs to be elucidated.

1279 SPERM RNA AMPLIFICATION FOR GENE EXPRESSION PROFILING BY DNA MICROARRAY TECHNOLOGY.

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DNA microarray technology has been widely used to detect differential gene expression and to generate gene expression profiles useful for studying drug or environmental effects on animals or humans. In order to establish methods for monitoring effects of environmental chemicals on the male reproductive system, we have collected rat (pool of ten 75-90 day Sprague-Dawley rats) epididymal sperm and isolated total RNA. Due to the limited number of sperm from individual animals, the amount of total RNA purified from rat sperm was not sufficient for microarray probe labeling. Therefore, RNA amplification was used to generate sufficient quantities of antisense RNA (aRNA) for array hybridization. Two rounds amplification of 200ng rat sperm RNA generated 25 to 35ug aRNA. Only one round of amplification was necessary to obtain similar amounts of aRNA from 200ng testis RNA. After indirect labeling with Cy3 or Cy5, aRNAs were hybridized to glass-slide microarrays with 70-mer oligonucleotides probes for 4, 370 rat genes. Preliminary analysis identified hundreds of genes/mRNAs in both the testis and sperm samples (e.g., SP22 sperm protein). These results indicate that RNA amplification can be used to generate gene expression profiles from tissue samples with limited amounts of RNA. More detailed studies of sperm RNA profiles from rats following chemical exposure are underway. Results from these toxicology studies promise to complement studies of sperm RNA profiles in humans. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1280 A PRACTICAL EXPERIMENTAL DESIGN TO CORRECT FOR DYE BIAS IN DUAL-LABELED CDNA MICROARRAY EXPERIMENTS WITHOUT SACRIFICING PRECISION.

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A significant limitation to the use of dual-labeled spotted cDNA microarrays is the introduction of signal variance by dye bias presumably due to sample incorporation of two distinctly different chemical dyes. Several approaches were used to assess and minimize the effects of dye bias on fluorescent signal hybridizations. Human TK6 cells were exposed to vehicle or (0.01 to 1.0 uM) benzo[a]pyrene diol epoxide (BPDE) for 4 or 24h and total RNA was isolated, labeled with Cy3 or Cy5, and hybridized to Human 350 Microarrays (Phase-1, Santa Fe, NM). Dye bias alone accounted for a significant component of measured differences and introduced unacceptably high numbers of both false positive and false negative statistically significant signals. However, we have found that within a given set of concurrently processed hybridizations, the bias is remarkably consistent and, therefore, measurable and correctable. Paired replicate dye-swap corrections are commonly performed to eliminate dye bias but can be costly to end-users, can introduce statistical error, and may therefore generate false negative signals when statistical criteria are used to identify gene expression alterations. We demonstrate a practical experimental design to measure and mathematically eliminate this dye bias for each set of concurrently processed microarrays by including split control sample hybridizations (Control Cy3 vs. Control Cy5). The number of replicate hybridizations required for confidence in data interpretation can thus be minimized. We show that approximately 25 - 30% of the genes identified as statistically significantly (paired t-test, $p<0.001$) altered by BPDE treatment on a pair of microarrays can differ depending on the approach chosen to eliminate dye-bias effects. Incorporating split control microarrays within a set of concurrently processed hybridizations to measure dye bias specifically can eliminate the need for dye-swaps and improve experimental precision.

1281 EVOLUTIONARILY CONSERVED RESPONSES TO ARSENIC IN YEAST AND HUMAN CELLS.

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Human exposure to arsenic in the drinking water is associated with an increased incidence of neoplasia including skin cancer. However, the mechanisms in arsenite-mediated toxicity and carcinogenesis remain elusive, due in part to the lack of an appropriate animal model. Microarrays have provided important information about the response to environmental toxicants. Previous gene expression studies in normal human epidermal keratinocytes (NHEK) suggested that arsenite (AsIII) was associated with oxidative stress, altered DNA repair, and increased proliferation (Hamadeh et al, 2002). To investigate the importance of arsenite-modulated genes in the etiology of arsenic-related effects, we are comparing gene expression profiles in yeast and human cells. Our hypothesis is that arsenic will lead to a conserved response in all eukaryotic cells. Yeast is an excellent model for characterizing and interpreting genomic expression data because of extensive understanding of its biology and genome. Our goal is to assess whether a yeast model for AsIII toxicity can identify human-yeast orthologs modulated by arsenite. Preliminary results of multiple biological experiments with four hybes/experiment and analysis of statistically validated outlier genes at 99% confidence show stress response genes (e.g. heat shock proteins, thioredoxins, metallothioneins, glutathione transferase) are induced in both yeast and human cells after non-toxic doses of AsIII. Additional candidate human genes are being discovered through the analysis of yeast orthologs, using the 6200 gene set. AsIII treated NHEK RNA will be subjected to RT-PCR to quantify the levels of target genes identified using yeast gene expression and deletion analysis, and help discover new genes involved in human arsenite-mediated toxicity and carcinogenesis.

1282 TEMPORAL GENE EXPRFESSION PROFILIES OF HUMAN FETAL ASTROCYTE SVG CELLS TREATED WITH A NEUROTOXICANT, 6-AMINONICOTINAMIDE.

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6-Aminonicotinamide is a nicotinamide analogue that is known to induce severe neurotoxic effects to both developing and adult animals. Several histopathological and biochemical analyses suggest that astrocytes are its major targets. This chemical is considered to affect the pentose phosphate pathway, resulting in the inhibition of NADPH production. However, the molecular mechanism leading to brain disorders remains unknown. In this study, the effects of 6-aminonicotinamide on global gene expression were examined in SVG, an immortalized human fetal astrocyte cell line. Cells were treated with 6-aminonicotinamide at doses ranging from 0.1 to 10 μM for 24h. After treatment, RNA was isolated from each treatment and vehicle-treated control group and the temporal changes in gene expression were measured using a custom, SVG-specific cDNA microarray (3, 372 genes). Data were background corrected, and subsequently normalized by z-score normalization using the GenePix Post Processing (GP3) script. Data were filtered using a General Linear Mixed Model to model the microarray data, followed by pairwise comparisons using a t-test. The expression of numerous genes were affected following treatment, including a series of genes known to be responsive to oxidative stress as well as apoptosis-related genes. The temporal gene expression profiles suggest that 6-aminonicotinamide induces oxidative stress, followed by adaptive responses and events leading to apoptosis of SVG cells. Supported by ES 04911-12.

1283 ALTERATIONS IN GENE EXPRESSION BY TRIVALENT CHROMIUM IN HEPG2 CELLS AND MOUSE LIVER.

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Trivalent chromium [Cr(III)] is used as a dietary supplement to lose weight and is the dominant toxicant at several Superfund sites around the United States, including an estuarine site in South Carolina. However, the effects of Cr(III) are not well

understood and studied because of its low bioavailability. Therefore, we have attempted to characterize the effects of Cr(III) on gene expression in male mouse liver and HepG2 cells. HepG2 cells were exposed to Cr(III), as chromium chloride hexahydrate to determine their toxicity. Cr(III) did not cause cell death at any dose tested, but significantly decreased growth at 100uM. Cells were treated with 20uM Cr(III) for 6 hours or five days, RNA was extracted and Clontech™ arrays were incubated and analyzed. ResGen Pathways Universal Microarray Analysis Software™ was used for microarray analysis and alterations in gene expression were considered statistically significant by the Chen Test at $p<0.05$. Several genes involved in protein turnover showed altered expression due to Cr(III) in HepG2 cells, including α-1 antichymotrypsin, cathepsin D, apoptotic protease activating factor 1, and ACl proteasome subunit a3. Furthermore, several genes that have previously been shown to be altered by oxidative stress were changed in HepG2 cells, as were some growth related genes (IGF-BP1). RT-PCR was performed to confirm differential expression of some of these genes. In comparison, mice were treated with Cr(III) in their drinking water at a concentration of 200mg/L for two weeks. Mouse liver arrays also demonstrated changes in gene expression in oxidative stress and protein turnover related genes. However, most of the alterations in gene expression in mouse liver were in either growth related genes (Erbb2, IGFBP-1, STAT6, Myc) or genes involved in the immune system (TDAG51, IL6R). Many of the genes altered are involved in tumor progression and metastasis and some are involved in Alzheimers disease. A thorough comparison of changes between short and long-term exposure to cells and treatment of mice will be presented.

1284 CHARACTERIZATION OF TCDD-RESPONSIVE GENES IDENTIFIED BY CDNA MICROARRAY ANALYSIS.

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2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a powerful teratogen capable of disrupting development of the cardiovascular system, kidney, brain, craniofacial structures, and endocrine system. The molecular mechanisms of TCDD embryotoxicity are poorly understood, but likely involve AHR-mediated transcriptional regulation. Previously, we have reported the use of cardiovascular-specific cDNA microarrays to identify alterations in gene expression in zebrafish embryos exposed to TCDD. Here we present more detailed analyses of genes of interest identified by these studies. cDNA clones manifesting 2-fold or greater differential regulation by TCDD were sequenced and found to represent 12 genes of known function, as well as 21 ESTs. The normal roles of the known genes include xenobiotic metabolism, cardiovascular function, hormone synthesis, and fatty acid binding. In order to better understand their roles in embryotoxic processes, we have used RT-PCR to characterize the dose- and developmental time- dependence of TCDD-regulated changes in expression of these genes. In addition, a combination of *in situ* hybridization, RT-PCR, and morpholino knock-down is being utilized to begin functionally characterizing novel TCDD-responsive ESTs. This work promises to provide important insights into the molecular pathways affected during TCDD-induced embryotoxicity. NIH #P42-ES07381

1285 DIFFERENTIATION BETWEEN SENSITIZERS AND IRRITANTS IN THE LOCAL LYMPH NODE ASSAY USING A MINIMAL TRANSCRIPT SET.

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Previous studies utilized the LLNA in conjunction with DNA microarrays to evaluate expression profiling as a method to distinguish between structurally diverse sensitizers and irritants and to identify potential transcript biomarkers specific for each compound class. Eight transcript discriminators were identified: mucin 10 (Muc10); T-cell death associated gene (Tdag); T-cell receptor gamma V4, (Tcrg-V4); gap junction membrane channel protein β2; lymphocyte-activation gene 3 (Lag3); RAB4A; and kallikreins 9 and 26 (KIK9 and KLK26). The objective of this study was to confirm and/or refine the 8 potential transcript biomarkers using real-time PCR. Analyses were performed on auricular lymph node total RNA isolated from 8-week-old female CBA/JHsd mice (n=10-15/group) dosed with either 4:1 acetone/olive oil (vehicle) or one of three dermal sensitizers (0.25% dinitrochlorobenzene, DNCB; 0.05% oxazolone, OX; 25% α-hexylcinnamaldehyde, HCA) or one of four irritants (25% chlorobenzene, CB; 5% phenol, PH; 20% benzoic acid, BA; 0.5, 1, or 2% benzalkonium chloride, BAC) according to standard LLNA protocol. Experiments were performed using the TaqMan® technology and primer/probe sets of each specific biomarker. Consistent with the array results, Lag3 was upregulated in mice treated with the sensitizers and unchanged in mice from the irritant groups. Evaluation of sensitizer-specific signatures revealed a strong induction of cell cycle and inflammatory response transcripts that are unaltered by irritant treatment. These clusters of potential biomarkers effectively confirm proliferation-based scoring in the LLNA. Expression profiling performed on higher doses of BAC that typically score positive in the LLNA, revealed a similar induction of many of these transcripts, as well as the proinflammatory serine protease, kallikrein 6 providing a molecular basis for high-dose irritant scoring in the LLNA. These results revealed a minimal transcript biomarker set that has the potential to distinguish sensitizers from irritants in the LLNA.

1286 COMPARATIVE GENE EXPRESSION PROFILING IN FEMALE RATS TREATED SUBCHRONICALLY AND CHRONICALLY WITH PCB126, PCB153, AND TCDD.

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PCB126 (3, 4, 5, 3', 4'), PCB153 (2, 4, 5, 2', 4', 5'), and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) represent persistent environmental contaminants that elicit a variety of toxicological responses. Disparate affinities for the Aryl Hydrocarbon Receptor (AhR) partially explains differences in the toxicology of PCB126 (high affinity), TCDD (high affinity) and PCB153 (little or no affinity), but little is known about how these chemicals influence other molecular pathways. The purpose of the present study was to characterize global gene expression profiles in female Sprague-Dawley rats treated with vehicle, 100 ng/kg/day TCDD, 1000 ng/kg/day PCB126, or 1000 ug/kg/day PCB153 for 13 or 52 weeks (NTP cancer bioassay). Total RNA from toxin treated and time matched, vehicle treated liver tissue was reverse transcribed in the presence of amino-allyl dUTP and subsequently coupled to Cy5 or Cy3 esters, respectively. Triplicate labeling reactions were applied to cDNA microarrays fabricated at the Institute for Genomic Research (TIGR, http://pga.tigr.org/RatText.shtml). Each array contained over 26 k genes from 13 different tissue libraries. Poor quality spots were removed from further analysis and the remaining datasets were normalized by lowess and subjected to cluster analysis to identify dose, time and toxicant-related patterns in gene expression. Additionally, Sprague-Dawley rat tissue slices were incubated for 24 hr in dynamic organ culture containing either 10 nM dioxin or vehicle and analyzed by microarray in order to identify biomarkers for acute exposure. We discovered a number of genes exhibiting time and/or compound-specific alterations in expression and selected changes were validated by RT-PCR. These data may provide new biomarkers for exposure to PCBs and TCDD and elucidate new biological mechanisms for their toxicity. (Supported in part by NIEHS ES09440, SOT, and University at Buffalo).

1287 ESTROGEN INCREASES DOPAMINE BETA HYDROXYLASE ACTIVITY AND PROTEIN EXPRESSION IN CATH.A CELLS.

H. L. Rincavage and C. M. Kuhn. *Pharmacology, Duke University, Durham, NC.* Sponsor: T. Slotkin.

We have previously found that the locus coeruleus-derived cell line Cath.a expresses Estrogen receptor (ER) beta protein, but does not express ER alpha protein. The expression of ER beta in this cell line may have important ramifications for the regulation of estrogen-dependent genes involved in noradrenergic function. To test the possibility that estrogen modulates noradrenergic function, we investigated the effects of estrogen on two enzymes involved in norepinephrine synthesis, dopamine beta hydroxylase (DBH) and tyrosine hydroxylase (TH). In order to assess the effects of estrogen on DBH activity, cells were treated with estrogen (10 pM and 100 nM) for 24 hrs, then lysed and freeze-thawed to release DBH. DBH was semi-purified by adsorption onto concavalin A Sepharose 4B beads. Enzyme activity was assayed in the presence of a saturating dopamine concentration (3 mM). Norepinephrine was extracted with alumina and detected by HPLC. Estrogen increased the activity of DBH in a concentration-dependent manner. Western blot analysis showed that DBH protein expression increased with estrogen treatment as well. Protein expression of tyrosine hydroxylase (TH) did not change with estrogen treatment. These findings indicate that estrogen may regulate mood by affecting central noradrenergic mechanisms. Supported by MH-65093

1288 USE OF A PLANAR MICROELECTRODE ARRAY SYSTEM TO MEASURE TOXICANT INDUCED CHANGES IN THE ELECTRICAL ACTIVITY OF ACUTE BRAIN SLICES FROM BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*).

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Existing electro-chemical water monitoring systems recognize only specific threats or categories of threats. The present study used a commercially available, planar microelectrode array system (Panasonic MED64) to develop a flexible system for

water supply monitoring. It was first necessary to determine that fish brain would provide viable slices for use with the MED64 System, previously demonstrated to work only with mammalian brain slices. Initially, thirty two 500 uM slices obtained from the optic tectum of bluegill sunfish were attached to 64-electrode microelectrode arrays and bathed in continuous perfusion of fish artificial cerebrospinal fluid (F-CSF). The brain slices were attached to the array so that it maximally covered the areas believed to be the most active for sensory input integration. Electrode sites were successively stimulated with 50 uA biphasic pulses with a pulse duration of 100 uS. The presence of evoked response activity was then assessed at each of the 63 remaining electrode sites. This process was repeated until the entire array was mapped. Test electrode pairs were then chosen for each brain slice. The gain in evoked response amplitude averaged 5-10x for all of the slices. The biological nature of the responses was tested by treatment with 20 uMol of the sodium channel blocker tetrodotoxin (TTX). TTX reversibly eliminateed the evoked responses. Subsequently, slices were treated with ethanol (20-80 uMol), and dose dependent reductions in evoked response amplitude were observed. Slices treated with the GABA-A chloride channel suppressor (TMPP) (10-30 uMol) exhibited dose related increases in the amplitude of the evoked responses, as did slices treated with the GABA-B agonist baclofen (10-20 uMol). Phaclofen, a GABA-B antagonist, was found to reduce the amplitude of the responses.

1289 USE OF A PLANAR MICROELECTRODE ARRAY SYSTEM TO MEASURE EFFECTS OF CHEMICAL CHALLENGES ON AN ACUTE TISSUE SLICE PREPARATION FROM MOUSE CEREBELLUM.

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To date, several publications have reported use of acute tissue slice preparations mounted on 8-64 electrode microarrays to evaluate neurotoxicant or pharmaceutical challenges. These experiments have utilized hippocampal, cortical or suprachiasmatic nucleus (SCN) preparations dissected from rat brain. To date, no published research has used either acute tissue slice preparations from mouse brain, or cerebellar preparations from any species. Because the cerebellum subserves important fine motor control and associative learning functions, development of a cerebellar preparation is of obvious importance to military toxicology. The present experiment used cerebellar slices from young adult mice and typically included the interpositus and other deep cerebellar nuclei. Slices were mounted for up to 6 hr on MED64 (Panasonic) microarrays perfused with mouse ACSF. Slices were challenged with one of four compounds active at the GABA-A receptor [TMPP (5-15 uMol), PTZ (20-80 uMol), picrotoxin (25 uMol), or ethanol (50 uMol)] or with either a GABA-B agonist [baclofen (15 uMol)] or antagonist [phaclofen (20 uMol)]. Each chemical challenge provided a "response profile" to electrical stimulation, as compared to baseline, that was unique to that chemical. It is suggested such "profiles" can be used to identify chemical challenges with unknown compounds whose mechanism of neural action is identical or similar.

1290 POLYCHLORINATED BIPHENYLS INHIBIT DOPAMINE UPTAKE IN HUMAN NEUROBLASTOMA CELLS STABLY EXPRESSING THE HUMAN DOPAMINE TRANSPORTER.

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Polychlorinated biphenyls (PCB) are persistent environmental contaminants that have been reported to produce neurological dysfunction in both humans and laboratory animals. Studies by Seegal and co-workers have shown that sub-chronic or chronic exposure of non-human primates and rodents to the PCB mixtures Aroclor 1016 or 1254 results in long-term depletion of dopamine levels in the striatum and substantia nigra. Recently, Mariussen and Fonnum reported that the PCB mixtures Aroclor 1242 and 1254 inhibited dopamine uptake into whole rat brain synaptosomes. To determine if inhibition of dopamine uptake observed in rat brain synaptosomes was due to direct inhibition of the dopamine transporter (DAT), we generated a human neuroblastoma line stably expressing the human dopamine transporter. Human DAT cDNA was inserted into the plasmid pcDNA 3.1 and transfected into SK-N-MC neuroblastoma cells using Lipofectamine. Cells were cultured in growth medium containing G418, resistant colonies screened by western immunoblotting and dopamine uptake, and a stable cell line expressing high levels of DAT was used for further study. Cells were plated in 24-well trays at a density of 100, 000 cells per well and assays conducted two days later. For determination of IC_{50} values, cells were incubated with various concentrations of Aroclor 1016 or 1254 for 15 min. Dopamine uptake was initiated and allowed to proceed for 10 min. Non-specific uptake was determined in the presence of 10 μM GBR12935, a specific inhibitor of DAT, and IC_{50} values were estimated by nonlinear regression. Aroclor 1016 and 1254 inhibited dopamine uptake with IC_{50} values of 7.9 and 8.6 μM, respectively. These concentrations are in the same range as those shown to elicit dopamine release and deplete dopamine levels in striatal slices. Therefore, these results suggest that a possible mechanism for depletion of dopamine levels by PCBs may be inhibition of DAT mediated reuptake of released dopamine.

1291 THE INDUCTION OF MITOCHONDRIAL SP60 IN RAT HIPPOCAMPAL ASTROCYTES BY DIETHYLDITHIOCARBAMATE.

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Diethyldithiocarbamate (DDC) and its parent compound Disulfiram have been shown to cause alterations in mitochondria. These include alterations in their outer membrane and cristae, the formation of myelin whorls and the accumulation of electron dense material and condensation of material within the mitochondrial matrix. Mitochondrial SP60 and SP10 belong to a family of highly conserved proteins, termed chaperonins, that occur in prokaryotes and in mitochondria and chloroplasts of eukaryotic cells. They function in the biogenesis of mitochondria and their constitutive role is the assembly of newly imported, matrix localized proteins. They are also involved in the assembly of proteins of that are encoded by the mitochondrial genome and translated on the mitochondrial ribosomes. In conditions of stress, SP60 and SP10 act as molecular chaperones by binding to hydrophobic protein surfaces that are transiently exposed during various denaturing processes. This binding may inhibit incorrect interactions that may lead to protein aggregation, misfolding, or the production of any non-functional structures. The present report investigated the effects of DDC on the induction of SP60 and SP10 in rat hippocampal astrocytes. Cultures of rat hippocampal astrocytes were treated with 150 μM DDC for one hour and samples were collected at 0, 4, and 6hrs post-DDC treatment. Immunoblot analysis revealed significant increase in SP60 at 0 and 6 hrs. post-DDC treatment. Preliminary investigation of SP10 induction did not show any changes.

1292 INTERLEUKIN-1β ENHANCES NMDA RECEPTOR-MEDIATED $[CA^{2+}]_I$ INCREASE IN PRIMARY RAT HIPPOCAMPAL NEURONS: ROLE IN NEUROTOXICITY.

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Interleukin (IL)-1β and the over-activation of NMDA receptor have often been implicated in several cases of neurotoxicity and in the pathogenesis of various CNS diseases. In this study, we investigated the effect of IL-1 βon NMDA-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) increase in primary rat hippocampal neurons. We found that 0.025-0.1ng/ml IL1b rapidly and dose-dependently enhances 10 μM NMDA-induced increases of $[Cal^{2+}]_i$ by 50% on average thus suggesting a facilitation of NMDA-receptor function. This effect occurred only when neurons were pretreated with IL-1β while it was absent if IL-1β and NMDA were simultaneously applied. The effect of IL-1β was mediated by IL-1 type I receptors since it was abolished by 1μg/ml IL-1 receptor antagonist. Facilitation of NMDA-induced $[Cal^{2+}]_i$ increase by IL-1β was prevented by 500 nM staurosporin and 500nM levendustin A, suggesting an involvement of src tyrosine kinase. These data suggest that IL-1β increases NMDA receptor function. Since over-activation of this receptor increases $[Ca^{2+}]_i$, that can activate downstream signaling pathways leading to cell death, the observed effect may play a role in glutamate-mediated neurodegeneration.

1293 ACCUMULATION OF THE PERSISTENT ENVIRONMENTAL TOXICANTS METHYLMERCURY OR POLYCHLORINATED BIPHENYLS IN *IN VITRO* MODELS OF RAT NEURONAL TISSUE.

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Polychlorinated biphenyls (PCBs) and methylmercury ($CH3Hg^+$) are known toxicants which persist in the environment and accumulate in tissue. Studies to identify mechanisms of action associated with these toxicants have largely been conducted *in vitro*, and dosimetry comparisons across *in vitro* preparations and *in vivo* dosing conditions have not been well defined. The present study examined the accumulation of

the PCB mixture Aroclor 1254 (A1254) or CH_3Hg^+ in commonly used *in vitro* neuronal models: primary cultures of rat neocortical cells and nerve growth factor differentiated pheochromocytoma (PC12) cells. Cells were exposed to A1254 (0, 3 μM) or to CH_3Hg^+ (0, 10, 100, 1000 nM) in serum-free media for 1 or 24 hr. In addition, cells were exposed to 3 μM A1254 in double the media (2x) volume for 1 hr. After each exposure cells were washed, centrifuged, cell pellet weighed, and total PCB or Hg^+ content measured by dual column gas chromatography with electron capture detection or by cold vapor atomic absorption, respectively. Neocortical cells exposed to A1254 contained 20.6, 24.1, and 55.4 ppm PCB after 1 hr, 24 hr, and 2x volume respectively. PC12 cells exposed to A1254 contained 66.7, 103.8, and 103.9 ppm for 1 hr, 24 hr, and 2x volume. Neocortical cells exposed to 0, 10, 100, 1000 nM CH_3Hg^+ contained 0.023, 0.149, 1.206, and 11.498 ppm and 0.032, 0.136, 1.177, and 7.708 ppm after 1 and 24 hr. PC12 cells exposed to CH_3Hg^+ incorporated 0.128, 0.266, 2.171, 17.473 ppm after 1 hr and 0.086, 0.408, 2.806, 21.855 ppm after 24 hr at 0, 10, 100, and 1000 nM, respectively. These results demonstrate that these neurotoxicants readily accumulate in neuronal tissue *in vitro*. These results will help to facilitate comparison of *in vitro* exposures to these compounds with tissue levels observed following *in vivo* exposure. (This abstract does not necessarily reflect USEPA policy).

1294 PERCHLOROETHYLENE (PERC) INHIBITS FUNCTION OF VOLTAGE-GATED CALCIUM CHANNELS IN PHEOCHROMOCYTOMA CELLS.

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The industrial solvent perchloroethylene (PERC) is listed as a hazardous air pollutant in the 1990 Ammendments to Clean Air Act and is a known neurotoxicant. However, the mechanisms by which PERC alters nervous system function are poorly understood. In recent years, it has been demonstrated that volatile organic solvents such as toluene and trichloroethylene disrupt the function of ligand and voltage-gated ion channels in neurons, including voltage-gated calcium channels (VGCC). The present experiments examined the hypothesis that PERC also disrupts ion channel function by examining PERC effects on Ca^{2+} currents (I_{Ca}) in nerve growth factor differientiated pheochromocytoma (PC12) cells using whole cell patch clamp techniques. PERC (100-1000 μM) inhibited I_{Ca} rapidly in a concentration-dependent and reversible manner; 1000 μM PERC completely suppressed I_{Ca}. When I_{Ca} was elicited by a step potential from -70 to +10 mV, 500 μM PERC decreased peak I_{Ca} to 56 ± 3% and end I_{Ca} to 39 ± 6% of control amplitude (n=7), suggesting that end current is more sensitive to PERC effects than is peak current. PERC (500 μM) also shifted the voltage of half-maximal activation ($V_{½}$) of I_{Ca} from -1.6 mV to -15.8 mV (n=5). As such, the current-voltage (I/V) relationship in the presence of PERC demonstrated increased I_{Ca} amplitude at hyperpolarized test potentials (-20 and -10 mV), but decreased I_{Ca} amplitude at test potentials greater than 0 mV (n=5). In addition, peak of the I/V relationship was shifted by approximately 10 mV to more hyperpolarized potentials. These results demonstrate that PERC blocks I_{Ca} at low micromolar concentrations, and suggest that effects on VGCC could contribute to the neurotoxicity of this solvent. (This is an abstract of a proposed presenatation and does not necessarily reflect EPA policy)

1295 INHIBITORY EFFECTS OF PERCHLOROETHYLENE ON HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS.

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Perchloroethylene (PERC) is a volatile organic solvent with a variety of industrial uses. PERC exposure has been shown to cause adverse neurological effects including deficits in vision and memory in exposed individuals. Despite knowledge of these effects, the mechanisms by which PERC may modulate central nervous system function are relatively unknown. Studies with other volatile organic compounds (VOCs) such as alkylbenzenes and 1, 1, 1-trichloroethane have correlated neurobehavioral changes to modifications in brain receptor function. Specifically, the nicotinic acetylcholine receptor (nAChR), which is can play a role in cognition and memory, has recently been shown to be sensitive to VOCs, including toluene, as well as volatile anesthetic gases. Therefore, we examined whether nAChRs were sensitive to PERC. Using two electrode voltage clamp techniques, acetylcholine-induced currents were measured in recombinant human α4β2 and α7 nAChRs expressed in Xenopus oocytes. PERC (100-500 μM) inhibited nAChR function in a concentration-dependent and reversible manner. PERC (500 μM) decreased α4β2-mediated acetylcholine responses by 50 percent (n=3) and α7 responses by 60 percent (n=5) compared to control current amplitudes. Toluene (1 mM), an alkylbenzene solvent, was used as a positive control and also inhibited receptor responses (α4β2, 24.2 percent, n=4; α7, 18.0 percent, n=2) as has been previously shown in rat nAChRs. These results show that nAChRs represent a potential target for solvents such as PERC in the central nervous system and suggest that nAChR inhibition could contribute to neurotoxic effects associated with this compound. Furthermore, the data implies that human nAChRs are more sensitive to inhibition by PERC than toluene. (This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.)

1296 BROMINATED FLAME RETARDANTS; OXIDATIVE STRESS AND CELL DEATH USING CEREBELLAR GRANULE CELLS AND GRANULOCYTES.

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Studies have shown that brominated flame retardants have an effect on behavior in laboratory animals. We have therefore studied the effect of these compounds on neurons in culture. We have found that pentabromodiphenylether (PBDE), tetrabromobisphenol-A (TBPPA) and hexabromocyclododecane (HBCD)in low micromolar concentration induce cell death to cerebellar granule cells in culture. The effect on cell death were all reduced by the NMDA receptor blocker MK-801 and by 50μ vitamin E.This indicated that calcium influx through the NMDA receptor and free radical formation could play a role in cell death. TBBPA was the only compound that lead to free radical production using the fluorescent probe DCF-DA.The results on free radical formation was confirmed with human granulocytes.The free radical formation in both cases was inhibited by U0126, a MAPK inhibitor. In granulocytes we have further shown by Western blotting that TBBPA will induce phosphorylation of ERK.

1297 ORTHO-SUBSTITUTED 2, 2', 3, 3', 4, 4', 5-HEPTACHLOROBIPHENYL (PCB170) ALTERS NEUROPLASTICITY IN ACUTE HIPPOCAMPAL SLICE.

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There is mounting evidence that exposure to non-coplanar PCB's is associated with significant changes in locomotor activity, spatial learning and memory in rodents. In the current study, PCB 170-induced changes in synaptic transmission of rat hippocampal slices were monitored by electrophysiological measurements. Field excitatory postsynaptic potential (fEPSP) was evoked by single pulse stimulation of Schaffer Collateral/commissural fibers at striatum radiatum of the CA1 region in the hippocampus. Following exposure to 100 nM PCB 170, time-dependent changes in the slope and amplitude of fEPSP were seen, with pases of enhancement and depression. To investigate the contribution of inhibitory neurons in the actions of PCB 170, hippocampal slices were pre-treated with the GABAa receptor antagonist, picrotoxin (PTX, 100 uM). Pre-treatment with PTX resulted in negligible change in EPSP slope elicited by single pulse stimuli. Importantly PCB170 (10 nM) introduced in the presence of GABAa blockade enhanced EPSP slope (200-300%) revealing a significant facilitation of synaptic transmission. Non-coplanar PCBs therefore influence both excitatory and inhibitory pathways in CA1 that can mask their potent effects. These results demonstrate that blockade of inhibitory inputs with PTX can unmask the potent actions of ortho-substituted PCB 170 toward facilitating excitatory transmission. Moreover the measured effect of PCB 170 on neuroplasticity is a summation of actions on inhibitory and excitatory pathways in hippocampus, possibly stemming from a common mechanism.

1298 ORGANOPHOSPHORUS COMPOUNDS SELECTIVELY INHIBIT ESTERASE ACTIVITY AND GROWTH FACTOR-INDUCED CELL GROWTH IN AN *IN VITRO* BLOOD BRAIN BARRIER MODEL.

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The blood brain barrier (BBB) is a structural and functional interface between the circulatory system and the brain. The BBB protects the free passage of substances into the brain as well as maintains homeostasis of the central nervous system. Organophosphorous (OP) compounds such as chlorpyrifos (CPF) are lipophillic and are able to pass through the BBB and may disrupt BBB integrity and function. To determine possible mechanisms of action for CPF toxicity, we used an *in vitro* BBB model where bovine microvascular endothelial cells (BMEC) and neonatal rat astrocytes are co-cultured. We examined the hypothesis that CPF and CPF-oxon have potential to disrupt the BBB by selectively altering esterase activity as well as affecting growth factor signaling. The co-culturing of BMEC and astrocytes re-

sulted in tight junction formation, which was evident by electron microscopy and western blot analysis of ZO-1, a marker of tight junctions. Vascular endothelial cell proliferation and migration are critical events for angiogenesis and are regulated by vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Following 24hr exposure to VEGF, FGF or VEGF/FGF, cell number increased by 113%, 121% and 141% respectively in serum-free conditions. Concurrent exposure of various concentrations of CPF (0.01, 0.1 and 1 mM) diminished VEGF-induced (15%, 52% and 50%), FGF-induced (4%, 56% and 56%) and VEGF/FGF-induced (18%, 59% and 63%) proliferation. It has been suggested that carboxylesterase (CaE) was important in detoxication of OP compounds and inhibition of this enzyme may potentiate OP neurotoxicity. At final concentrations of 0.1, 1 and 10 mM, CPF inhibited CaE activity (43%, 50%, and 100%). At all tested concentrations, CPF-oxon totally inhibited CaE activity. These data suggest the possibility that CPF and CPF-oxon may alter growth factor signaling as well as the inhibition of esterases leading to a disruption on BBB structure and function.

1299 *IN SITU* EFFECTS OF ORGANOPHOSPHATE (OP) COMPOUND EXPOSURE ON ATP PRODUCTION IN HUMAN NEUROBLASTOMA CELLS.

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In situ study of cell energetics has benefits in allowing for evaluation under conditions that require fewer cells and cause less organelle damage than studies using isolated mitochondria. We examined ATP production in digitonin permeabilized SH-SY5Y human neuroblastoma cells depleted of residual ATP by a 30 min, 37 degree preincubation of harvested cells in phosphate buffered saline (PBS). Reactions mixtures included 40 mcg/ml digitonin and 1 mM ADP with and without 10 mM each of glutamate and malate (mitochondrial respiratory chain complex I substrates). A complex I inhibitor (rotenone 20 mcg/ml) and the OP compound phenyl saligenin phosphate (PSP), 0.01 mM, were included in some incubates as well. After incubations of 5 to 25 min, ATP was extracted from neutralized trichloroacetic acid precipitates and quantified, based on mg protein, using a luciferase enzyme assay. Results demonstrated increased production of ATP with time and addition of complex I substrates. The inhibitory effects of rotenone were exaggerated 50% - 70% by the presence of 0.01 mM PSP at time points of 15 min or more. These results suggest that PSP may have an effect on cellular energetics of neuroblastoma cells when evaluations are done in situ.

1300 cAMP AND RETINOIC ACID INDUCED DIFFERENTIATION OF HUMAN SVG CELLS: MORPHOLOGIC AND TRANSCRIPTIONAL EFFECTS.

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Previous work has shown that treatment of human SVG cells with 5μM forskolin (F) and 200μM 3-isobutyl-1-methylxanthine (I) increases cAMP levels resulting in differentiation and dramatic morphologic changes. Gene expression profiles were analyzed over time (2, 4, 6, 12, 24hrs) during F/I induced differentiation, using a microarray spotted with approximately 3, 000 cDNAs. Data was normalized and clustered before and after filtering by General Linear Mixed Model with post hoc t-test (GLMM-T) or Shannon Entropy. Genes involved in the regulation of morphology, especially regulation of actin dynamics, were identified as having been affected by F/I treatment over time. GLMM-T filtering of the data identified a cluster of genes downregulated in vehicle treated cells at 6, 12, and 24hrs following exposure that may represent a vehicle or a response to the transfer of cells to serum free media. cAMP induction of retinoid X receptor α (RXRα) suggested that retinoic acid treatment may interact with cAMP-induced differentiation. Cotreatment of SVG cells with F/I and all-trans retinoic acid (ATRA; 0.05, 0.5, 5μM in ethanol) resulted in a significant increase in the percentage of differentiated cells over time ($p < 0.001$) compared to F/I and F/I + ethanol groups, but no dose-dependent response to ATRA was observed. These results indicate that ATRA may enhance F/I related gene expression effects regulating actin dynamics. This work is supported by EPA Grant ES 04911-12 and NIH grants ES 011777 and T32 ES07255.

1301 MODULATION OF THIOL STATUS BY MANEB IN NEURONAL CELLS.

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Recent attention has focused on exposure to environmental chemicals as risk factors for Parkinson's disease (PD). The dithiocarbamates are a class of synthetic organic chemicals that have various agricultural, clinical, and industrial applications. They can act as chelating agents to alter the redox status of cells by generating a state of oxidative stress within exposed cells. Maneb (MB) is a manganese-complexed ethylene-bis-dithiocarbamate fungicide that is used to control early and late blights on potatoes and tomatoes. Case studies of agricultural workers exposed to MB have shown the development of an extrapyramidal syndrome reminiscent of PD. The goal of this study was to determine whether MB alters thiol status in neuronal cells. PC12 cells and primary ventral mesencephalic cultures treated with MB show elevated intracellular glutathione (GSH) and heme oxygenase-1 (HO-1) levels, which may serve as a first line of defense against oxidative stress. No changes were observed in glutatione peroxidase enzymatic activity and oxidized GSSG, suggesting that MB could directly act on γ-glutamyl-cysteine synthetase (γGCS) to increase intracellular GSH levels. To examine this possibility, PC12 cells were pretreated with 50μM BSO (an inhibitor of γGCS) for 6h, which induced a 60-80% depletion in intracellular GSH. Cells were subsequently treated with varying concentrations of MB (50-2500ng/mL) and harvested at 0, 6, 12, 24, or 48h after MB treatments to measure GSH recovery. While lower MB concentrations (<500ng/mL) resulted in a rapid recovery of GSH levels, higher concentrations (>500ng/mL) delayed recovery. The lack of GSH recovery was also accompanied by increased cell death, as indicated by decreased membrane integrity and a decline in metabolic function. These studies confirm that MB regulates thiol and ultimately redox status within neuronal or PC12 cells, thus, underlying the importance of GSH in the detoxification of reactive oxygen species that can be generated from pathways that lead to the progression of neurodegenerative diseases such as PD. (T32 ES07026)

1302 DOPAMINERGIC TOXICITY OF THE HERBICIDE ATRAZINE IN *EX VIVO* STRIATAL SLICES.

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A link between Parkinson's Disease and pesticide exposure has been suggested and recently is was shown that the herbicide atrazine (ATR) modulates catecholamine metabolism in PC12 cells. Because ATR might affect basal ganglia function and, at present, there are no data addressing that, the objective of this study was to determine if ATR is capable of modulating dopamine (DA) metabolism in *ex vivo* striatal tissue slices. Striata from adult male Sprague-Dawley rats were incubated with up to 500 μM ATR in a metabolic shaker bath at 37 °C and an atmosphere of 95% O_2 and 5% CO_2 for 4 h. At the end of incubation, samples were collected for both tissue and media levels of DA and its metabolites (3, 4-dihydroxyphenylacetic acid, DOPAC and homovanillic acid, HVA), which were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Lactate dehydrogenase (LDH) release into the medium was used as an index of non-specific cytotoxicity. To gain a mechanistic insight how ATR affects DA metabolism, several pharmacological manipulations were performed. Striata exposed to ATR, at concentrations of 100 μM and greater had a dose-dependent decrease of tissue levels of DA. At doses of ATR 50 μM and greater, the DOPAC+HVA/DA ratio was dose-dependently increased. Tyrosine hydroxylase (TH, the rate-limiting enzyme in DA synthesis) activity was not affected by ATR treatment. However, high potassium induced DA release into the medium was decreased, whereas the increase in media DA observed in the presence of the DA uptake inhibitor nomifensine was dose-dependently increased even further by ATR. All of these effects of ATR were observed at levels that were not toxic to the tissue. Taken together, results from this study suggest that ATR decreases tissue DA levels not by affecting TH activity, but by increasing DA turnover. The increased DA turnover may be due to ATR effects on (*i*) vesicular storage of DA, (*ii*) cellular uptake of DA, (*iii*) monoamine oxidase activity, or (*iv*) any combination of these mechanisms.

1303 THE DITHIOCARBAMATE PROPINEB DEPOLYMERIZES ACTIN AND INCREASES ACETYLCHOLINE RELEASE IN DIFFERENTIATED PC12 CELLS.

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The dithiocarbamate Propineb is widely used in Europe as fungicide, and due to its large spectrum of action on fungi an expansion of the market is expected. Neurological complications as well as movement disorders characterized by plastic

rigidity, muscle twitch and paralysis are the prevailing symptoms in chronically exposed animals and humans. Recently we showed that propineb interferes with cholinergic transmission *in vivo* and this study was designed to investigate *in vitro* the molecular mechanisms involved. Propineb 0.001-100 nM released acetylcholine (ACh) from rat pheochromocytoma cells (PC12) differentiated with NGF. Ach release dose-dependently increased up to propineb 1 nM, to decrease for the highest concentrations (10-100 nM). *In vivo*, propineb induced-potentiation of cholinergic transmission seemed to be partly dependent on the activation of ganglionic nicotinic receptor. However, mecamilamine 1 or 50 μM, an antagonist of neuronal nicotinic receptor, did not reduce ACh release induced by propineb in PC12 cells suggesting the involvement of different mechanisms. The increase in ACh release was not associated with alterations of intracellular Ca^{2+} homeostasis and was not prevented in Ca^{2+} free conditions. Nevertheless, treatment of PC12 cells with propineb 1 nM induced a significant actin depolimerization within 30 sec from the exposure. Actin, as ACh release, was not affected by propineb 100 nM. These data suggest the involvement of cytoskeletal structure in the modulation of propineb effect independently by intracellular calcium.

1304 CHLORPYRIFOS INHIBITS AXON OUTGROWTH IN PRIMARY CULTURES OF PERIPHERAL NEURONS THROUGH INHIBITION OF THE MORPHOGENIC ACTIVITY OF ACHE.

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While there is increasing evidence that perinatal exposure to organophosphorus containing pesticides (OPs) is linked to cognitive and behavioral problems, the mechanism(s) by which this can occur is not known. One possibility is that OPs disrupt neuronal morphogenesis through interaction with AChE *via* either inhibition of the catalytic activity of AChE or inhibition of the morphogenic activity of AChE. To investigate these possibilities, we have utilized two *in vitro* model systems: neurons from the superior cervical ganglia (SCG), which are cholinoceptive in culture and neurons from the dorsal root ganglia (DRG), which are neither cholinergic nor cholinoceptive in culture. Neurons were treated with either chlorpyrifos (0.01 – 10μM) or its oxon metabolite (0.01 – 10 nM) for 24 or 72 hr. In both culture systems, we see a decrease in axon outgrowth in neurons treated with concentrations of the OP chlorpyrifos that do not decrease the catalytic activity of AChE. Thus the effect does not appear to be dependent on acetylcholine signaling. Furthermore, the decrease in axon outgrowth can be seen following either short-term (24 hr) or long-term (72 hr) exposure in SCG cultures. In an effort to determine if chlorpyrifos mediates its effects through the morphogenic domain of AChE, we used neurons from the AChE knockout mouse. Specifically we chose dorsal root ganglia (DRG) neurons because these neurons are known to express AChE during the period of axon outgrowth *in vivo*. We have found that in culture, DRG from AChE -/- animals do not extend axons as rapidly as DRG from AChE +/+ animals and that the DRG from AChE +/+ animals put out shorter axons in the presence of chlorpyrifos as compared to vehicle control. In contrast, the DRG from AChE -/- animals appear to be unaffected by treatment with the pesticide. These data support the hypothesis that OPs disrupt axon outgrowth through inhibition of the morphogenic activity of AChE.

1304a 1, 3-DINITROBENZENE INTERACTION WITH nNOS, PEROXYNITRITE FORMATION AND APPARENT INCREASE IN CEREBELLAR TYROSINE NITRATION.

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1, 3-dinitrobenzene (DNB) is a cerebellar neurotoxicant. Co-localized in the cerebellum, the enzyme neuronal nitric oxide synthase (nNOS) produces nitric oxide ($NO^{\bullet}$) and L-citrulline. Recently, we initiated a series of studies aimed at determining the mechanism(s) of interaction of 1, 3-DNB with nNOS. Previous *in vitro* studies in our laboratory have shown that the DNB's redox-cycle in the presence of nNOS, resulting in an accelerated rate of NADPH oxidation. The increased NADPH oxidation results in superoxide radical ($O_2^{\bullet-}$) formation. The $O_2^{\bullet-}$ traps the enzymatically-produced $NO^{\bullet}$, thus resulting in increased nNOS activity due to the release of $NO^{\bullet}$-mediated auto-inhibition of nNOS. Most importantly, the 1, 3-DNB-mediated generation of $O_2^{\bullet-}$ by nNOS leads to peroxynitrite ($ONOO^-$) production, converting nNOS from a NOS to an enzyme that produces the powerful oxidant $ONOO^-$. Furthermore, $ONOO^-$ production is blocked by inclusion of either SOD or nitroarginine, demonstrating the requirement for both $O_2^{\bullet-}$ and $NO^{\bullet}$, respectively. $ONOO^-$ is known to nitrate tyrosine residues. In our studies, an increase in tyrosine nitration relative to the controls supports the hypothesis that a mechanism involving the interaction of 1, 3-DNB with nNOS, resulting in the generation of $ONOO^-$, may occur *in vivo* and play a role in 1, 3-DNB-mediated cerebellar neurotoxicity. (Supported by the Research Challenge Trust Fund of the University of Kentucky)

1304b EFFECTS OF ORGANOPHOSPHORUS TOXICANTS ON G-PROTEIN COUPLED RECEPTOR KINASE 2-MEDIATED PHOSPHORYLATION OF M2 RECEPTORS.

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We studied the possible effects of chlorpyrifos, parathion and their oxons on the phosphorylation of muscarinic M2 receptors by G-protein coupled receptor kinase 2 (GRK2). Reconstituted purified recombinant human M2 receptor was incubated with varying concentrations of paraoxon, chlorpyrifos oxon or the parent insecticides along with GRK2, G protein beta-gamma subunits, and [32P]ATP in the presence of the agonist carbachol (1 mM). Paired tubes contained the muscarinic antagonist atropine (10 μM) or the GRK inhibitor heparin (10 μM). Proteins were separated by electrophoresis, gels were dried and autoradiograms developed. Bands containing M2 receptor were excised and counted by liquid scintillation. Both atropine and heparin completely blocked agonist induced M2 receptor phosphorylation. Chlorpyrifos oxon inhibited phosphorylation of muscarinic M2 receptors by GRK2 with an IC50 of approximately 70 μM. Chlorpyrifos also inhibited M2 receptor phosphorylation, but was less potent and efficacious than chlorpyrifos oxon. Interestingly, paraoxon and parathion had little effect on receptor phosphorylation under the same conditions. To evaluate further modulation of M2 receptor phosphorylation by chlorpyrifos oxon, we purified M2 receptors from porcine brain by using ABT-agarose affinity chromatography. Purified porcine brain M2 receptor was detected as one primary band with a molecular weight of 68, 000 by Coomassie Blue staining. CPO had relatively similar concentration-dependent effects on the GRK2-mediated phosphorylation of porcine M2 receptors. Chlorpyrifos oxon and chlorpyrifos may therefore alter GRK2-mediated regulatory pathways for M2 receptors, either by direct interaction with the M2 receptor or GRK2. These differential actions could contribute to selective differences in toxicity following exposure to chlorpyrifos compared to other OP toxicants.

1305 INCREASED UBIQUINATION OF THE KINASE TAK1 FOLLOWING As (III) EXPOSURE IN HEK293 CELLS.

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Previous studies have shown that low-level As (III) treatment (0.1 μM- 10 μM) increases the level of ubiquitin (Ub)-protein conjugates in HEK293 cells. In the current study, the effect of As (III), a known activator of NF-kB and AP-1 complexes, on ubiquitination of the kinase TAK1 was examined. TAK1 is a member of the mitogen-activated kinase kinase kinase (MAPKKK) family that stimulates downstream activation of JNK and p38 MAP kinase. While ubiquitination of proteins often acts as a signal for proteolytic degradation, ubiquitination of TAK1 has been shown to activate this kinase, leading to phosphorylation of the downstream substrates MKK6 and IKK. The kinase activity of TAK1 can be activated by IL-1, TGF-b, ceramide, and lipopolysaccharide. The objective of this study is to determine if As (III) may be an additional activator of TAK1. For these studies, HEK293 cells were transfected with epitope-tagged HA-TAK1 or pcDNA3 vector as a control. Cells were treated with As (III) for 4 hr with concentrations ranging from 0.1 μM to 50 μM. Immunoprecipitations were performed with antibodies to the HA-epitope and proteins separated by SDS-PAGE. Western blots were performed to probe for the presence of high-molecular weight Ub-protein conjugates with antibodies to Ub, TAK1, and the HA-epitope. Results from these experiments show that high concentrations of As (III), 25-50 μM, initiated a robust increase in the ubiquitination of TAK1. Lower concentrations of As (III), 1-10 μM, cause subtle increases in the ubiquitination of TAK1. High-molecular weight Ub-conjugates were not seen in immunoprecipitations from pcDNA3-transfected cells. Ongoing studies seek to understand whether As (III) is capable of activating the kinase activity of TAK1. The current results demonstrate that As (III) has a specific effect on the Ub-modification status of TAK1 that may help explain some of the stress signaling associated with As (III) exposure. (NIH ES 04940, NIH ES 12007)

1306 ARSENIC IMPACTS NRF2 TRANSCRIPTION FACTOR AND RELATED GENE EXPRESSION IN CULTURED KERATINOCYTE CELLS.

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Arsenic is a known human skin carcinogen. Oxidative stress resulting from an imbalance between antioxidants and oxidants during arsenic metabolism has been implicated as a possible etiologic factor in arsenic toxicity and carcinogenesis. Nrf2, a

leucine zipper transcription factor, plays a central role in the regulation of expression of antioxidant enzymes through binding to antioxidant or electrophile response elements in the promoter regions of the target genes. Thus, the effects of acute inorganic arsenite (As^{3+}) exposure on Nrf2 expression and translocation, as well as expression of Nrf2 related genes, were studied in HaCaT cells, an immortalized human keratinocyte cell line. When HaCaT cells were exposed to As^{3+} (0, 2.5 or 10 μM) and fractionated, Nrf2 protein accumulated in the nuclear fraction in a time and dose-dependent fashion which reached a peak at 6 hr. Total cellular Nrf2 protein and cytosolic Nrf2 increased in a similar fashion with As^{3+} exposure but both reached peaks at 12 hr. These results suggest As^{3+} up-regulates Nrf2 expression and induces Nrf2 translocation from the cytosol to the nucleus. Additional study showed the expression of the manganese superoxide dismutase (MnSOD) gene, a possible target of Nrf2, increased after As^{3+} exposure. The levels of MnSOD RNA were correlated with Nrf2 protein levels in nuclear extract, suggesting Nrf2 mediated MnSOD up-regulation by As^{3+}. When HaCaT cells were pre-treated with Tiron, a scavenger of superoxide radical, As^{3+}-induced increases in Nrf2 levels in all cellular fractions were suppressed, indicating As^{3+}-induced superoxide formation is a possible regulator of Nrf2. These results suggest that As^{3+} alters Nrf2 expression and cellular localization, and activates expression of an Nrf2-related gene (MnSOD) in HaCaT cells. These actions appear related to oxygen radical formation.

1307 P53 SIGNALING PATHWAY INVOLVED IN ARSENITE-INDUCED HOS CELL TRANSFORMATION AND ITS PREVENTION BY CAFFEIC ACID PHENETHYL ESTER(CAPE).

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Chronic arsenic exposure is of concern mainly because of its carcinogenicity, but its mechanism is not well understood. Many studies have focused on the expression of the p53 gene in response to acute exposure to arsenite, but very little data concerning the role of p53 in response to chronic arsenic exposure are available. HOS (human osteoblast-like) cell transformation assay was used to mimic chronic exposure to arsenic and explore the mechanism of the p53 signaling pathway using genearrays. After HOS cells were continuously exposed to low concentrations of arsenite (NaAsO2, 0.3 uM) for 8 weeks, transformed HOS (As-HOS) cells were cloned on agar. mRNA expression related to p53 signal pathway in As-HOS cells was significantly down regulated as compared to parental HOS cells. The average fold decreases for p53, mdm2, gadd45, gadd45b, p21, pig8, bax, and TRPM2 were 5.4, 10.5, 32.2, 8.1, 5.9, 7.1, 6.2, and 16.3, respectively. When HOS cells were incubated together with arsenite and CAPE (0.5 uM) for 8 weeks, the cell transformation was suppressed and the expression of above genes increased 3.1, 3.1, 7.7, 3.1, 7.3, 2.5, 1.7 and 12.2 fold, respectively, compared to those expressed by As-HOS cells. Since we also found that CAPE induces As-HOS cell death by apoptosis but causes no damage to parental HOS cells, we conclude that the deficiency in p53 and its target genes, such as gadd45 (growth arrest gene), p21 (cyclin-dependent kinase inhibitor), bax (apoptosis gene), mdm2 (p53 binding protein), Pig8 (p53 interaction gene), and PRPM2 (apoptosis gene), caused by arsenite prevents the HOS cells from entering cell cycle arrest or apoptosis, and leads to cell transformation instead. Since the lower expression of p53 mRNA made As-HOS cells more vulnerable to an anticancer agent CAPE, enhancing the p53 signaling pathway might be helpful in preventing arsenite-induced cell transformation. [Supported in part by NIH grants, ES10344, ES 00260, and CA 37858]

1308 EFFECT OF CADMIUM ON P53 AND MITOGEN-ACTIVATED PROTEIN KINASES IN A MURINE MACROPHAGE CELL LINE: RELATION TO APOPTOSIS.

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Cadmium (Cd) is a persistent metal pollutant prevalent in the environment. It is immunotoxic *in vivo* and induces apoptosis in many cultured cells. P53 and mitogen-activated protein kinases (MAPKs) are important regulators of apoptosis; however, the Cd-modulated p53 and MAPKs signaling mechanisms leading to cellular toxicity on macrophages have not been investigated. Present study was designed to determine the impact of cadmium on cell proliferation, cell cycle and apoptosis, and to investigate the possible involvement of p53 and MAPKs signaling pathways in J774A.1 murine macrophages. Cd inhibited cell proliferation *via* cell cycle arrest and induced apoptosis in a dose-dependent manner. Cd at 20 μM markedly increased cells in G2/M and hypodiploid sub-G1 phases of the cell cycle suggesting cycle arrest and cell death. Treatment with Cd at 20 and 50 μM induced phosphorylation of extracellular signal-regulated kinase (ERK), but did not alter p53 mRNA expression or the activation of p38 MAPK and c-Jun N-terminal MAPK. ERK inhibitor, PD98059, suppressed Cd-induced ERK activation. Inhibition of ERK suppressed DNA synthesis and had an additive effect with Cd-inhibited proliferation suggesting that Cd-induced ERK activity is not responsible for the G2/M arrest and subsequent inhibition of cell proliferation. Instead, the increase in ERK activation in cells treated with Cd may reflect a stress response. Pretreatment with PD98059 increased Cd-induced cytotoxicity suggesting that ERK activation may be a survival response in the present cell system. Cycloheximide, an inhibitor of protein synthesis, did not alter Cd-induced cytotoxicity indicating that J774A.1 cell death by Cd is independent of *de novo* protein synthesis including p53. Results suggested that p53 is not involved in Cd-induced cell cycle arrest and apoptosis in J774A.1 cells. ERK activation by Cd is not related to decreased proliferation of macrophages but may play a protective role against Cd-induced cytotoxicity.

1309 MITOGEN AND STRESS SIGNAL TRANSDUCTION PATHWAYS CONTRIBUTE TO SODIUM ARSENITE-INDUCED CYCLOOXYGENASE-2 EXPRESSION IN NORMAL HUMAN EPIDERMAL KERATINOCYTES.

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Arsenic is an atypical human skin carcinogen whose mode of action is not defined. Based on evidence that arsenic influences inflammatory events that are involved in skin carcinogenesis and dermatotoxicity, this study addressed the ability of sodium arsenite to regulate the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) in normal human epidermal keratinocytes (NHEK). It was hypothesized that arsenite regulates COX-2 expression in NHEK; an effect dependent on specific mitogen- and stress-related signal transduction pathways. NHEK were exposed to sodium arsenite in a dose- and time-dependent manner and COX-2 expression/activity, DNA synthesis, and mitogen activated protein kinase (MAPK) phosphorylation quantified. Specific inhibitors of p42/44 and p38 MAPK pathways were used to examine the contribution of mitogen and stress signaling to arsenite-induced COX-2 expression. Non-cytotoxic concentrations of arsenite ($\leq$ 5μM) elevated COX-2 expression in a dose-dependent manner at the gene and protein level, as well as increased prostaglandin E2 (PGE_2) secretion; a functional measure of COX-2 activity. Arsenite also initiated an acute and delayed increase in the phosphorylated/activated form of p42/44 MAPK, but did not stimulate p38 MAPK phosphorylation. Pharmacological inhibition of mitogen-activated protein kinase kinase (MEK) using the inhibitor PD98059 partially attenuated arsenite-induced *COX-2* mRNA expression whereas the p38 MAPK inhibitor SB202190 had minimal effect on the induction of *COX-2*. In contrast, elevation of COX-2 protein by arsenite is reduced by both PD98059 and SB202190 treatment. These results demonstrated that sodium arsenite modulates COX-2 in NHEK at the transcriptional, translational, and functional level; effects dependent in part on MAPK signaling. Because COX-2 plays a critical role in skin cancer, this enzyme and/or the pathways that regulate its expression could be key components involved in arsenic skin carcinogenesis.

1310 ACTIVATION OF ERK SIGNALING PATHWAY AND AP-1 IN UROTSA CELLS BY INORGANIC AND METHYLATED TRIVALENT ARSENICALS.

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Arsenic is classified as a human carcinogen. The molecular mechanism by which arsenic induces cancer is not well understood. In humans, inorganic arsenic (iAs) undergoes oxidative methylation, yielding trivalent and pentavalent mono- and dimethylated species. Recent studies have shown that trivalent methylated arsenicals are more cytotoxic, genotoxic and more potent enzyme inhibitors than trivalent iAs, arsenite (iAsIII). Mono- and dimethylated trivalent arsenicals were detected in the urine of individuals chronically exposed to iAs. It is generally accepted that transactivation of the transcription factor AP-1 is required for tumor promotion. In this study, we examined signaling pathways that are responsible for AP-1 activation in SV40-immortalized normal human urothelial (UROtsa) cells exposed to iAsIII, monomethylarsine oxide (MAsIII) or iododimethylarsine (DMAsIII). One-hour exposures to 0.1 to 5.0 μM iAsIII, MAsIII or DMAsIII significantly increased DNA-binding activity of AP-1. The EMSA/supershift and Western blot analyses showed that p-c-Jun and Fra-1 were the major AP-1 constituents. MAsIII was the most potent activator of AP-1 followed by DMAsIII and iAsIII. The activation of AP-1 by trivalent arsenicals was associated with an increased phosphorylation (activation) of ERK1, 2 and ERK5. Pretreatment of UROtsa cells with PD98059 or U0126 (specific inhibitors of the MEK/ERK signaling pathway) completely suppressed phosphorylation of ERK1 and 2, but only partially decreased the phosphorylation of ERK5 in cells exposed to MAsIII or DMAsIII. Similarly, treatment with either inhibitor decreased, but not abolished the phosphorylation of c-Jun in exposed cells. Neither arsenical induced activation of JNK1, 2 or p38 under these exposure conditions. These results indicate that trivalent arsenicals induce AP-1 DNA-binding activity in UROtsa cells through ERK-mediated phosphorylation of c-Jun and that ERK5 may be an essential component of this mechanism.

1311 ARSENIC TRIOXIDE-INDUCED APOPTOSIS REQUIRES JNK ACTIVITY.

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Despite arsenic's reputation as an environmental contaminant, it is currently an effective chemotherapy used to treat acute promyelocytic leukemia (APL). In fact, APL cells are uniquely sensitive to arsenic and undergo apoptosis rapidly after treatment. This apoptosis has been linked to accumulation of free radicals and the resulting state of oxidative stress. Therefore, we've focused our investigations on the stress-activated protein kinase, c-jun N-terminal kinase (JNK) and it's role in arsenic signaling pathways. Using the arsenic-sensitive, APL cell line, NB4, we found that arsenic increased the expression of phospho-JNK1 and to a lesser degree, phospho-JNK2, although the total levels of JNK proteins did not change. JNK activity also increased after arsenic treatment. Previously, we and others have shown that buthionine sulfoximine (BSO), which depletes glutathione and alters the redox state of the cell, enhanced arsenic's cytotoxicity. In correlation with increased apoptosis, phospho-JNK protein expression and JNK activity could be further enhanced by co-treatment with BSO. In arsenic-resistant NB4 subclones established in our laboratory, which have normal levels of JNK1 protein, arsenic alone did not induce JNK activity, although BSO co-treatment restored JNK activation and cell death in response to arsenic. We used chemical and genetic approaches to determine whether JNK activation was required for apoptosis. Dicumarol, a pan-JNK inhibitor, blocked arsenic-induced apoptosis significantly in APL cells. To confirm and extend this result, we utilized SEK1-/- fibroblasts, which are deficient in the upstream regulator of JNK, SEK1. Although SEK1-/- cells express normal levels of JNK protein, arsenic-induced JNK activity and apoptosis are impaired significantly in SEK1-/- cells as compared to wild-type counterparts. In contrast, doxorubicin induced more apoptosis in SEK-/- cells than in wild-type controls. Therefore, we conclude that JNK activation is required for arsenic-induced cytotoxicity of APL cells and may be necessary for effects of environmental arsenic exposure as well.

1312 INHIBITION OF NUCLEAR FACTOR κB (NF-κB) PROMOTES APOPTOSIS OF KIDNEY EPITHELIAL CELLS VIA MITOCHONDRIAL CYTOCHROME C RELEASE AND CASPASE 3 ACTIVATION.

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NF-κB is a pleiotropic transcriptional activator that promotes survival and prevents apoptosis in numerous cell types. We previously reported (TAAP 173:176, 2001;EHP 110S5, 2002) that mercuric ion (Hg^{2+})at < 10 μM impairs NF-κB activation in normal rat kidney epithelial (NRK52E) cells and increases their sensitivity to apoptosis by LPS, TNF-α and other agents to which these cells are otherwise resistant. To further demonstrate that increased sensitivity to apoptosis incurred by Hg^{2+} pretreatment is associated specifically with inhibition of NF-κB, we conducted studies to determine if comparable increased sensitivity could be elicited by treatment with sepcific NF-κB inhibitors. Pretreatment of cells with Bay 11-7082 (0-10 μM, a specific inhibitor of IκB kinase, attenuated NF-κB activation by TNF (3 ng/ml) in a dose-related manner, and this corresponded to a subsequent increase in sensitivity to TNF-induced apoptosis, as measured by TUNEL assay (7.5% of untreated or TNF alone versus 28% and 56% of cells pretreated with 2.5 or 5 μM, respectively, Bay 11 before TNF). Comparable results were obtained after pretreatment of cells with SN-50, a synthetic polypeptide that blocks nuclear translocation of NF-κB, before TNF. Increased sensitivity to TNF-induced apoptosis following attenuation of NF-κB activity was characterized by translocation of mitochondrial cytochrome c and activation of caspase 3. The latter was completely prevented by constitutive p65 expression. These findings support the hypothesis that sensitivity to apoptosis induced by Hg^{2+} (and other NF-κB inhibitors) is regulated at the level of NF-κB in kidney epithelial cells and that this effect is mediated *via* a mitochondrial and caspase 3 pathway. Supported by ES04696, ES07032 and ES07033.

1313 ARSENITE INDUCED ACTIN CYTOSKELETON AND VINCULIN DISRUPTIONS CAN BE BLOCKED BY PROTEIN SYNTHESIS INHIBITORS.

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Arsenite produces a variety of stress responses including metabolic inhibition and eventually apoptosis in many cell types. Morphological alterations in arsenite- exposed cells suggest that disruption of cytoskeletal structure elements are responsible for cellular integrity, shape, and locomotion change seen in many disease processes. In this study, we found that protein synthesis inhibitor cycloheximide or RNA synthesis inhibitor actinomycin D can block the actin cytoskeleton and vinculin disruptions induced by sodium arsenite (25 micromolar) in Balb/c 3T3 cells. In immunoblot assays, cycloheximide and actinomycin D can not block PAK, JNK, phospho-JNK, p38, and phospho-p38 expressions induced by arsenite. Since protein synthesis are physiological processes related to the maintenance of homeostasis, down-stream effectors of EGFR-PI3K signaling pathway related to the protein synthesis, including transcription and translation processes, are proposed to play a major role for arsenite-induced toxicity. Our present studies demonstrated that the JNK and p38 activations are not affected. We have earlier reported that tyrosine kinase inhibitor genistein, EGFR inhibitor 4, 5-dianilinophthalimide or PI3K inhibitor wortmannin completely blocked these toxic responses induced by arsenite (Suramana et al., 2002). The role of specific anchoring protein which may be attached to enzymes requires actin cytoskeleton and focal adhesion protein are being investigated.

1314 DNA DAMAGE INDUCED BY METHYLATED TRIVALENT ARSENICALS IS MEDIATED BY REACTIVE OXYGEN SPECIES.

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Arsenic is a human carcinogen; however, the mechanisms of arsenic's induction of carcinogenic effects have not been identified clearly. We have shown previously that monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) are genotoxic and can damage supercoiled ϕX174 DNA and the DNA in peripheral human lymphocytes in culture. These trivalent arsenicals are biomethylated forms of inorganic arsenic and have been detected in the urine of subjects exposed to arsenite and arsenate. We show here by molecular, chemical, and physical methods that reactive oxygen species (ROS) are intermediates in the DNA-damaging activities of MMA^{III} and DMA^{III}. Using the ϕX174 DNA nicking assay we found that the ROS inhibitors Tiron, melatonin, and the vitamin E analog Trolox inhibited the DNA-nicking activities of both MMA^{III} and DMA^{III} at low micromolar concentrations. The spin trap agent 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) also was effective at preventing DNA nicking by MMA^{III} and DMA^{III}. ESR spectroscopy studies using DMPO identified a radical as a ROS intermediate in the DNA incubations with DMA^{III}. This radical adduct was assigned to the DMPO-hydroxyl free radical adduct on the basis of comparison of the observed hyperfine splitting constants and line widths with those reported in the literature. The formation of the DMPO-hydroxyl free radical adduct was dependent on time and the presence of DMA^{III} and was completely inhibited by Tiron and Trolox and partially inhibited by DMSO. Using electrospray MS, micromolar concentrations of DMA^{V} were detected in the DNA incubation mixtures with DMA^{III}. These data are consistent with the conclusions that the DNA-damaging activities of DMA^{III} are mediated by ROS formed concomitantly with the oxidation of DMA^{III} to DMA^{V}. The data suggest that ROS generated from DMA^{III} may play an important role in the carcinogenic activity of arsenic.

1315 DEFEROXAMINE SYNERGISTICALLY ENHANCING IRON-INDUCED STIMULATION OF ACTIVATOR PROTEIN-1 THROUGH INCREASED ERKS PHOSPHORYLATION.

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Deferoxamine (DFO) is the only approved drug in the US for iron overload treatment. Studies have demonstrated the ability of DFO to remove iron bound to low molecular weight (LMW) chelators and those bound to proteins, such as ferritin. Because of its ability to sequester LMW iron, DFO is known as an antioxidant. Recently, the DFO-Fe complex ferroxamine and even DFO alone were shown to act as pro-oxidants. In the present study, we have shown in mouse epidermal JB6 cells that ferrous sulfate transactivated activator protein-1 (AP-1) and nuclear factor of activated T cells (NFAT), two transcription factors responsive to oxidative stress. Interestingly, pretreatment of cells with DFO, followed by iron treatment, synergistically increased AP-1 activity, a ten-fold increase over the control as compared to iron alone, a 3-fold increase. In contrast, DFO prevented iron-induced NFAT activation and significantly inhibited iron-induced ferritin and lipid peroxidation in human liver HepG2 cells. We have further shown that iron stimulated the phosphorylation of extracellular signal-related kinases (ERKs) and p38 mitogen-activated protein kinases (MAPK) but not c-jun NH2 terminal kinases (JNKs) after 15-90 min treatments. The increased phosphorylation was lessened by the pretreat-

ment of cells with N-acetyl-L-cysteine, an antioxidant. DFO inhibited iron-induced ERKs phosphorylation within 1 h of treatment, whereas it further enhanced ERKs phosphorylstion in longer periods of treatment (over 6 h). These results suggest that the phosphorylation of ERKs and p38 MAPK by iron is a stress response through oxidative pathways, and the increased phosphorylation of ERKs by DFO may be due to the imbalance between phosphatases and kinases. It was previously shown that DFO inhibited alkaline phosphatase. Whether DFO inhibits the tyrosine and serine/threonine phosphatases leading to the synergistic increase in AP-1 is being studied. The clinical implication of this observation may be important because many genes are regulated by AP-1.

1316 INVOLVEMENT OF OXIDATIVE STRESS IN THE METALLOTHIONEIN SYNTHESIS INDUCED BY MITOCHONDRIAL INHIBITORS.

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Antimycin A and 2, 4-dinitrophenol (DNP) are potent inhibitors of respiration and radical generators in mitochondria. We previously reported that metallothionein (MT) was induced by antimycin A or DNP. However, the involvement of oxidative stress in the induction of MT by antimycin A or DNP remains obscure. Therefore, we examined the involvement of oxidative stress in MT synthesis by the inhibitors. Administration of mitochondrial inhibitors elevated the level of lipid peroxidation in not only the liver but also hepatic mitochondria, indicating the production of mitochondrial oxidative stress. Antimycin A treatment elevated the levels of lipid peroxidation and MT in the liver time dependently, and the increase in hepatic MT and lipid peroxidation levels occurred in parallel. In addition, under the condition of glutathione depletion, due to pre-administration of L-buthionine-SR-sulfoximine (BSO), MT synthesis by mitochondrial inhibitors was further elevated, although the BSO treatment did not alter the hepatic MT concentrations. The data suggest that inducement of MT synthesis in the liver of antimycin A- or DNP-treated mice is due to the enhancement of lipid peroxidation. The occurrence of oxidative stress was confirmed by the fact that antimycin A or DNP increased hydrogen peroxide formation in mouse fibroblast cells. Furthermore, intracellular accumulation of hydrogen peroxide caused by the actions of mitochondrial inhibitors was greater in MT-null than in wild-type fibroblast cells, suggesting that MT plays a role as a radical scavenger against intracellular reactive oxygen species (ROS) caused by mitochondrial stress. The results suggest that mitochondrial oxidative stress induces the synthesis of MT, which may contribute to regulation of mitochondrial ROS production.

1317 EFFECTS OF CADMIUM ON THIOREDOXIN AND THIOREDOXIN REDUCTASE IN HUMAN PULMONARY FIBROBLASTS.

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The heavy metal Cadmium (Cd) is a known carcinogen that has been shown to induce oxidant stress. Glutathione (GSH) and thioredoxin (Trx) are major thiol-containing constituents implicated in a number of cellular functions including protection against oxidative stress and maintaining redox balance. Our previous research has shown that GSH and enzymes involved in GSH metabolism are up-regulated by Cd in acute and adapted pulmonary models. Trx expression is known to be induced by a variety of stress factors including UV radiation, hyperoxia, and hydrogen peroxide. The objective of the present study was to determine if exposure to Cd also up-regulated the activity and levels of expression of thioredoxin and cytosolic thioredoxin reductase (cTR), an enzyme that reduces oxidized thioredoxin. To test this hypothesis, sub-confluent normal human lung fibroblasts (IMR-90) were exposed to 10 µM CdCl2 for different durations up to 24 hrs, total RNA was extracted and purified, and mRNA levels of Trx and cTR were examined following RT-PCR. Basal gene expression of cTR was approximately double that of Trx. Following exposure to Cd, Trx transcript levels increased, peaking around 16 hrs at ~ 3-fold that of control levels, and remained high after 24 hrs. cTR mRNA levels also peaked around 16 hrs at ~ 2.15-fold that of control levels, but dropped to ~ 1.5-fold by 24 hrs. Similar elevations in the enzymatic activity of Trx and cTR were also observed in extracts from cells exposed to Cd. Studies are continuing to determine whether Trx and cTR levels are also altered in cells adapted to Cd and to further characterize the relationship between Cd and the thioredoxin system.

1318 CALCITE IS A LIKELY ANTAGONISTIC FACTOR AGAINST BIOAVAILABLE IRON-CONTAINING COAL-INDUCED TOXICOLOGICAL EFFECTS.

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Coal mining causes chronic obstructive pulmonary diseases such as pneumoconiosis and emphysema. The disease is most common in the Pennsylvania (PA) coal miners and least common in miners from Utah (UT). We have previously shown that levels of bioavailable iron (BAI) in the coals correlated well with the prevalence of coal worker's pneumoconiosis (CWP) from that coalmine region. PA coals contained large amounts of BAI enhanced the coal dust-induced lipid peroxidation and cytokine formation in A549 cells, which were dimished by the pretreatment of cells with DFO, a specific iron chelator. UT coals had the least amount of BAI due to the presence of calcite (CaCO3) in the coals, which makes the iron less bioavailable. In the present study, pre-determined percentages of calcite (0, 2, 5, 10% w/w) were added to three PA coals. After suspending the mixtures in an aqueous solution (phosphate-buffered saline, 10 mM, pH 4.5), which mimics the phagolysosomal conditions of cells, the levels of pH as well as calcium ions (Ca2+) were increased as a function of time. In contrast, levels of BAI (both Fe2+ and Fe3+) were decreased at day one and had completely disappeared after one-week incubation. The attenuating effects were calcite dose-dependent. A549 cells as well as primary rat alveolar macrophages were then treated with the 10% calcite-PA coal mixture for 24 hours. We have found that the mixture significantly decreased the PA coal-induced ferritin formation, lipid peroxidation, as well as interleukin-6 release. Calcite alone had no significant toxicological effects on the cells. Our results suggest that calcite may play a protective role by inhibiting the bioavailability of iron in the coals, and thus limiting the toxicological effects of BAI. Our studies suggest that the addition of calcite into the water spray when mining BAI-containing coal may be an effective tool for preventing the occurrence of such diseases. Supported by OH03561.

1319 THE EFFECT OF TRACE ELEMENT MIXTURES ON THE INDUCTION OF δ-AMINOLEVULINIC ACID:A 90-DAY DRINKING WATER STUDY IN RATS.

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δ-Aminolevulinic acid (ALA) is a heme precursor that can generate reactive oxygen species (ROS) upon iron-catalyzed aerobic oxidation. ROS such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$) can cause oxidative damage to proteins, subcellular structures, and DNA. This study investigated whether subchronic exposure to single trace elements (lead, cadmium, or arsenic) or trace element mixtures increased urinary ALA excretion. Male Sprague-Dawley rats were administered 25 ppm lead (Pb)(as lead acetate), 10 ppb cadmium (Cd)(as cadmium chloride), 5 ppm arsenic (As)(as sodium arsenite), or trace element mixtures (PbxCd, PbxAs, CdxAs, PbxCdxAs) in deionized drinking water for 90 days. Mean urinary ALA concentrations were significantly increased among 7 of the 8 groups compared to the control: 97.2 (control), 126 (Pb), 148 (Cd), 134 (As), 74.1 (PbxCd), 113 (PbxAs), 146 (CdxAs), and 141 (PbxCdxAs) µg ALA/24 hours. On a percentage basis, urinary ALA concentrations (µg/24 hours) were +29%, +53%, +38%, -18%, +16%, +50%, and +45% different than the control, respectively. The accumulation of ALA is due to inhibition of heme pathway enzymes, notably ALA dehydratase (ALAD). As expected, ALAD activities were inhibited in both the kidney and blood. Inhibition of kidney ALAD activity was observed among all groups compared to control (-51%, -22%, -5%, -24%, -54%, -5%, and -8%). Inhibition of blood ALAD activity was observed among all groups compared to control (-62%, -57%, -59%, -67%, -88%, -54%, and -54%). This experiment demonstrates that accumulation of ALA is occurring in response to subchronic administration of trace elements and trace element combinations, and is a first step towards elucidating the effect of oxidative stress upon trace element toxicity. Experiments are underway to characterize the heat shock protein-32 (heme oxygenase-1) response to subchronic administration of trace element mixtures [Supported by USEPA Star Grant R827161-01-0].

1320 DISTRIBUTION OF IRON IN TISSUES AND CELLS OF RATS EXPOSED TO SILICA.

J. Stonehuerner and A. Ghio. *USEPA, Chapel Hill, NC.* Sponsor: M. Madden.

The stable iron isotope 57Fe has been used to study the distribution of iron in tissues, bronchial alveolar lavage fluid (BALF), and alveolar macrophages (ma) of rats in- stilled with silica relative to saline. Sixty-day old male Sprague-Dawley rats were instilled intratracheally with 10 mg silica in 0.5 mL normal saline (NS). Controls were simi- larly instilled with 0.5 mL NS. Animals were immediately injected intraperitoneally with 1 mL 1.0 mM 57FeCl3 in PBS. Animals were sacrificed 1, 24, or 96 hrs. after silica installation. The distribution of iron was determined by ICP/MS analysis of acid digests of tissues, BALF, and ma for 57Fe. 57Fe distribution in lung and liver tissue was remar- kably similar for samples and controls. Plasma 57Fe levels were increased in both groups at the 1 hr. time point and remained elevated at 96 hr. although silica-treated rats exhibited a higher initial [57Fe] accompanied by a time- dependent decrease. The biggest differences between treated animals and controls were in BALF and ma where silica- treated animals exhibited increases in 57Fe at all time points with the highest level observed at 24 hr. These results demonstrate the utility of 57Fe analyses in elucidating the

metabolic fate of iron compounds known to cause a variety of negative health effects. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1321 FEASIBILITY OF SCROTAL ULTRASOUND EVALUATION IN THE CYNOMOLGUS MONKEY.

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The cynomolgus monkey (Macaca fascicularis) represents a suitable nonhuman primate model for human reproductive physiology and is frequently used in toxicity studies. The purpose of this investigation was to evaluate the feasibility of scrotal sonography in the cynomolgus monkey model. In man, testicular sonography allows repeated and safe imaging diagnostics of the scrotal contents and determination of testicular volume without side effects. Thus it has become an important diagnostic tool in andrology. As in humans, measurements of testis volume were performed in the cynomolgus monkey with a 7.5-megahertz sector scanner (Sonoline Versa Pro, Siemens, Erlangen, Germany). For all interventions, the monkeys were sedated with an intramuscular injection of ketamine hydrochloride. In the cynomolgus monkey, as in man, normal testis and epididymis displayed homogenous parenchymal echogenicity. Furthermore, similar to man, the rete testis could be easily identified in the monkey by ultrasound due to its higher echogenicity, and prepubertal animals testes were characterized by hypoechogenicity. Testicular volume determination by scrotal ultrasonography was compared to caliper measurements. The total coefficient of variation for all sonographic measurements was 10.3%. The corresponding coefficient of variation for caliper testicular volume was 15.9%. Both methods of volume determination were highly significantly ($p < 0.001$) correlated with each other. In conclusion, scrotal sonography in the cynomolgus monkey revealed a similar echogenicity to that of man and provides an appropriate method for determination of testicular volume.

1322 ANALYSIS OF ANDROGEN- AND EGF-RECEPTOR EXPRESSION IN THE FETAL RAT PHALLUS AFTER EXPOSURE TO VINCLOZOLIN.

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Improper development of the male phallus during fetal sexual differentiation resulting in hypospadias is at least partially due to impaired androgen receptor (AR) function. Other mediators may be involved since hypospadias in humans is not usually associated with mutations of the AR. Epidermal growth factor (EGF) is suspected since it has been implicated in other developmental processes in the reproductive tract as an AR-dependent gene. The fungicide vinclozolin (V) is an AR inhibitor that induces cleft phallus and hypospadias in male rats exposed during fetal sexual differentiation. We investigated the ability of V to alter the expression of AR and EGF receptor (EGFR) in the fetal phallus at a dose that induces cleft phallus in the 100% of male offspring. Pregnant rats were dosed (po) with either corn oil (2.5 ml/kg) or V (400 mg/kg) on gestational days (GD) 14-18. On GD 18 phalluses were collected (3 phalluses/ 3 litters/ 3 treatment groups: control female, control male and V-treated male), fixed in 3% paraformaldehyde, dehydrated in ethanol and paraffin embedded. 6 µm sections were placed on slides in matrix fashion with 1 representative per litter per treatment group per slide. Immunohistochemistry for AR or EGFR was performed by the avidin-biotin-peroxidase technique. Densitometric analysis of sections was performed blind using ImagePro Plus software. Mean density and area of the epithelial layers of the epidermis and the urethral groove, and number and summed area of receptor-positive cells in the mesenchyme was measured. We found a slight reduction in both AR and EGFR expression in control females as compared to control males, but no change in AR or EGFR expression by V. These results imply that events other than AR number or EGFR number mediate V-induced hypospadias. Future studies will focus on differences in gene expression induced by V that contribute to hypospadias. This abstract does not necessarily reflect EPA policy.

1323 EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS (EDCS) ON FETAL TESTES HORMONE PRODUCTION.

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Exposure to EDCs during critical periods of fetal sexual development can have profound effects on the male reproductive system. We examined effects of gestational exposure to eight EDCs on testes testosterone (T) and progesterone (P4) production. Chemicals included fungicides (Procymidone (PD), Vinclozolin (V), Prochloraz (PZ)), phthalate esters (DEHP, DBP, BBP) and herbicides (Linuron (L), Neburon (N)). Doses of 200, 200, 250, 1000, 1000, 1000, 100, 100 mg/kg/day respectively were administered daily GD 14-18 to SD rats and chosen based on previous studies indicating that the majority of fetuses would be affected. PD and V are androgen receptor (AR) antagonists, while the phthalates alter fetal Leydig cell differentiation and reduce fetal T production. L and PZ have been reported to display multiple endocrine activities, including AR antagonism and inhibition of fetal T synthesis. N has been shown to bind AR *in vitro*, but it is unknown if it alters sexual differentiation. Dams were sacrificed on GD 18 and fetal testes were incubated in M199 medium supplemented with 10% DCC serum for 3 hours. Medium were collected for hormonal analysis. L, N, DEHP, DBP, and BBP all caused a significant reduction in fetal testes T and P4 release. As anticipated, V and PD treatment did not reduce the release of either hormone. PZ exposure resulted in a 10-20 fold increase in P4 and a slight decrease in T versus the control, suggesting a specific inhibition of CYP450 conversion of P4 to the androgens. Other studies are ongoing to examine the direct effects of these chemicals *in vitro*. Gene expression will also be examined in the testes using microarrays and PCR. These studies provide background from which we can further investigate the mechanisms by which these chemicals affect fetal testes hormone production after gestational exposure. Abstract of a proposed presentation and does not necessarily reflect EPA policy.

1324 PROGRESSIVE LOSS OF SPERMATOGENESIS IN LYSOPHOSPHATIDIC ACID RECEPTORS KNOCKOUT MICE.

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Lysophosphatidic acid (LPA; 1-acyl-2-sn-glycerol-3-phosphate) can evoke many cellular and biochemical responses in a variety of cell types. The LPA-induced responses are mediated through three G protein-coupled LPA receptors: LPA1, LPA2, and LPA3. To elucidate their *in vivo* function, each of the LPA receptor genes was targeted for deletion in mice. We demonstrate that LPA receptors are involved in mouse spermatogenesis. Mice lacking one, two and three LPA receptors show gene dose-response suppression of spermatogenesis. The suppression of spermatogenesis is progressive. Consequently, the male knockout mice have decreased sperm counts and a reduced rate of fertilization. Since there is no significant difference between knockout and wild type mice in serum testosterone levels, it is likely that the deficit in spermatogenesis in LPA receptor(s) null mice is not caused by endocrine but local defects. Indeed, increased apoptosis and decreased cell proliferation as well as impaired signaling are detected in the testes of LPA receptor(s) null mice. Our study has established a novel *in vivo* function of LPA receptors in spermatogenesis.

1325 IDENTIFICATION OF PROTEINS INVOLVED IN TESTICULAR TOXICITY INDUCED BY HALOACID BY-PRODUCTS OF DRINKING WATER DISINFECTION.

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Dibromoacetic acid (DBA), a prevalent disinfection by- product in drinking water, perturbs spermiogenesis in adult rats suggesting that Sertoli-germ cell communication is compromised. When isolated seminiferous tubules from rats exposed to DBA *in vivo* were cultured, quantitative analysis of autoradiograms following two-dimensional gel electrophoresis (2D SDS-PAGE) revealed that the synthesis of multiple cytosolic proteins in tubules representing stages I-V was diminished; four of these cytosolic proteins were also diminished by *in vitro* DBA exposure. We sought to identify these four proteins to determine whether Sertoli cells and/or germ cells are target cells in haloacid-induced toxicity. Seminiferous tubules representing stages I-V of spermatogenesis were isolated from adult rats and cultured overnight. Tubule homogenate was centrifuged and supernatant was concentrated and assayed for protein. Proteins resolved by 2D SDS-PAGE gels were stained with SyproRuby and gel punches of each of the four proteins were frozen at -70 °C. After tryptic digestion and extraction, proteins were resolved and analyzed by electrospray ionization /ion trap mass spectrometry. Data were analyzed using the Sequest search algorithm. The major proteins represented were: 14-3-3 Epsilon, 14-3-3 Theta/ Zeta, cAMP-dependent Protein Kinase Regulatory Subunit, and 1-Cys Peroxiredoxin. While cAMP-dependent Protein Kinase Regulatory Subunit is in-

volved with FSH-mediated signaling in Sertoli cells, 14-3-3 Theta and 14-3-3 Epsilon modulate protein kinase C activity and are also found in Sertoli cells. 1-Cys Peroxiredoxin and 14-3-3 Zeta have phopholipase A2 activity. These data reveal disruption in Sertoli cells and imply that germ cell compromise may be secondary to Sertoli cell insult.

1326 DI(*n*-BUTYL) PHTHALATE INTERFERES WITH FETAL TESTICULAR STEROIDOGENESIS AT THE LEVEL OF CHOLESTEROL TRANSPORT AND CLEAVAGE.

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The phthalate ester di(*n*-butyl) phthalate (DBP) produces antiandrogenic effects on male reproductive development in rats. In the fetus, these effects are mediated, not by interaction with the androgen receptor, but rather through diminution of testosterone (T) production by the testes. Previous studies have shown that several genes involved in cholesterol transport and steroidogenesis are downregulated at the mRNA level following *in utero* exposure to DBP. The purpose of this study was to make a functional determination of the points in the cholesterol transport and steroidogenesis pathways affected by DBP. We cultured fetal testis explants with T precursors and assessed cholesterol uptake and T production. Pregnant Sprague-Dawley rats were treated with 500 mg/kg DBP or corn oil control *via* oral gavage from gestational days 12 to 19. Following the final treatment, testes were removed from the fetuses and cultured for 3 h with ^{3}H-cholesterol, leuteinizing hormone (LH), Bt_2-cAMP, 22(R)-hydroxycholesterol, pregnenolone, progesterone, or 17-hydroxyprogesterone. T production in unsupplemented cultures of DBP-exposed testis was roughly 10% of that seen in corn oil controls (164.7 ± 32 pg/h vs. 1684.1 ± 347 pg/h). Both control and treated explants could be stimulated by LH or Bt_2-cAMP, but T production by DBP-treated testes remained less than 50% of control levels. Incorporation of ^{3}H-cholesterol by mitochondria of DBP-treated explants was 67% of that observed in controls, although this difference was not statistically significant ($p = 0.08$). Pregnenolone, progesterone, and 17-hydroxyprogesterone all significantly increased T production compared to unsupplemented DBP-treated explants. However, there was no significant difference between the unsupplemented explants and those treated with the membrane-permeable 22(R)-hydroxycholesterol. These data indicate that the toxic effects of DBP on the fetal testis are mediated at the level of cholesterol cleavage by $P450_{scc}$ and possibly at the level of cholesterol transport into the mitochondria.

1327 EFFECTS OF METHOXYCHLOR (M) OR ITS ACTIVE METABOLITE, 2, 2-BIS(*p*-HYDROXYPHENYL)-1, 1, 1-TRICHLOROETHANE (HPTE), ON TESTOSTERONE (T) FORMATION BY CULTURED NEONATAL (FETAL) LEYDIG CELLS (LC).

E. P. Murono and R. C. Derk. *Pathology and Physiology Research Branch, CDC/NIOSH, Morgantown, WV.* Sponsor: V. CASTRANOVA.

M is a pesticide developed as a replacement for dichlorodiphenyltrichloroethane (DDT). Its active metabolite is thought to be HPTE. Both M and HPTE have been reported to exhibit weak estrogenic or antiandrogenic activities, their proposed mechanism(s) of action. In the present studies, we examined the effects of M or HPTE on T biosynthesis by cultured LC from neonatal rats, which represent fetal LC. Increasing concentrations of M or HPTE (100-1000 nM) caused a progressive decline in both basal and 10 mIU/ml human chorionic gonadotropin (hCG)- or 1 mM 8 Br-cAMP-stimulated T following exposure for 4 or 24 h, although the declines with HPTE were greater. To localize the site(s) of action of HPTE, LC were exposed to HPTE (100-1000 nM) for 24 h (both alone or with hCG), then fresh media containing steroid precursors of T were added to assess their conversion to T over 4 h. The conversion of 0.01 mM pregnenolone, progesterone or androstenedione to T was unaffected by prior exposure to HPTE; however, the conversion of 22(R)hydroxycholesterol to T progressively decreased, suggesting that among the enzymes involved in converting cholesterol to T, P450 cholesterol side-chain cleavage activity is inhibited by HPTE. The concomitant inclusion of the "pure" estrogen antagonist, ICI 182, 780, did not alter the inhibitive effects of HPTE, suggesting that the effects of HPTE are not mediated through the estrogen receptor (ER) pathway. Furthermore, the antiandrogen 4-hydroxyflutamide (100-1000 nM) had no effect on hCG-stimulated T following 24 h exposure, suggesting that HPTE is not acting as an antiandrogen through the androgen receptor (AR). These results suggest that the two prevailing proposed modes of action of MC/HPTE in altering male reproductive function (weakly estrogenic through the ER or antiandrogenic through the AR) do not apply with respect to their inhibitive effect on T formation by fetal LC.

1328 EVIDENCE FOR THE PRESENCE AND ACTIVITY OF SOLUBLE EPOXIDE HYDROLASE IN THE RAT EPIDIDYMIS AND SPERM.

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Soluble epoxide hydrolase (sEH) is proposed to play a role in mediating ion balance in the kidney through the catalytic transformation of bioactive arachidonic acid metabolites. The epididymis and kidney are related embryonically. In addition, the epididymis regulates ion concentrations through diffusion, active transport, and leaky epithelium much like the kidney. Based on these similarities, the present study used immunohistochemistry, Western blotting, and enzyme assays to address the possibility that sEH is present in the epididymis. Tissue sections probed with an antibody to sEH showed immunoreactive proteins in the clear cells of the caput, corpus, and cauda epididymis. The sEH antibody also reacted with proteins on the ventral acrosome and the principal piece of sperm. In Western blots, immunoreactive proteins in kidney cytosol were consistent with a 65 kD recombinant sEH standard. The same antibody recognized a single 75 kD protein in epididymal segments and a single 71 kD protein in sperm. Epoxide hydrolase activity was measured in kidney, epididymal, and sperm samples using [^{3}H]-*trans*-diphenylpropene oxide (tDPPO) as a substrate. Results indicate that all portions of the epididymis as well as sperm are capable of generating diols from the tDPPO epoxide. The specific activity of the corpus epididymis (110 pmol/min/mg) was similar to the activity in the kidney cortex (113 pmol/min/mg). Evaluation of *in vitro* arachidonic acid metabolism showed that the epididymis can generate epoxides and diols. In addition, epididymal metabolism was chemically inhibited by cyclohexyldodecyl urea, a potent sEH inhibitor. The present study indicates the presence of epoxide hydrolase activity in the epididymis at levels similar to the kidney. Because the protein detected in the epididymis is larger than the known mass of sEH, immunoinhibition assays and protein sequencing studies are planned to further elucidate the nature and identity of epoxide hydrolase in the epididymis.

1329 IDENTIFICATION OF TRICHLOROETHYLENE AND ITS METABOLITES IN HUMAN SEMINAL FLUID OF WORKERS EXPOSED TO TRICHLOROETHYLENE.

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We have investigated the potential of the male reproductive tract to accumulate TCE and its metabolites including chloral, trichloroethanol (TCOH), trichloroacetic acid (TCA)and dichloroacetic acid (DCA). Human seminal fluid and urine samples from eight mechanics diagnosed with clinical infertility and exposed to TCE occupationally were analyzed. In *in vivo* studies, TCE and its metabolites were determined in epididymis of mice exposed to TCE (1000 ppm) by inhalation for 1 to 4 weeks. In other studies, incubations of monkey epididymal microsomes were performed in the presence of TCE and NADPH. Our results showed that seminal fluid from all 8 subjects contained TCE, chloral and TCOH. DCA was present in samples from 2 subjects, and only 1 contained TCA. TCA and TCOH were also identified in urine samples from 2 subjects. TCA, chloral and TCOH were detected in murine epididymis after exposure to TCE for 1 to 4 weeks. Levels of TCE and chloral were similar throughout the exposure period. TCOH levels were similar at 1 and 2 weeks, but increased significantly after 4 weeks. Chloral was identified in microsomal incubations with TCE in monkey epididymis. CYP2E1, a P450 enzyme with a major role in TCE metabolism, was localized in human and monkey epididymal epithelium and testicular Leydig cells. These results indicated that TCE is metabolized in the reproductive tract of the mouse and monkey. Furthermore, TCE and its metabolites were identified in seminal fluid, and suggested associations between TCE metabolites, reproductive toxicity and impaired fertility. Supported by Health Canada/Environment Canada.

1330 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) INHIBITS PROSTATIC EPITHELIAL BUD FORMATION IN THE UROGENITAL SINUS (UGS) OF C57BL/6J MICE VIA MESENCHYMAL BUT NOT EPITHELIAL ARYL HYDROCARBON RECEPTOR (AHR).

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We previously reported that *in utero* TCDD exposure prevents prostatic epithelial buds from forming in the ventral region of the UGS and reduces the number of buds formed in the dorsal and lateral regions of the UGS. These effects of TCDD

are AhR dependent. However, whether TCDD acts on UGS mesenchyme, UGS epithelium, or both was unknown. The objective of this study was to test the hypothesis that TCDD inhibits bud formation by acting through AhR in UGS mesenchyme. First, we established a tissue separation and recombination method and *in vitro* culture system in which 10^{-8} M 5α-dihydrotestosterone (DHT) induced bud formation in recombined gestation day 15 UGS, as shown by scanning electron microscopy. We then found that 10^{-9} M TCDD severely inhibited DHT-dependent bud formation when ventral mesenchyme was recombined with ventral plus dorsal epithelium (VDE), but not when dorsal mesenchyme was recombined with VDE. When ventral mesenchyme/VDE recombinants were implanted under the renal capsule of nude male mice, prostatic tissue developed, as determined histologically and by high expression of mRNA for MP25, a ventral prostate-specific gene. To further refine the site(s) at which TCDD acts to inhibit bud formation, ventral mesenchyme and VDE were prepared from wild-type and AhR null mutant (AhRKO) mice. All possible combinations of wild-type and AhRKO ventral mesenchyme and VDE were prepared and incubated with 10^{-8} M DHT and either 0.1% DMSO or 10^{-9} M TCDD. TCDD significantly reduced the number of buds in recombinants containing mesenchymal AhR, regardless of epithelial AhR status. But bud formation was not inhibited by TCDD in recombinants lacking mesenchymal AhR, even when epithelial AhR was present. We conclude that TCDD inhibits prostatic epithelial bud formation in the UGS by interacting with AhR in the mesenchyme. (Supported by NIH Grant ES 01332)

1331 EFFECTS OF METHYL MERCURY AND CADMIUM ON STRESS SIGNALING AND UBIQUITINATION PATHWAYS IN A PRIMARY SERTOLI CELL-GONOCYTE CO-CULTURE SYSTEM.

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We have recently developed and optimized a primary Sertoli cell-gonocyte co-culture system from neonate rat using modified extracellular matrix culture techniques. In order to apply this system for improving our understanding of the mechanisms of reproductive toxicity, we exposed the Sertoli cell-gonocytes to two known toxicants: methyl mercury (MeHg) and cadmium (Cd). Specific or non-specific proteasomal inhibitors:Lactacystin (Lact) and MG 132 (MG)were also included, since the disruption of specific ubiquitin-proteasome degradation may be associated with resultant toxicity. The effects on the morphology, MAPK, AKT signaling and ubiquitination pathway were examined. To establish these cultures, a sequential enzymatic digestion of testicular tubules from 5-day-old rats was used to obtain a cell suspension. This was plated with an addition of ECM overlay (150 μg/ml medium). MeHg (2.5μM), Lact (2.5 μM), MG (2.5 μM) and Cd (40 μM) were added to the culture medium 72h after plating. Morphological changes were monitored and photographed. Cells were harvested at 4, 8, and 24h after treatments and western blotting was performed using polyclonal antibodies to Phospho-p38 MAPK, p38 MAPK, AKT, Phospho-AKT, Phospho-SAPK/JNK, c-kit, and PARP. Exposure to Cd markedly increased the ubiquitin-conjugated proteins and also caused time-dependent activations of P-p38, P-SAPK/JNK and P-AKT. MeHg also increased ubiquitin-conjugated proteins and induced the P-p38 MAPK in a time dependent manner and marginally induced the P-SAPK/JNK phosphrylation. MG and Lact induced differential stress and ubiquitin signaling responses. The above results suggested that although the four chemicals increased the accumulation of ubiquitin-conjugated proteins, the molecular mechanisms appeared to be different. This study further confirmed that this co-culture system is useful for studying the molecular mechanism of the effects of developmental toxicants on Sertoli cells and/or spermatogonia. Supported by NIH ES 07033, ES09601-02, USEPA R826886-01

1332 RECOMMENDED APPROACHES FOR THE EVALUATION OF TESTICULAR AND EPIDIDYMAL TOXICITY.

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Histopathology is acknowledged as the most sensitive endpoint for detecting testicular toxicity. The tissues must be appropriately and consistently collected, fixed, trimmed, sectioned, stained, and presented to the pathologist for evaluation. The pathologist must have an adequate understanding of the organization and dynamics of spermatogenesis in the species under investigation. Understanding and using consistent terminology for the changes observed and grading the severity of those changes are also important issues in the reporting and interpretation of testicular histopathology. As such, this poster is intended to provide practical recommendations made by the Society of Toxicologic Pathology on how to use routine histopathology to promote accurate and consistent evaluation of the testis for toxicological changes. Most of the information relates to the rodent as the most common species used for evaluating male reproductive toxicity. Information pertaining to other less commonly used species such as the dog and nonhuman primate is included where appropriate. Our key recommendations are as follows. (1) The use of modified Davidson's fixative is the preferred fixative for testes with Bouin's fixative as a second choice. (2) The counting of stages of the spermatogenic cycle is valuable only in very specific circumstances, while evaluation of the testis by a pathologist who knows how each stage should appear (i.e., "stage-aware") is much more valuable. (3) Hematoxylin and eosin stains are appropriate for studies longer than 28 days, and PAS-H is recommended for shorter studies. (4) The nomenclature and severity grading for disturbances in spermatogenesis may vary on a case-by-case basis depending on the specificity of the findings.

1333 MALE RATS EXPOSED TO LORATADINE FROM GESTATION DAY 12 TO POSTNATAL DAY 4 DO NOT EXHIBIT ALTERATIONS IN ANDROGEN-MEDIATED REPRODUCTIVE DEVELOPMENT.

B. McIntyre, P. Vancutsem, K. Treinen and R. Morrissey. *Schering-Plough Research Institute, Lafayette, NJ.*

Male rats exposed to antihistamines (H_1 antagonists) exhibit species-specific testicular atrophy that is characterized by hypospermatogenesis, suggesting a potential antiandrogenic action. Since normal pre- and postnatal male reproductive development and function is dependent upon testicular androgen production and is sensitive to antiandrogenic perturbations, it was of interest to determine if loratadine had the potential to alter androgen-mediated reproductive development. Loratadine was administered orally by gavage to pregnant Sprague-Dawley rats (n = 20/group) at doses of 4, 12 or 24 mg/kg from gestation day 7 to postnatal day 4, the period encompassing androgen-dependent male reproductive development. Vehicle control rats received 0.4% aqueous methylcellulose. Dams were allowed to deliver naturally and rear their offspring until postnatal day 21. On postnatal day 21 male offspring were retained for postweaning measurements and the female offspring were euthanized and their sex confirmed internally. Males were necropsied on postnatal day 72 to 85. Dams administered 24 mg/kg of loratadine exhibited a transient 45% decrement in maternal body weight gain at the initiation of dosing (gestation days 7 to 9). Mean pup body weight on postnatal days 1 and 4 were approximately 4% lower than controls. No other effects on offspring growth were observed. Anogenital distance on postnatal day 1 was unaffected by loratadine exposure. Loratadine exposure did not induce the retention of nipples in male rats. Exposure to loratadine did not affect preputial separation or induce hypospadias. Seminal vesicle and prostate weights were unaffected by loratadine exposure. These data clearly demonstrate that loratadine is not an antiandrogen as evidenced by the absence of alterations or malformations in androgen-dependent reproductive tissues in loratadine-exposed male rats.

1334 DECREASED ANOGENITAL DISTANCE (AGD) AND UNDESCENDED TESTES IN FETUSES OF RATS GIVEN MONOBENZYL PHTHALATE (MBEP) DURING PREGNANCY.

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We previously reported that administration of butyl benzyl phthalate (BBP) to pregnant rats during late pregnancy caused decreased AGD and undescended testes in male offspring. The present study was conducted to determine the adverse effects of MBeP, a major metabolite of BBP, on development of the reproductive system in male offspring following maternal administration during late pregnancy. Pregnant rats were given MBeP by gastric intubation at 167, 250, or 375 mg/kg on days 15 to 17 of pregnancy and fetuses were examined on day 21 of pregnancy. Maternal body weight gain and food consumption during the administration period were significantly decreased at 167 mg/kg and higher. No increase in the postimplantation loss was found after administration of MBeP. The body weights of male and female fetuses were significantly lower at 375 mg/kg. A significant decrease in the AGD of male fetuses was observed after administration of MBeP at 250 mg/kg and higher. The AGD/cube root of body weight ratio in male fetuses was also significantly reduced at 250 mg/kg and higher. The AGD and AGD/cube root of body weight ratio of female fetuses in the MBeP-treated groups were comparable to those in the control group. A significant increase in the incidence of fetuses with undescended testes was found at 250 mg/kg and higher. These findings indicate that MBeP during late pregnancy produces adverse effects on development of the male reproductive system and suggest that MBeP participates, at least in part, in the induction of the adverse effects of BBP.

1335 MOLECULAR CHARACTERIZATION OF THE DEVELOPING RAT WOLFFIAN DUCTS FOLLOWING *IN UTERO* EXPOSURE TO DI(*n*-BUTYL) PHTHALATE.

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Di(*n*-butyl) phthalate (DBP), a common plasticizer and solvent, disrupts androgen-dependent male reproductive development in rats. *In utero* exposure to 500 mg/kg/day DBP on gestation days (GD) 12 to 21 inhibits androgen biosynthesis, resulting in a decrease in fetal testicular testosterone (T) levels. One consequence of prenatal DBP exposure is malformed epididymides (~50%) in adult rats. Reduced fetal T levels may be responsible for the malformation since T is required for stabilization of the Wolffian ducts and development into epididymides. Currently, little is understood about the molecular mechanisms of Wolffian duct differentiation. The objective of this study was to investigate the molecular mechanisms of DBP-induced morphological disruption of the developing Wolffian ducts. Pregnant Crl:CD(SD)BR rats were gavaged with corn oil vehicle or 500 mg/kg/day DBP from GD 12 to termination on GD 19 or 21. There was no observable morphological difference between control and DBP-exposed Wolffian ducts on GD 19. On GD 21, 89% of male fetuses in the DBP dose group had underdeveloped Wolffian ducts with decreased coiling. RNA was isolated on GD 19 and 21 and analyzed using cDNA microarrays. These analyses identified several genes involved in tissue differentiation that may be associated with the morphological changes observed on GD 21. Significant changes in mRNA expression within the insulin-like growth factor (IGF) and fibroblast growth factor (FGF) pathways, matrix metalloproteinase (MMP) family, and components of the extracellular matrix were confirmed by real-time RT-PCR. Gene expression of IGF1, IGF2, and members of the MMP family was significantly increased with DBP exposure compared to controls on both GD 19 and 21. The mRNAs for FGF10, FGFR2, collagen, and fibronectin were increased only on GD 21. This study provides valuable insights into the molecular mechanisms of Wolffian duct differentiation and the effects of antiandrogens on duct development.

1336 EFFECTS OF *IN UTERO* EXPOSURE TO FINASTERIDE ON ANDROGEN-DEPENDENT REPRODUCTIVE DEVELOPMENT IN THE MALE RAT.

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Finasteride is a specific inhibitor of type II 5α-reductase, the enzyme that converts testosterone to the more potent androgen receptor (AR) agonist dihydrotestosterone. Finasteride induces similar reproductive and developmental effects as those observed following *in utero* exposure to AR antagonists. Previous reports have concluded that decreased anogenital distance (AGD) and increased nipple retention observed in early postnatal life are not maintained in adulthood following *in utero* exposure to finasteride. In contrast, other antiandrogens have been shown to induce permanent effects on these androgen-sensitive end points. The objective of this study was to correlate changes in androgen-dependent end points in early postnatal life and at sexual maturity in the same animal over a range of doses. Pregnant Crl:CD(SD)BR rats (n = 5-6/group) were gavaged with either corn oil vehicle or finasteride at 0.01, 0.1, 1.0, 10, or 100 mg/kg/day on gestation days 12 to 21. All male offspring were monitored individually until necropsy on postnatal day (PND) 90. In early postnatal life, decreased AGD (on PND 1) and increased areolae-nipple retention (on PND 13) were significantly different from controls in all finasteride-exposed animals. Significant effects on decreased AGD and retained nipples were still seen on PND 90 in the animals exposed to finasteride at 0.1 mg/kg/day and above. Dorsolateral and ventral prostate weights were significantly decreased at and above 10 mg/kg/day on PND 90. Absence of prostates and bulbourethral glands was observed at the higher dose levels. In the highest dose group, the incidence of hypospadias and ectopic testes was 88 and 73%, respectively. The incidence of undescended testes was much higher than previously reported. The NOAEL for finasteride based on permanent effects in this study was 0.01 mg/kg/day. This is the first report to demonstrate that *in utero* exposure to finasteride results in a significant and permanent retention of nipples and reduction in AGD in sexually mature animals.

1337 EFFECTS OF *IN UTERO* EXPOSURE T0 LINURON ON RAT WOLFFIAN DUCT DEVELOPMENT.

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Prenatal exposure to linuron, a weak competitive androgen receptor (AR) antagonist, perturbs androgen-dependent male rat reproductive development, resulting in epididymal malformations in combination with testicular atrophy in adulthood. Exposure to 50 mg/kg/day linuron on gestation days (GD) 12 to 21 results in epididymal malformations (~25% of rats) that can be observed on postnatal day (PND) 7. Studies were undertaken to identify potential molecular mechanisms associated with antiandrogen perturbation of Wolffian duct development into the epididymis. Pregnant Sprague-Dawley CD rats were gavaged with corn oil vehicle or linuron (50 mg/kg/day) from GD 12 to 21. Gene and protein expression was examined in testes on GD 21 and epididymides on GD 21 and PND 7. On GD 21, ~40% of epididymides from linuron-exposed rats showed decreased ductal coiling or were malformed. Linuron-mediated changes in testicular and epididymal mRNA expression were identified using cDNA microarrays and alterations in gene expression confirmed by real-time RT-PCR. Linuron exposure did not reduce mRNA expression of the AR or that of several steroidogenic enzymes in the fetal testis, which is in agreement with a previous study demonstrating that linuron exposure did not reduce fetal testosterone production. Significant changes in mRNA expression in GD 21 and PND 7 epididymides were identified in the bone morphogenetic protein, insulin-like growth factor, epidermal growth factor, fibroblast growth factor, and notch signaling pathways. These signaling pathways have been shown to be involved in tissue morphogenesis. Changes in the immunolocalization of AR and insulin-like growth factor receptor were observed in epididymides from linuron-exposed rats. This study has identified several highly conserved signaling pathways that are disrupted by linuron exposure, suggesting that they are involved in testosterone-mediated development of the Wolffian duct.

1338 EFFECTS FROM GESTATIONAL EXPOSURE TO A MIXTURE OF ATRAZINE AND ITS BIOLOGICAL METABOLITES IN MALE LONG-EVANS RATS.

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In order to characterize the potential developmental effects of atrazine (ATR) metabolites, we tested an environmentally-based mixture (EBM) of ATR (25%), and its metabolites hydroxyatrazine (20%), diaminochlorotriazine (35%), deethyl atrazine (15%) and deisopropyl atrazine (5%), based on published maximum ATR and metabolite concentrations in ground and surface water (estimated at 25 ppb, 1X). Pregnant Long Evans rats (N>8/dose) were gavaged with 0, 0.09, 0.9 or 8.729 mg/kg/d EBM on gestation days 15-19. These doses were based on estimated adult consumption and were 100, 1000, or 10000X, the 1X basis of total chlorotriazines. In male offspring, body weight (BW) did not differ by treatment on PND4, 21, or 120. Preputial separation (PPS) in controls occurred on PND 41.9±0.4, and EBM PPS was statistically delayed (means ranged from PND 43.0±0.5 to 43.6±0.7; P=0.05). BW at PPS of EBM-exposed males (range of means 238.6± 5 to 254.1± 6) was greater than control males (223.6±5.2g; P<0.01). At PND 120, EBM exposed males exhibited a dose-dependent increase in anterior pituitary gland weight when compared to controls (range of means 10.6±0.2 to 11.3±0.4 vs. 9.3±0.7mg; P<0.001). There was a dose-dependent decrease in ventral prostate weight due to EBM exposure (range of means 410±20 to 380±20 vs. 450±20mg; P=0.01), although the lateral prostate, testes, and seminal vesicle weights were unaffected when compared with controls. Also at PND 120, the EBM-exposed males (range of means 45.6±7.7 to 47.9±5.5 ng/ml) displayed a significant increase in serum estrone vs. control (28.9±2.5 ng/ml; P<0.01). Concentrations of serum and pituitary prolactin, serum estradiol, testosterone, and thyroid stimulating hormone were not different among dose groups. These data suggest that EBM delayed puberty without effect on body weight, and was capable of altering endocrine and reproductive tract endpoints. *(This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Supported by NHEERL Minority Training Agreement).*

1339 UVA EXPOSURE INCREASES XANTHOTOXIN-INDUCED GAMETE DNA DAMAGE IN MALE RATS.

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Previous research has shown that Wistar rats dosed with xanthotoxin (8-methoxypsoralen) have low birth rates when either the male or female in a mated pair is exposed. Reduced sperm counts, reduced litter size and birth defects were also exhibited when only the males were dosed and mated with undosed females, suggesting a direct adverse effect on the sperm. This was accompanied by teratogenic effects in offspring of naive females mated to dosed males. Therefore, the current study was initiated to see if xanthotoxin exposure would damage the DNA of rat spermatozoa. A comet assay protocol was developed to check the DNA of Spermatozoa for damage. Spermatozoa samples were collected from the testes of mature rats and reacted with varying concentrations of xanthotoxin (0, 2, 4, 6, 8, 10 mg/ml Acetone solution). Some of the treated samples were exposed to long-range UVA radiation and some were not. Samples were then digested with proteinase K in 1% SDS and TES buffer for 60 minutes. The cells were placed in a low melting point gel and then lysed. DNA damage in the samples was checked by Comet assay. The images

were gathered using a fluorescence microscope and analyzed by computer. The moments (ratio of nucleus to tail for each gamete) collected from groups of comets were statistically tested and means separated at the 5% level. The results indicate that the treated, irradiated sperm samples sustained significantly more damage than untreated samples. The assay also illustrates that the treated, non-irradiated sperm samples sustained significantly more damage than the untreated samples. The damage in these sperm samples occurred in a dose-dependant manner. The results suggest that the psoralens could compromise reproductive function in a naive female by indirect effect through the seminal fluid.

1340 FETAL TESTICULAR GENE EXPRESSION FOLLOWING *IN UTERO* EXPOSURE TO DI(*N*-BUTYL) PHTHALATE: ALTERATION OF KEY ANDROGEN-RELATED GENES.

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Di(n-butyl) phthalate (DBP) has been shown to alter male reproductive development by decreasing fetal testicular testosterone (T) when fetuses are exposed on gestation days (GD) 12 to 21. Earlier findings indicated that there were changes in gene expression for several enzymes in the steroidogenic pathway and for a variety of other androgen-related genes. The objective of this study was to build on the previous data by using increased numbers of dams and fetuses to confirm these findings and to examine changes in gene expression for additional genes in the T biosynthetic pathway. Pregnant Crl:CD(SD)BR rats (n = 6-7 dams/group) were dosed by gavage with corn oil vehicle or DBP at 500 mg/kg/day on GD 12 to 19. Testicular mRNA isolated from three random individual GD 19 fetuses per litter was used for real-time quantitative PCR for the following genes: scavenger receptor (SRB1), steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α-hydroxylase/17, 20-lyase (P450c17), 17β-hydroxysteroid dehydrogenase (17β-HSD), androgen receptor (AR), c-kit receptor (c-kit), proliferating cell nuclear antigen (PCNA), and testosterone-repressed prostate message-2 (TRPM-2). Following DBP exposure, there was downregulation of the following genes: SRB1, StAR, P450scc, 3β-HSD, P450c17, and c-kit; there was upregulation of TRPM-2. 17β-HSD, AR, and PCNA were not significantly changed. Downregulation of genes in the testosterone biosynthetic pathway confirms and extends previous findings and supports diminished T synthesis as the cause of reduced fetal testicular T observed following *in utero* exposure to DBP. A decrease in androgen levels was further supported by altered expression of other androgen-related genes such as TRPM-2. These data strongly support a generalized effect on steroidogenesis in fetal Leydig cells versus blockade of a single enzyme or protein in the steroidogenic pathway.

1341 TOXICOLOGY: ETHICAL, LEGAL, AND SOCIAL ISSUES.

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Through an emphasis on exposure/response and mechanistic based research, the toxicological sciences exert an increasing influence on bioethical thinking and societal decision-making. Results from toxicology research have motivated many regulatory and legal actions, and public policy decisions, including the banning of some pesticides, reducing exposure in the workplace, and lowering of acceptable blood lead levels in children. Ongoing advances in the genomic and toxicological sciences continue to emphasis the importance of considering individual sensitivity and particularly the susceptibility of children. This session will explore the ethical, legal and social issues raised by the toxicological sciences by examining some of the difficult policy and research decisions that must be made as we address our need for additional information about the health effects of chemicals on adults and children and the impact of having this information. The objectives of this session are: 1) to explore the contributions and implications of toxicological research on bioethical thinking and public policy, and 2) to provide a forum for discussion of ethical issues raised by current and future toxicological and genomic based research. This session will be of interest to toxicologists concerned about the broader impact of our research on bioethical and societal decision-making.

1342 GENOMIC AND PROTEOMIC ANALYSIS OF SURROGATE TISSUES FOR ASSESSING TOXIC EXPOSURES AND DISEASE STATES.

D. J. Dix and J. Rockett. *Reproductive Toxicology, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

Genomics and proteomics have made it possible to define molecular physiology in exquisite detail, when tissues are accessible for sampling. However, many tissues are not accessible for human diagnostic evaluations or experimental studies, creating the need for surrogates that afford insight into exposures and effects in such tissues. Surrogate tissue analysis (STA) incorporating contemporary genomic and proteomic technologies may be useful in determining toxicant exposure and effect, or disease state, in target tissues at the pre- or early clinical stage. In this symposium, speakers will discuss various applications of STA, including the use of peripheral blood lymphocytes (PBLs) as a source of biomarkers for radiation exposure; the use of PBLs and hair follicles to monitor the impact of toxicants on organs such as liver and testis; the use of mRNA in sperm to determine genetic and environmental effects on male fertility; and the use of serum protein profiles to monitor for ovarian cancer. The symposium will conclude with a discussion of the challenges of validating surrogate tissue fidelity and sensitivity. This is an abstract of a proposed symposium and does not necessarily reflect EPA policy.

1343 GENE EXPRESSION PROFILING OF ACCESSIBLE SURROGATE TISSUES TO MONITOR MOLECULAR CHANGES IN INACCESSIBLE TARGET TISSUES FOLLOWING TOXICANT EXPOSURE.

J. C. Rockett, C. Blystone, A. Goetz, R. Murrell and D. J. Dix. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

Gene expression profiling (GEP) offers the potential to classify toxicant exposures, predict clinical outcome of such exposures, and provide mechanistic data useful for risk assessments. It could ultimately provide a vehicle for developing early diagnostic and preventative measures in at-risk populations or individuals. However, using GEP to monitor the impact of toxicant exposures on inaccessible tissues is a difficult prospect, since direct biopsy of such tissues is not feasible. A less invasive method must therefore be developed. We propose that gene expression changes in accessible (surrogate) tissues (e.g. blood) often reflect those in inaccessible (target) tissues, thus offering a convenient biomonitoring method to provide insight into the effects of environmental toxicants on target tissues. GEP of surrogate and target tissues have compared following exposure of animal models to model compounds and environmental toxicants. In an initial pilot study, gene expression changes in peripheral blood lymphocytes (PBLs) were compared to those in the uteri of adult rats to identify genes that were altered in both tissues following estradiol treatment. Ovariectomized rats were treated with either 17-b-estradiol or vehicle control for 3 days. PBL and uterine RNAs were hybridized to arrays containing 1185 genes. A number of genes (e.g. jun-D, phospholipase A2, thymidine kinase) were found to demonstrate a similar degree of expression change that was treatment-, but not tissue-specific. This indicates that accessible tissues such as PBLs can serve as surrogate tissues for observing gene expression changes in the uterus following chemical exposure, and provides supportive evidence for the surrogate tissue analysis concept. This presentation will also discuss data from a follow-up study, currently in progress, in which GEP of a variety of surrogate and target tissues is being conducted in rodent models exposed to conazoles. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1344 THE MALE GAMETE AS A PATERNAL MARKER OF GENETIC INSULT.

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The primary goal of systems biology is to understand the mechanisms of development in the normal and diseased states. This reflects the selective activation of the 40, 000-80, 000 genes that are encoded by our genome. The self renewing system of spermatogenesis provides an excellent model to address how this program is articulated. The recent demonstration that ejaculated spermatozoa contain sufficient mRNA for analysis uniquely positions us to begin to address the direct impact of the environment on the paternal genome. We know that environment, e.g. cigarettes, alcohol, caffeine and heavy metals, has a direct effect. This raises the question: what is the genetic load that the sperm will bring to a new being? Answering this question will fill a significant gap in our understanding of the healthy child. We will review the results of our methods and analyses showing that human sperm do indeed carry a wide spectrum of RNAs to the egg. This will include a microarray survey of 20, 002 expressed sequences. Analysis of this data using Onto-Express has revealed that some sperm mRNAs may impact post-fertilization. With this enabling foundation in place, we will also explore the use of sperm RNA as a biomarker of the "fitness and exposure of the male gamete." Data will be presented from our ongoing human studies and complementary toxicological studies profiling sperm mRNAs in rodents exposed to effective doses of reproductive toxicants.

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1345 GENE EXPRESSION IN RESPONSE TO LOW DOSE IONIZING RADIATION: A FUNCTIONAL GENOMICS APPROACH.

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Gene expression changes occur in both human and murine cells in response to doses of radiation of 10 cGy and less. Using a quantitative single-probe hybridization method to accurately measure increases in mRNA levels relative to untreated cells, we have demonstrated a linear non-threshold response for multiple stress genes in the human p53-wt myeloid ML-1 line, and in human peripheral blood lymphocytes (PBL) irradiated *ex vivo*. Similar responses are also emerging in mice irradiated *in vivo*. cDNA microarray hybridization analysis was used to identify radiation-regulated genes that could potentially serve as biomarkers of radiation exposure. Initial studies identified several genes significantly up-regulated in human PBL between 24 & 72 hours after *ex vivo* irradiation. Three of these genes, DDB2, CDKN1A (CIP1/WAF1) and XPC, were induced in a linear fashion between 0.2 and 2 Gy at 24 & 48 hours after treatment, with less linearity at earlier or later times. These and other strongly radiation responsive genes are p53-regulated, indicating a major role for p53 in mediating radiation gene responses in PBL (and probably other primary cells). These results support the use of peripheral blood cells as an accessible and sensitive indicator of radiation exposure, and begin laying the foundation for expression profiles that may someday provide signatures for past radiation exposure. Reducing the dose-rate of exposure of ML-1 cells across 3 orders of magnitude shows a trend toward protection from apoptosis, and attenuated induction of GADD45A and CDKN1A. In contrast, there was no change in induction of MDM2. Clustering of cDNA microarray analysis results obtained from irradiations carried out at different dose-rates indicates 2 distinct groups of responding genes: a group of genes with attenuated induction at low dose-rates, and a group of genes with similar or possibly enhanced induction by low dose-rate exposure. Further analysis of these 2 classes of responding genes may provide key insights into the molecular mechanisms of radiation response and tolerance.

1346 SERUM PROTEOMIC PATTERN DIAGNOSTICS: USE OF ARTIFICIAL INTELLIGENCE BIOINFORMATICS TO DISCOVER SURROGATE MARKERS FOR EARLY DISEASE.

E. Petricoin. *Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD.* Sponsor: J. Rockett.

The need for specific and sensitive surrogate markers for the presence of ovarian cancer is critical. Currently there is no effective screening options available for the patients with the ovarian cancer. CA125, the most widely used biomarker for ovarian cancer does not have a high positive predictive value and it is only effective when used in combination with other diagnostic tests. Unfortunately, ovarian cancer presents at a late clinical stage in over 80% of patients and is associated with an abysmal 5-year survival of 35%. In contrast, the 5-year survival for stage I ovarian cancer patients is in excess of 90%, with most patients cured of their disease by surgery alone. In contrast to past strategies where a single biomarker is used as a discriminator, we propose a new paradigm for biomarker discovery and profiling that is based on a multitude of proteomic patterns as the diagnostic endpoint in and of itself. We speculated that pathologic changes within an organ might be reflected in the serum as a surrogate proteomic patterns. Low-molecular weight proteins and peptides may provide the most target rich information archive since these molecules are most likely aberrantly secreted, clipped or shed as a cause or consequence of the disease and are deposited in the circulating serum. Serum analysis coupled with high-throughput robotic handling stations and rapid mass spectroscopy proteomic pattern generation allowed us to utilize artificial-intelligence based systems to uncover hidden diagnostic signatures. We have achieved a 99% sensitivity and 99% specificity in over 250 patient samples analyzed to date, including 100% of the 35 early stage I cancer patients, making this technique a clinical possibility.

1347 FUNDAMENTALS OF PROTEIN ALLERGENICITY: WHY ARE SOME PROTEINS ALLERGENIC?

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For a variety of reasons the toxicology of protein allergenicity has assumed greater significance. There is an increasing prevalence of atopic diseases in westernized populations, the use of protein and peptides in therapeutics and in consumer products is growing, and evaluation of potential allergenicity is a major issue in the safety assessment of novel foods, including foods derived from genetically modified crops. The questions that must be addressed are whether proteins and peptides have the inherent potential to cause sensitization and whether under the likely conditions of exposure this intrinsic hazard will translate into a risk of human allergic disease. With respect to the first of these questions a pivotal consideration is definition of the characteristics that confer on proteins the ability to cause allergic sensitization, or alternatively, what distinguishes protein allergens from other foreign proteins that despite being potentially immunogenic fail to induce sensitization. Among the features that are believed to influence sensitizing properties are: size, stability, glycosylation status and the way in which the protein is recognized, internalized and processed by the immune system. The purpose here is to examine the contribution of these variables to inherent sensitizing potential as a basis for future safety assessment strategies. With respect to the second question, one must consider how the allergenic potential and potency of the protein in combination with exposure translates into a risk for developing allergy.

1348 IMMUNOBIOLOGY OF SENSITIZATION BY PROTEIN ALLERGENS.

I. Kimber and R. J. Dearman. *Syngenta Central Toxicology Laboratory, Macclesfield, CHESHIRE, United Kingdom.*

Allergy induced by proteins may take a variety of forms. In many (but not all) instances allergic reactions are provoked by IgE antibody-dependent mechanisms and the acquisition of sensitization requires the stimulation of an IgE antibody response. The initiation and maintenance of IgE production are highly regulated by functional subpopulations of T lymphocytes and their cytokine products. Type 2 T helper (Th2) cells secrete cytokines such as interleukins 4, 5, 10 and 13 that support IgE antibody responses and facilitate the elicitation of IgE-mediated allergic hypersensitivity reactions. In contrast, products of Th1-type, and of T cytotoxic type 1 (Tc1) cells, and in particular interferon gamma, antagonize IgE responses. The initiation of specific IgE antibody production and the development of allergic sensitzation are dependent upon the characteristics of the protein itself (size, stability, glycosylation status and the way in which it is processed and presented to the immune system), the extent, duration and route of exposure and the age at which exposure occurs, and genetic and acquired predisposition (and particularly inheritance of an atopic phenotype). The immunobiology of sensitization will be considered in the context of both the inherent allergenic potential of proteins and the variables that determine individual susceptibility to allergic disease.

1349 STRUCTURAL BIOLOGY OF PROTEIN ALLERGENS.

R. C. Aalberse. *Immunopathology, Sanquin Research at CLB, Amsterdam, Noord Holland, Netherlands.* Sponsor: I. Kimber.

Size and solubility.The requirement for physical contact between protein and cell defines requirements for solubility and size, particularly allergens that enter the body by crossing a mucosal barrier (airways, gastro-intestinal tract). Typically, such allergens are soluble in aqueous buffers and have a molecular mass between 10 and 100 kD. Larger proteins may become allergenic upon limited proteolytic degradation. Smaller molecules may become allergenic upon linkage to a carrier protein. Stability is an issue primarily for food allergens (resistance to thermal denaturation and to gastric/intestinal juice). Glycosylation affects allergenicity in two ways. Glycans may influence stability. Moreover, glycans may be contact sites for IgE antibodies. Glycan allergenicity is mainly an issue in relation to non-mammalian glycoproteins, particularly plants and invertebrates. A common glycan structure is called the Crossreactive Carbohydrate Determinant (CCD). Since glycans are attached *via* post-translational mechanisms, their presence in recombinant glycoproteins depends on the expression system. Cross-reactivity may result in allergic symptoms without a prior contact. In some cases in-vitro tests indicate that a protein has IgE-binding activity, whereas in-vivo exposure does not result in symptoms. This discrepancy may be due to cross-reactivity that is apparent only *in vitro* (e.g. because of low affinity or because only a single cross-reactive epitope is present whereas biological activity requires multiple epitopes). Two proteins will be cross-reactive only if they (or their domains) belong to a similar protein fold family. However, a similar fold does not suffice: a substantial degree of amino acid identity is required, usually more than 60%. A crucial feature of IgE-mediated allergy is the involvement of TH2 cells. Since human IgG4 antibodies are also TH2-dependent, the induction of IgG4 antibodies can be used as surrogate marker for allergenic potential. An important feature of allergenic source materials is that they do not contain substances that stimulate TH1 responses (such as bacterial cell wall material), which down-regulate TH2 responses.

1350 FOOLING MOTHER NATURE: CAN PROTEIN ALLERGENS BE MADE HYPOALLERGENIC?

F. A. Harding. *Immunology, Genencor International, Palo Alto, CA.* Sponsor: K. Sarlo.

The critical role for CD4+ T cell activation in the induction of respiratory allergy has been clearly shown. T cell activation is dependent on a number of variables including the quality of the T cell receptor (TCR) signaling and the microenvironmental milieu. The component of antigen recognized by CD4+ TCRs is 10+/-2 amino acid fragments derived from the linear sequence. Peptides are bound by their receptor, HLA class II, and are displayed on the surface of antigen-presenting cells. A tri-molecular complex of HLA, peptide and TCR must form in order for antigen-specific signaling to occur. Interfering with this assembly by disabling any one of the components would obviate antigen-specific CD4+ T cell signaling, and by extension, any subsequent immune response including the induction of allergy. A number of mechanisms can be envisioned to interfere with the assembly of the tri-molecular complex. The derivation of peptide fragments from the antigen is a process that involves a number of well-characterized proteolytic enzymes. Modifying the protein sequence to inhibit or upregulate enzymatic processing is one technique currently under study. A second method is the modification of immunogenic peptide epitopes to interfere with either binding to HLA or to the TCR. In order to create hypo-immunogenic proteins, we are currently using the latter approach, while investigating the utility of the former. We have designed a functional assay that allows for the localization of immunodominant CD4+ T cell epitopes in any protein of interest. This information makes it possible to create protein variants with reduced capacity to induce immune responses. In our assay, CD4+ T cell epitopes are determined on a human population basis. Using this knowledge coupled with our ability to efficiently and rationally modify, express and screen protein variants, we have created active protein variants with reduced immunogenic potential that are specifically designed for the human population. Examples of this technique will be shown, using *in vivo* and *in vitro* methods to validate our approach. We think that yes, it is possible to fool Mother Nature!

1351 PROTEIN ALLERGENICITY: CHALLENGES FOR THE TOXICOLOGIST.

K. Sarlo. *Procter & Gamble Co., Cincinnati, OH.*

To assess the risk of developing allergy to protein, one must understand the inherent allergenic potential of the protein, how individuals will be exposed to this protein and the likelihood that this exposure can lead to allergy. *In vitro* tests to assess allergenic potential are in their infancy and *in vivo* animal models have not been fully developed and validated for a wide range of proteins. The questions of choice of species, which endpoints to measure, route of exposure and use of adjuvant all add complexity. For enzymes used by the detergent industry, animal models have provided data on inherent allergenic potency of enzymes and enzyme mixtures. These data were generated on relevant endpoints (allergic antibody) using a relevant route of exposure (respiratory tract) and have been used to set exposure guidelines to minimize sensitization among workers. Prospective surveillance of the worker population confirmed the assessments made in the animal models. By keeping exposure low, the rate of sensitization is low and this reduces the risk of disease among workers to near zero. This points to another area of complexity for protein allergy: how does one identify the thresholds for sensitization vs. disease and how does one use this information to manage protein allergens? We have some knowledge on the sensitizing and non-sensitizing exposure conditions for enzymes used in laundry products and personal cleansing applications. Via retrospective and prospective clinical evaluation of exposed populations, we are beginning to understand how intensity of exposure is related to sensitization and disease. Incorporating hazard information on an enzyme with the exposure assessment, we can compare to known exposures to predict the likelihood of sensitization. Prospective clinical testing is used when there is uncertainty in the comparisons. This risk assessment approach has supported the safe use of enzymes. Whether this approach can be applied to other proteins or to other modes of exposure (e.g., ingestion) remains to be seen.

1352 DOSE-DEPENDENT TRANSITIONS IN TOXIC MECHANISMS.

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As experience with dose-response and mechanism expands, it is evident that multiple mechanisms may exist for any given agent as the full dose-response curve is explored. It is highly likely that critical, limiting steps in any given mechanistic pathway may become overwhelmed with increasing exposures, signaling the emergence of new modalities of toxic tissue injury at these higher doses. Therefore, dose-dependent transitions in the principal mechanism of toxicity may occur and could have significant impact on the interpretation of data sets for risk assessment. The purpose of this Workshop is to serve as a reference around the principle that each saturable or inducible process that occurs as part of the overall chemical disposition and biological response represents a potential point of departure from linearity in the dose-response relationship. Major objectives are to document and establish methods for identifying that dose-dependent transitions in mechanism can occur and to discuss potential impacts of this phenomenon on the risk assessment process. Individual case studies that exemplify this phenomenon including butadiene, vinyl chloride, vinylidene chloride, vinyl acetate, formaldehyde and progesterone will be presented. The impact of these and other examples on risk assessment procedures will be presented. This timely topic should be of interest to basic researchers, modelers and risk assessors.

1353 DOSE, TISSUE AND SPECIES TRANSISTIONS IN BIOMARKERS.

J. A. Swenberg. *Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

DNA and hemoglobin adducts represent biomarkers of exposure that integrate bioactivation, detoxication and DNA repair. As such, they provide excellent data for understanding mechanisms that drive the carcinogenic process and for improving risk assessment. The two chemicals highlighted in this presentation, vinyl chloride (VC) and butadiene (BD), have been extensively studied. VC is metabolized by CYP2E1 in a saturable manner. It exhibits two transitions of interest. Its metabolism is linear at low concentrations and becomes saturated at concentrations >100 ppm. Since the ultimate carcinogen, 2-chloroethylene oxide, is highly reactive and unstable, it does not get transported to tissues distant to the site of metabolic activation. Thus, liver, but not brain DNA becomes alkylated. However, all tissues examined to date from non-exposed animal and human tissues contain identical DNA adducts that arise endogenously from lipid peroxidation. Interpolation of data from control and VC-exposed rat livers (10 ppm, 4 wks, 6 hr/day) demonstrated that low exposures typical of current occupational exposure would represent 5% and 50% increases over the identical endogenous DNA adducts for exposures to 0.1 and 1.0 ppm VC, respectively. BD is much more complex, with at least four different electrophiles being formed, which vary in mutagenic potency by at least 200-fold, and differ greatly by species. Both linear and nonlinear responses exist over a wide range of exposures. Furthermore, the epoxide metabolites are relatively stable and circulate to distant tissues. We have developed a battery of biomarkers for the metabolites of BD. These have been applied to tissues and globin from rats and mice. In addition, we have examined the same biomarkers in globin in humans. It is critical that a correct understanding of species differences be utilized in scientifically-based risk assessment.

1354 CASE STUDIES OF FORMALDEHYDE AND HYDROXYFLUTAMIDE: EVIDENCE FOR DOSE-DEPENDENT TRANSITIONS IN MECHANISMS DUE TO ALTERED HOMEOSTASIS AND RECEPTOR MEDIATED INTERACTIONS.

R. Conolly. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Nonmonotonic dose-response curves, including thresholds and J-shapes, can arise when a chemical exerts different effects at different doses. With formaldehyde, a J-shaped curve for tumor formation in F344 rats may occur when cell division is depressed at low inhaled concentrations (≤ 2 ppm) but increased at higher, cytolethal concentrations. Depression of the division rate is associated with formation of DNA-protein cross-links (DPX) that may invoke cell cycle checkpoint controls or may physically block the replication complex. At higher concentrations, formaldehyde is cytolethal and regenerative proliferation drives the division rate above control levels. The summation of these low- and high-concentration effects provides an overall J-shaped dose response for cell division. When this dose-response is linked to tumor formation through a 2-stage clonal growth model, a J-shaped dose-response for tumor formation is predicted, even in the presence of a low dose linear, directly mutagenic effect whose magnitude is proportional to the DPX burden. J-shaped dose-response behaviors can also arise when two chemicals interact within a single mode of action. Dihydrotestosterone (DHT) and hydroxyflutamide (OHF) together produce a J-shaped curve for gene expression mediated by the androgen receptor (AR) in a HepG2 cell line using a luciferase reporter gene. DHT and OHF are both substrates for the AR. 10E-7 M DHT is used to generate a maximal rate of gene transcription, and the J-shaped dose-response for OHF arises in the presence of the DHT. The J-shape may be explained by a relative inefficiency of DHT-OHF heterodimers for promotion of gene transcription given efficient promotion by DHT and OHF homodimers. These two examples illustrate alternative mecha-

nisms whereby significant dose-dependent transitions can occur. The common theme in these examples is the need for at least two effectors of the dose-dependent transition, either two interacting modes of action or two chemicals interacting within a single mode of action.

1355 DOSE-DEPENDENT TRANSITIONS IN VINYL ACETATE AND VINYLIDENE CHLORIDE TOXICITY: TWO CASE EXAMPLES SUGGESTIVE OF IMPACTS ON BIOLOGICAL RESERVE CAPACITY.

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Non-linear dose response curves in toxicology studies suggest modes of action in which a shift in the dominance of critical kinetic or dynamic determinants has occurred. Vinyl acetate and vinylidene chloride (VDC) are two examples where sharp changes in the shape of the dose response curve occur over a discrete dose range. Although the modes of action and target organs for toxicity are very different for these two compounds, together they illustrate the fundamental concept that alterations of cellular defenses beyond homeostatic bounds can yield the observed transition in dose response behavior. Vinyl acetate causes nasal olfactory degeneration, cellular proliferation and nasal tumors at high exposure levels. The mode of action is believed to be related to intracellular acidification, which results from metabolic formation of acetate, followed by cytotoxic or mitogenic cellular proliferation. Proliferation, in the presence of metabolically formed acetaldehyde, provides the driver for mutation and eventually cancer. All of these critical steps are present only at high exposure levels. Modeling and *in vitro* studies suggest that intracellular pH must be perturbed beyond homeostatic bounds to permit the subsequent mechanistic steps. Vinylidene chloride (VDC) is metabolized to an epoxide and 2-chloroacetylchloride both of which bind covalently to hepatic protein and deplete tissue glutathione (GSH). Dose-response curves for hepatotoxicity and lethality demonstrate abrupt changes despite a relatively linear dose response curve for GSH depletion and covalent binding. These two case studies suggest that perturbation of critical biochemical defenses (e.g. intracellular buffering mechanisms, glutathione stores, etc.) beyond homeostatic bounds can result in dose-dependent transitions in mechanism. These cases further suggest that research is needed to better define the limits of cellular defense mechanisms and functional reserve capacity and should raise regulatory awareness of changes in toxic mechanisms imparted by high dose (MTD) testing.

1356 DOSE-DEPENDENT TRANSITIONS IN TOXIC MECHANISMS: IMPLICATIONS FOR RISK ASSESSMENT.

D. W. Gaylor. *Gaylor and Associates, Little Rock, AR.* Sponsor: W. Slikker.

Reference doses associated with negligible health risks for non-cancer effects are established by dividing a point-of-departure (no or low observed adverse effect level or benchmark dose) by uncertainty factors to account for extrapolation from effects in animals to humans, duration of exposure, and allowance for inter-individual sensitivity. Cancer risk estimation generally has employed low-dose linear extrapolation to zero. If the shape of the dose response can be established, it may be possible to dispense with linear extrapolation and/or modify the point-of-departure relative to dose-dependent transitions for risk assessments of cancer and non-cancer effects. Comparisons of dose-dependent transitions across and within species may support the reduction of the size of one or more uncertainty factors. The statistical design and analysis of experiments to demonstrate dose-dependent transitions and determine the point-of-departure will be discussed.

1357 STRATEGIES TO IDENTIFY SATURABLE AND/OR INDUCIBLE KINETIC AND DYNAMIC STAGES OF DOSE-DEPENDENT TRANSITIONS IN TOXIC MECHANISM.

K. B. Wallace[1] and W. Slikker[2]. *[1]Biochemistry & Molecular Biology, University of Minnesota, Duluth, MN and [2]Division of Neurotoxicology, NCTR/FDA, Jefferson, AR.*

The ultimate expression of toxicity following exposure to foreign substances is dictated by a variety of saturable kinetic processes, both in the disposition of the chemical and biological expression of the pathogenic response. Examples of such processes include the absorption of the chemical in question, its transport and accumulation within specific tissues or cellular compartments, metabolic transformation to metabolites of differing activities, interaction on the biologically active form with specific tissue receptors, elicitation of defined intracellular signaling pathways to affect the ultimate biological response, various protective or reparative pathways that counteract the adverse biological effect, and altered molecular regulation to establish new homeostatic states in response to the biological stress imposed as a result of the chemical exposure. Saturation of any one of these steps redirects the disposition, metabolism or expression of biological activity for that compound. Consequently, as one explores the range of exposures it is not uncommon to encounter inflections in the dose-response relationships, which signify saturation of one or more essential steps in the pathogenic mechanism. This, then, brings into question assumptions associated with extrapolating mechanism-based risk assessments across the full range of exposures. Several examples will be presented in this workshop that illustrate transitions between mechanisms of toxicity that occur as a function of exposure dose. The goal will be to identify key determinants of dose-dependent transitions and to develop guiding principles for applying these phenomena to risk-assessment paradigms.

1358 QUESTIONS SURROUNDING DEPLETED URANIUM TOXICITY: ANSWERS FROM THE CLINIC AND THE LABORATORY.

K. S. Squibb[1] and D. Jacobson-Kram[2]. *[1]Program in Toxicology, University of Maryland School of Medicine, Baltimore, MD and [2]BioReliance, Rockville, MD.*

The use of depleted uranium (DU) by US military forces in the Gulf War created a unique exposure scenario by which soldiers were exposed to a metal well known for its chemical toxicity in workers in the nuclear industry. However, exposure during the Gulf War occurred either for a short period of time *via* inhalation of aerosolized uranium oxide, or as a chronic exposure in injured soldiers left with embedded uranium fragments. Questions regarding the long-term health effects of these two types of exposures have fueled considerable debate, raising questions regarding continued use of DU weapons by the military and liability of the government for health problems in the Gulf War veteran population. DU, used in munitions because of its high density and low cost, is a by-product of the uranium enrichment process. DU has a lower University^{235} content than natural uranium, with a University^{235}/U^{238} ratio of 0.245 *versus* 0.721 for natural uranium. Research to address questions of exposure and toxicity has moved forward on a number of fronts, including clinical surveillance of vets with DU shrapnel, ICP-MS exposure assessment techniques for differentiating between DU *versus* natural University excretion, *in vivo* animal toxicity and carcinogenicity studies using embedded DU metal, and genotoxicity studies of the chemical versus radiological hazards associated with DU. Results of these studies provide mechanistic and clinical toxicity information needed for assessing risk associated with exposure to DU during the Gulf War and emphasize the importance of the basic toxicological principles of dose, duration and route of exposure. Mechanistic studies begin to provide an understanding of the chemical versus radiological toxicity of this unique metal.

1359 HEALTH EFFECTS OF DEPLETED URANIUM ON EXPOSED GULF WAR VETERANS.

M. A. McDiarmid. *Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Medical surveillance of a group of US Gulf War veterans (N=63) who were victims of depleted uranium (DU) 'friendly fire' has been carried out since the early 1990s. Urine uranium concentration has been used as a measure of systemic exposure with health outcomes and organ system function evaluated as a dependent variable. DU possesses only 60% of the radioactivity of natural uranium, having been 'depleted' of much of its most highly radioactive University^{234} and University^{235} isotopes. Radiation dose estimates calculated from whole body radiation counting using the ICRP 30 Biokinetic model for University yielded upper limits of 0.1 rem/yr (the public dose limit) and 5.3 rem/50 yrs (with the annual occupational limit being 5 rem/yr as a comparison). Therefore, the focus has been on uranium's chemical, heavy metal character, rather than its radiologic toxicity. Findings to date reveal the persistent elevation of urine uranium, more than 10 years after exposure, in those veterans with retained shrapnel fragments, presumably from on-going mobilization of DU from fragments oxidizing *in situ*. Other clinical outcomes related to uranium measures have revealed few abnormalities. Renal function is normal despite the kidney's expected involvement as the 'critical' organ of uranium toxicity. Subtle perturbations in some proximal tubular measures may suggest early, albeit not statistically significant, effects of uranium. A mixed picture of genotoxic outcomes is also observed. Observations from this chronically exposed cohort inform health predictions of future effects in other potentially-DU exposed populations and provide helpful data for hazard communication of future deployed personnel. *Supported by the Office of Public Health and Environmental Hazards, Department of Veterans Affairs, and the VA Maryland Healthcare System/Baltimore Division*

1360 DETECTION OF DEPLETED URANIUM (DU) IN URINES OF GULF WAR VETERANS.

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American soldiers involved in 'friendly fire' accidents during the Gulf War were injured with DU containing fragments or possibly exposed to DU *via* other routes such as inhalation or ingestion. To evaluate the presence of DU in these soldiers eight years later, the University concentration and DU content of urines were determined by an inductively coupled plasma mass spectrometry (ICP-MS) method in (a) DU exposed soldiers with shrapnel, (b) DU exposed soldiers with no shrapnel and (c) deployed soldiers who did not participate in combat. The minimum detectable fraction of DU in urine uranium by this method was 20% at a urine [U] of 21 ng/L, and the ability to detect DU in urine increased as urine uranium level increased. Many urines injected directly into the ICP-MS and measured at a mass resolution m/Δm of ~ 300 had University ratios enriched in ^{235}U. Analyses of the same urines at a mass resolution of 4000 showed that the apparent extra ^{235}U counts were due to an interference with a mass of 234.81 amu. Because no two-isotope combination could yield such light molecular weight, most likely the interference was a polyatomic molecule of more than two atoms. When analyses were done on uranium extracted from these urines the ^{235}U abundance was natural or depleted and the interference was eliminated. Urine uranium levels of soldiers with shrapnel were higher than those of the two other groups and DU was detected in 16 out of 17 subjects. In exposed soldiers with no shrapnel DU was detected in 10 out of 28 as opposed to 1 in 11 in the deployed but not in combat group, yet urine uranium levels of these two groups were not different. Using the criteria of presence or absence of DU in urine, uranium levels of DU bearing urines from groups (b) and (c) were significantly higher than uranium levels of urines with no DU. These results indicate that uranium uptake can occur *via* routes other than injury with DU bearing shrapnel and a one time event exposure can result in long term uranium excretion.

1361 *HPRT* MUTATIONS IN T-CELLS IN GULF WAR VETERANS EXPOSED TO DEPLETED URANIUM (DU).

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Gulf War veterans exposed to depleted uranium (DU) by "friendly fire" were studied for somatic cell genetic effects approximately ten years later. Urinary concentrations (urU) of uranium (U) were used to estimate current body burdens of DU. Some veterans harbor DU shrapnel visible on X-rays. Current mean age of the group is 35.1 ± 0.76 yr; all are male (56% Caucasian, 31% African American, 10% Hispanic and 3% "other"). The group was divided into high and low urU subgroups (> or < 10 mcg/gm creatinine). Mean frequencies of chromosome aberrations (CA) determined in peripheral blood T-lymphocytes by traditional methods were 0.01 ±0.005 and 0.02 ± 0.008 for the high and low urU groups, respectively, revealing no statistically significant difference and no DU effect (p=0.70). Mean frequencies of sister chromatid exchanges (SCE), however, were 6.35 ± 0.267 and 5.52 ± 0.182 for the high and low urU groups, respectively, showing a slight but statistically significant elevation in the high group (p = 0.03). *HPRT* mutant frequencies (MFs) in T-lymphocytes assessed by cloning assay ranged from 4.4 to 69.1 x 10-6 . There was a positive correlation between individual urU concentrations and MFs (r = 0.51) which remained statistically significant (p < 0.001) after adjusting for effects of age, cloning efficiency of the assay and smoking, thus revealing an unambiguous mutagenic effect of DU exposure. The lack of an increase in CA frequencies with an elevation of SCEs argues against ionizing radiation as the cause of the somatic cell genetic effects. It is reasonable to attribute the *HPRT* mutations, which could have either a radiation or chemical basis, to the latter. Chronic low-level high LET radiation could show this genotoxicity profile, however DU should provide only low intensity radiation. Characterization of molecular spectrum of the *HPRT* mutations will help assess these possibilities. Results suggest a need to more fully characterize DU genotoxicity and its public health implications.

1362 DEPLETED URANIUM METAL IMPLANTS ARE CARCINOGENIC IN RATS.

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The carcinogenicity of depleted uranium (DU) metal implants in muscle was determined in Wistar rats. Tissue reactions to DU implants were compared with implants of tantalum (Ta) metal and injections of Thorotrast® (Th®). Three sizes of DU fragments were surgically implanted, 5x5x1.5 mm, 2.5x2.5.1.5 mm or 2.0x1.0 mm diam. Four fragments were embedded in each rat, 2 in each thigh muscle. Other groups received Ta implants (5x5x1.5 mm) as controls for foreign-body materials, Th® injections *i.m.* as controls for radioactive materials, or sham surgery as negative controls. Fifty rats per group were observed for life span for the onset, incidence, and types of reactions at the site. At death, all major organ systems were microscopically examined for lesions. Radiographs of the DU implants showed marked corrosion on the surface as early as 3 mo. after surgery. Later, rarefactions in the radiographic profiles correlated with proliferative tissue reactions. Connective tissue capsules formed around the metal implants, but were much thicker around the DU. Soft tissue tumors arose from some capsules 16 to 20 mo. after implant. Over 90% were malignant. Tumor incidence was significantly increased around the larger squares of DU (18%) and the radioactive control, Th® (50%), when compared with the sham controls (0%). Incidence was slightly increased in rats with 2.5x2.5 mm DU squares (6%) and with 5x5 mm squares of Ta (4%). No tumors were seen in rats with the small DU pellets or in the sham controls. DU fragments of sufficient size cause localized proliferative reactions and soft tissue sarcomas in the muscles of rats that can be detected with radiography. Implanting materials *i.m.* in rats is a sensitive technique for detecting potentially carcinogenic materials in humans. These findings do not indicate, however, that DU fragments are necessarily carcinogenic in humans. Funding: US Army MRDC MIPR# KVFM5529 with US DOE, Coop. Agreement #DE-FC04-96AL76406. Research conducted using SOT criteria and in AAALAC-accredited facilities.

1363 RADIATION-INDUCED EFFECTS OF DEPLETED URANIUM *IN VITRO*: THE INVOLVEMENT OF BYSTANDERS AND INSTABILITY.

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Depleted uranium (DU) is a dense heavy metal used primarily in military applications. Published data from our laboratory have demonstrated that DU exposure *in vitro* to immortalized human osteoblast cells (HOS) is both neoplastically transforming and genotoxic. DU possesses both a radiological (alpha-particle) and chemical (metal) component. Since DU has a low-specific activity in comparison to natural uranium, it is not considered to be a significant radiological hazard. The potential contribution of radiation to DU-induced biological effects is unknown and the involvement of radiation in DU-induced biological effects could have significant implications for current risk estimates for internalized DU exposure. Several approaches were used to address this question. Using the same concentration of three uranyl nitrate compounds (isotopes) that have different uranium isotopic concentrations and therefore, different specific radioactivity, we examined their effect *in vitro* on neoplastic transformation, genotoxicity, mutagenicity, and induction of genomic instability. HOS cells were exposed to one of three-uranyl nitrate compounds (^{238}U-uranyl nitrate, specific activity 0.33 μCi/g; DU-uranyl nitrate, specific activity 0.44 μCi/g; and ^{235}U-uranyl nitrate, specific activity 2.2 μCi/g) delivered at a concentration of 50 μM for 24 hrs. Results showed, that at equal uranium concentration, there was a specific activity-dependent increase in neoplastic transformation frequency and other endpoints examined, suggesting that radiation plays a role in DU-induced biological effects *in vitro*. Lastly, using populations of DU-exposed, non DU-exposed cells, and a mixed population of DU-exposed and non DU-exposed cells, we provide the first evidence that cells that have taken up DU can induce a bystander transformation response in neighboring cells that neither took up DU nor were traversed by alpha particles (from DU).

1364 VANILLOID RECEPTORS: MEDIATORS OF RESPIRATORY INJURY.

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Vanilloid receptors (members of the superfamily of TRP non-voltage-gated cation channels) are the recently identified cation channels that are critical for sensory neuronal nociception to painful and tissue-damaging stimuli, including physicochemical (heat, cold, hypoosmolarity, and surface charge) and chemical (acids and capsaicinoids) insult. Cloning, expression, and characterization of a variety (at least six members) of related ion channel proteins, that are responsive to noxious environmental stimulants, have provided a cornucopia of molecular targets that mediate physiological responses. These receptors are highly expressed in dorsal root ganglia, and significant expression of several of the TRP proteins has also been demonstrated in respiratory tissues of animals and man. Activation of these respiratory receptors by a surprisingly diverse array of irritants, including particulate matter from ambient air, has been associated with toxic sequelae in the respiratory tract. However, the mechanisms responsible for receptor regulation, activation, and responsiveness in respiratory epithelial and neuronal cells have not been adequately elucidated. This workshop will present a highly focused, mechanistic evaluation of the chemical, biochemical, cellular, and toxicological factors that regulate vanilloid

receptor-mediated toxicities in the respiratory tract. Topics of the workshop will include structure/function characterization of TRPV receptors, human lung epithelial cell sensitization to capsaicinoids by over-expression of the recombinant human vanilloid receptor TRPV1, stimulation of respiratory sensory nerve reflex responses to inspired acidic aerosols in rodents, and TRPV1 receptor activation in human lung epithelial cells by particulate matter-mediated cytokine production that is correlated to surface charge. Mechanistic research into the activation and regulation of the vanilloid receptors may provide precise molecular descriptors of respiratory injury that can be used to achieve exciting new therapeutic modalities.

1365 TRPV ION CHANNEL PROTEINS: CANDIDATE MEDIATORS OF DIVERSE VISCERAL SENSORY PROCESSES.

M. J. CaterinaM. J. Caterina. *Biol. Chem. and Neurosc., Johns Hopkins University School of Medicine, Baltimore, MD.* Sponsor: G. Yost.

Among the molecules that detect irritant stimuli in cutaneous and visceral tissues is TRPV1 (VR1), a nonselective cation channel that can be activated by vanilloid compounds, protons, or heat, and is highly expressed in certain cutaneous and vagal sensory neurons. Recently, TRPV1 has also been found to be expressed in visceral epithelia, where it may mediate the indirect detection of irritants by afferent nerve fibers. At least six homologous TRPV genes have been identified. One, TRPV4, is highly expressed within tracheal epithelial cells and submucosal glands. This channel is unresponsive to vanilloid compounds or protons, but can be activated by certain other physico-chemical stimuli, such as hypoosmolarity. These findings suggest a number of possible roles for TRPV family members in physiological and pathophysiological respiratory function. Supported by grants from the American Cancer Society (RGS-01-063-01-CSM), The W. M. Keck Foundation, The Searle Scholars Program, and The Beckman Foundation

1366 ENHANCED CYTOTOXICITY OF CAPSAICINOIDS TO TRPV1-OVEREXPRESSING HUMAN LUNG CELLS.

G. S. Yost and C. A. Reilly. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

Capsaicinoids are the active components of pepper sprays, and exposure to these sprays have been associated with respiratory failure and death in animals and people. Although inhalation of pepper spray extracts in rats causes acute inflammation and damage to respiratory cells, the mechanisms responsible for cellular damage have not been elucidated. Recent characterization of irritant receptors, including the human vanilloid receptor type-1 (VR1 or TRPV1) that is a capsaicin-responsive cation channel receptor, have provided molecular targets that could be responsible for respiratory cell death. *In vitro* cytotoxicity assays demonstrated that human lung cells (BEAS-2B and A549) were more susceptible to necrotic cell death than liver (HepG2) cells. Transcription of TRPV1 was demonstrated in all three of these cell lines, and the relative transcript levels were correlated to cellular susceptibility to capsaicin. Surprisingly, the prototypical antagonists of this receptor were cytotoxic to BEAS-2B cells, and the antagonists did not ameliorate capsaicinoid-induced damage. Conversely, TRPV1 antagonists or calcium chelation ablated IL-6 production after capsaicin exposure. Recombinant human TRPV1 was cloned and stably over-expressed in a new BEAS-2B cell line, and these cells exhibited dramatically increased (100-fold) cellular susceptibility to capsaicin, and an apoptotic mechanism of cell death. The cytotoxicity of capsaicin to TRPV1 over-expressing cells was not inhibited by the antagonists, or by treatments that modified extracellular calcium. Therefore, capsaicin activated the TRPV1 receptor in human bronchiolar epithelial cells to produce the calcium-dependent release of cytokines and, conversely, calcium-independent cell death. Thus, capsaicinoid-induced respiratory toxicities may be mediated by activation of vanilloid receptors that are expressed in epithelial cells, through a process that is independent from cytokine production. Supported by the Colgate-Palmolive Post-Doctoral Fellowship in *In Vitro* Toxicology and a Department of Commerce Contract (#60NANBOD0006)

1367 IMMEDIATE RESPIRATORY TRACT RESPONSES TO INSPIRED IRRITANTS: SENSORY NERVES AND VANILLOID RECEPTORS.

J. B. Morris and P. T. Symanowicz. *Toxicology Program and Pulmonary Research Consortium, University of Connecticut, Storrs, CT.*

Stimulation of respiratory tract sensory nerves produces a range of local and centrally mediated reflex responses. The local responses, mediated *via* local neuropeptide release and/or parasympathetic nerve activation, include mucus secretion, vasodilation, and neurogenic edema. Centrally mediated responses include changes in breathing pattern and cough. The receptors responsible for irritant detection and initiation of these responses are not known, nor is it known if irritants stimulate nerves directly or indirectly through paracrine mediators released by epithelial or other cells. Numerous *in vitro* studies have documented an important role for the vanilloid receptor in acid-induced stimulation of sensory nerves. *In vitro* studies in our laboratory have shown that the potent cigarette smoke irritant acrolein can also directly stimulate sensory nerves. *In vivo* studies are focused on examining the role of vanilloid receptors in initiating respiratory reflex responses to inspired irritants. Inhalation exposure to the known vanilloid receptor agonist capsaicin results in the same spectra of responses as that induced by the inspired irritants acrolein, acetic acid or sulfuric acid. More importantly, the reflex responses to inspired sulfuric acid aerosol in the rat are diminished by pretreatment with the vanilloid receptor antagonist ruthenium red. Similarly, pretreatment with this antagonist decreases the sensory nerve-mediated respiratory tract responses to inspired acetic acid in the mouse. These results suggest the vanilloid receptor plays a key role in detection and initiation of respiratory tract responses to inspired irritants. This work supported by the NIH NIEHS R01-08765 and the University of Connecticut Pulmonary Research Consortium.

1368 THE SURFACE CHARGE OF PARTICULATE MATTER (PM) ACTIVATES VANILLOID (VR1) RECEPTORS.

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Exposure to PM produces a uniform degree of mortality in exposed populations, in spite of its diverse sources. This suggests a common mechanism of action. We have shown that PM initiates airway inflammation through activation of VR1 receptors carried on human respiratory epithelial cells (BEAS-2B). To determine what physicochemical feature of PM was culpable in this activation, the surface charges (i.e., zeta potentials) were measured on PM particles obtained from various sources. When measured in neutral pH vehicles, these PM particles carried zeta potentials of varying electronegativity. On exposure to BEAS-2B epithelial cells, each PM (60 mg/cm2) stimulated a differential release of IL-6. The zeta potential of individual PM samples significantly correlated (r2 = 0.98) with its respective level of IL-6 release, indicating that the surface charge carried on PM particles contributed to IL-6 release from target respiratory cells. To test whether surface charge itself could activate VR1 receptors, synthetic polystyrene micelles (SPM), charged with individual chemical groups (e.g., COOH-, SO2-, NH2+), were obtained and measured for their zeta potential. These SPM were next exposed to BEAS-2B cells and the resulting biological changes (e.g., membrane potential, intracellular calcium increases, IL-6 release) were measured. IL-6 release stimulated by the COOH- and SO2-charged SPM could be significantly depressed by capsazepine, an inhibitor of VR1 receptors. The rank order of the SPM's zeta potential and their respective IL-6 release also correlated. RT-PCR performed on SPM exposed BEAS-2B cell indicated that VR1-receptor transcript was only increased in BEAS-2B cells exposed to COOH- and SO2- charged SPM . Together, these data suggest that the negative surface charge carried on some colloidal particles is able to initiate airway inflammation through activation of the VR1 receptor and offers a common mechanism of action to explain PM toxicity.

1369 A DNA REPAIR ROLE FOR RECQ AND TOPOISOMERASES III AND IV IN *E. COLI*.

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Across the domains of life, genetic data imply that topoisomerase III may have a fundamental function in maintaining genomic integrity. Cells lacking topoisomerase III display a number of DNA instabilities including a mutator phenotype, deletion of short repetitive elements, and a failure to segregate recombinant products of meiosis. Alterations in human RecQ-family DNA helicases, which interact with topoisomerase III, have been linked to the Bloom, Rothmund-Thomson, and Werner Syndromes. Patients with these inherited disorders have elevated rates of cancer incidence and cells from these individuals show genomic instability. Despite these findings, the role of topoisomerase III is unknown for any organism. Using genetic techniques, we found that the genes that encode topoisomerase III and temperature sensitive mutants of topoisomerase IV are synthetically lethal at the permissive temperature in E. coli. Fluorescence microscopy data revealed that the double mutant is defective in DNA segregation. The overproduction of topoisomerase III rescued both the lethality of temperature sensitive topoisomerase IV mutants at the non-permissive temperature and the DNA segregation defects. Either recQ or recA null mutations rescued the synthetic lethality of the double topoisomerase mutant. We suggest a model whereby RecA and RecQ initiate a recombinational repair of DNA that is resolved by the topoisomerases. Failure to resolve is lethal. Finally, we found that the deletion of the gene that encodes topoisomerase III and a ruvC null mutation are synthetically lethal, which suggests that topoisomerase III disentangles Holliday junctions to maintain genomic stability.

1370 MECHANISMS OF DNA DAMAGE-INDUCED MUTAGENESIS.

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Replication of damaged DNA (translesion synthesis) requires specialized proteins and frequently leads to mutations. In the yeast S. cerevisiae, the polymerase zeta (Pol zeta) mutagenesis pathway was originally identified as the major mechanism of UV-induced mutagenesis. The Pol zeta pathway requires at least Rad6, Rad18, Rev1, Rev3, and Rev7 proteins. Based on the yeast genes, we cloned the human RAD18, REV1, and REV3 genes. Human REV1 gene codes for a dCMP transferase, which is able to respond to several types of DNA lesions by inserting a C opposite the lesion. Human Rev1 protein may thus contribute to dCMP insertion opposite many types of DNA damage during translesion synthesis. The REV3 gene codes for the catalytic subunit of DNA Pol zeta. The role of Pol zeta in translesion synthesis was examined using the yeast model system. Purified yeast Pol zeta performed limited translesion synthesis opposite several lesions. Furthermore, yeast Pol zeta catalyzed extension DNA synthesis from primers annealed opposite these lesions. Our results led to a dual-function model for Pol zeta. One function of Pol zeta is to catalyze nucleotide insertion opposite the lesion and subsequently extend DNA synthesis past the lesion. The second function of Pol zeta is to catalyze the extension DNA synthesis following nucleotide insertion opposite the lesion by other polymerases. Recently, the Y family of DNA polymerases was discovered. The Y family DNA polymerases are capable of DNA synthesis opposite various base lesions with either error-free or error-prone consequences, depending on the specific lesion and the specific polymerase. Our biochemical studies suggest that the Y family polymerases may be functionally connected to the Pol zeta pathway through a two-step two-polymerase mechanism in translesion synthesis of many types of DNA lesions. As indicated by genetic experiments, many types of DNA damage would not be able to induced mutations without the Pol zeta pathway. Therefore, the Pol zeta pathway plays a critical role in DNA damaged-induced mutagenesis.

1371 A REQUIREMENT FOR REPLICATION IN ACTIVATION OF THE ATR-DEPENDENT DNA DAMAGE CHECKPOINT.

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To maintain genomic stability, cells depend on the DNA damage and replication checkpoints. ATR (ATM and Rad3-related) and the proteins in the Rad1 complex, Rad1, Hus1 and Rad9, are believed to be upstream components of these checkpoints. It is not understood how these proteins respond to a diverse range of DNA damaging agents, including ultraviolet radiation, ionizing radiation and alkylating agents. We have investigated the involvement of DNA replication in activation of the DNA damage checkpoint in Xenopus egg extracts. We find that DNA damage caused by ultraviolet radiation or methyl methanesulfonate slows replication in a checkpoint-independent manner and is accompanied by the accumulation on chromatin of ATR and components of the Rad1 complex. We also show that the replication proteins RPA and Pol α accumulate on chromatin following DNA damage, suggesting that single-stranded DNA may accumulate. Importantly, we find that the accumulation of ATR, Rad1, RPA and Pol α is blocked by geminin, suggesting that their binding is dependent on the initiation of DNA replication. In addition, several components of the Rad1 complex become phosphorylated after activation of this checkpoint. These phosphorylations are dependent on the kinase activity of ATR and on initiation of DNA replication, but they are not required for the association of the Rad1 complex with chromatin. Finally, we show that the damage-induced phosphorylation of Chk1 and checkpoint arrest are abrogated when replication is inhibited. Taken together, these data suggest that replication is required for activation of the DNA damage checkpoint and may provide a unifying model for ATR activation by diverse lesions during S phase.

1372 I) MECHANISMS OF NITRIC OXIDE-INDUCED HOMOLOGOUS RECOMBINATION IN *E. COLI* AND II) FLUORESCENCE DETECTION OF HOMOLOGOUS RECOMBINATION IN MICE.

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Homology-directed repair defends somatic cells against potentially toxic and mutagenic DNA lesions. Although homology directed repair is generally a conservative process, mitotic homologous recombination can lead to deletions and loss of heterozygosity. We have investigated the molecular basis of damage-induced homologous recombination and developed tools for studying homologous recombination in mammals. Nitric oxide (NO·) is involved in neurotransmission, inflammation and many other biological processes. Exposure of cells to NO· leads to a wide variety of DNA lesions. Using a genetic approach, we have determined the relative contribution of excision and recombination pathways in defending *E. coli* against NO· toxicity. We found that recombination-deficient cells (*recBCD*) are very sensitive to NO· toxicity. Although DNA glycosylases that initiate the base excision repair (BER) pathway do not influence survival in wild type cells, when certain DNA glycosylases (Fpg and Ung) are inactivated in *recBCD* cells, survival is significantly enhanced. We present a model in which accumulated BER intermediates induce homologous recombination. These studies shed light on the underlying mechanism of NO·-induced homologous recombination. For studies of homologous recombination in mammals, we have developed a mouse model in which mitotic homologous recombination events can be quantified *via* a fluorescent signal. The FYDR (fluorescent yellow direct repeat) mice carry two mutant copies of an expression cassette for the enhanced yellow fluorescent protein (EYFP). Recombination frequency can be measured by flow cytometry of primary cells isolated from FYDR mice. When challenged with mitomycin C, we observe a 15 fold increase in the frequency of recombinant cells. This new mouse model can be used for dissecting genetic and environmental factors that put mammals at risk of deleterious homologous recombination events.

1373 MUTAGENESIS AND DNA STRAND SCISSION AT 2-DEOXYRIBONOLACTONE OXIDATIVE DNA DAMAGE LESIONS.

T. L. Sheppard and Y. Zheng. *Department of Chemistry, Northwestern University, Evanston, IL.* Sponsor: J. Kramarik.

DNA damage by toxic agents, including ionizing radiation and oxidizing species, leads to chemical alteration of the genome. DNA damage reactions may give rise to genetic mutations and DNA strand breaks, which represent molecular mechanisms for aging and the induction of human diseases. In particular, DNA damage by reactive oxygen species (ROS) presents a major pathway for the introduction of premutagenic lesions in genomic DNA. One form of oxidative DNA damage is the 2-deoxyribonolactone (or oxidized abasic site) lesion, which results from oxidation of the C-1 position of the sugar unit of a DNA nucleotide. Although the mechanisms that lead to these oxidized sites have been established, the biochemical consequences of C-1 damage remain unclear. This study reports the measurement of the biological half-life of these lesions, the characterization of the products of DNA strand scission at the damage sites, and the assessment of the mutagenic effects of lactone modifications. This laboratory previously developed a method for the selective photochemical generation of lactone lesions within DNA oligonucleotides. Synthetic lactone-modified DNA was prepared and used to measure a half-life of 21-50 hours for DNA strand scission at the lesion under simulated physiological conditions. The products of DNA strand scission at the lesion site were unambiguously characterized by gel electrophoresis and MALDI-TOF mass spectrometry. The mutagenicity of the oxidized abasic site was assayed using DNA polymerase primer extension assays with DNA templates containing the lactone lesion. The lactone lesion stalls the polymerase at the damage site and displays A-rule mutagenesis behavior. In summary, the deoxyribonolactone lesion displays a biological half-life of approximately one day and acts as a non-instructive mutagenic site during DNA replication. The significant lifetime of this lesion suggests that DNA repair will be required to avoid mutagenesis at lactone sites during replication or transcription. Accordingly, the oxidized abasic site may represent a genotoxic lesion resulting from oxidative DNA damage.

1374 MODULATION OF AROMATASE ACTIVITY IN PRIMARY CULTURE OF HUMAN MAMMARY FIBROBLASTS.

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Aromatase activity in human mammary tissue is mainly located in fibroblasts and a positive feedback loop is thought to be responsible for accelerating estrogen-responsive breast tumor growth through stimulation of estrogen synthesis. Tumor cells secrete factors such as prostaglandin E2 (PGE2) and interleukin-6 (IL-6) that are able to upregulate aromatase gene expression in tumor-surrounding fibroblasts. Various *in vitro* studies have shown that pharmacological agents and xenobiotics are able to interfere with the aromatase enzyme. In this study we examined the possibility of using primary human mammary fibroblasts as an *in vitro* screening tool for

compounds that may be involved in the development of estrogen-dependent tumors. Primary human mammary fibroblasts were exposed for 24h to 100 μM 8-Br-cAMP, 1 μM PGE2 or 100 nM dexamethasone (DEX), which resulted in a 3-fold, 43-fold or 600-fold increase in aromatase activity, respectively. Since the synthetic glucocorticoid DEX upregulated aromatase activity in a highly effective manner, compounds with anti-glucocorticoid properties would be expected to decrease dexamethasone induced aromatase activity. A 24h-exposure of fibroblasts to 100 nM DEX, together with 0.1, 1 or 10 μM 3-$MeSO_2$-CB-101, resulted in a 22, 26 or 55% reduction of aromatase activity. Cytotoxicity (20%) occurred only at the highest tested concentration. We are in the process of testing several persistent methylsulfonated metabolites of PCBs and DDT that are known to have anti-glucocorticoid properties.

1375 PRENATAL EXPOSURE TO THE FUNGICIDE PROCHLORAZ ALTERS THE ONSET OF PARTURITION IN THE DAM AND SEXUAL DIFFERENTIATION IN MALE RAT OFFSPRING.

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Prochloraz (PZ) is an imidazole fungicide reported to inhibit aromatase, as well as estrogen and androgen receptor mediated activities *in vitro*. Our laboratory demonstrated that PZ exposure from gestational day (GD) 14-18 decreased testosterone while increasing progesterone in fetal rat testes. Thus, PZ is interesting in that it may alter endocrine function through diverse mechanisms. We examined mechanisms of PZ action *in vitro* and conducted a preliminary study to investigate effects of gestational exposure on Sprague-Dawley dams and their offspring. *In vitro*, PZ displayed anti-androgenic activity in MDA-kb2 cells containing endogenous androgen receptor (AR) and stably transfected with a MMTV-luc reporter. Treatment above 1μM caused a dose-dependent inhibition of DHT-induced luc expression, and was cytotoxic at 100μM. Prochloraz inhibited R1881 binding to the rat AR (IC50 approx 60μM). Daily PZ administration (gavage) to pregnant dams from GD 14-18 at doses of 62.5, 125, 250 and 500 mg/kg bodyweight/day inhibited maternal weight gain in the 500 mg/kg group. Pup delivery was delayed by up to 30 hours in some high-dose treated dams and some pups were stillborn at 125 mg/kg/d and higher. No pups in the 500 mg/kg group survived to postnatal day 13. Prenatal PZ treatment reduced anogenital distance in 2 day old pups by about 8% in the highest dosage group (NS), and induced female-like areolas in male offspring at frequencies of 33%, 71% and 100% in 62.5, 125 and 250 mg/kg groups, respectively. Some males of the 250 mg/kg treatment group showed phallus abnormalities and delayed preputial separation. A larger scale follow-up is underway. Our results are consistent with published *in vitro* PZ effects, and indicate that PZ displays several forms of endocrine activity, resulting in delayed parturition and induction of male reproductive tract malformations. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1376 TRANSGENERATIONAL EFFECTS OF DI(2-ETHYLHEXYL) PHTHALATE IN THE MALE RAT.

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Phthalate esters such as di(2-ethylhexyl)- (DEHP), di(n-butyl)-, and benzyl butyl phthalate are widely used plasticizers that have been implicated in adverse developmental reproductive effects. They alter sexual differentiation by altering fetal Leydig cell development and hormone synthesis which in turn, results in abnormalities of the testis, gubernacular ligaments, epididymis and other androgen-dependent tissues. In spite of these concerns, studies of DEHP including relatively low dosage levels with developmental exposure and examination of sensitive endpoints in an adequate number of adult offspring have not been published. The current study was designed to begin to address these data gaps. Pregnant SD rats were dosed by gavage with DEHP from gestational day (GD) 8 to day 17 of lactation with 0, 11, 33, 100 or 300 mg/kg/d. On GD 18, two dams per group were euthanized and amniotic fluid was collected for analysis of MEHP levels. In half of the males (PUB cohort), dosing was continued from 18 to 63-65 days of age while the rest (IUL cohort) were not dosed directly. The PUB cohort was necropsied at 63-65 days of age while the IUL cohort was necropsied at full maturity. The 300 mg/kg/d, IUL group displayed permanent reductions in anogenital distance and reproductive organ weights, and permanent nipples. In the high dose group, about 25% of the males displayed testicular and/or epididymal abnormalities. These abnormalities also were displayed at a low incidence in the 11, 33 and 100 mg/kg/d dose groups along with subtle reductions in reproductive organ weights. In the PUB group, puberty (100 and 300) and reproductive organ weights (300 mg) were affected. Liver and adrenal weights were affected in all dose groups including 11 mg/kg/d (by one-tailed t-test), the lowest dose tested. Amniotic fluid levels of MEHP are being analyzed and a second study is reexaming the low dose effects of IUL DEHP exposure. Abstract of a proposed presentation and does not necessarily reflect EPA, CDC or CIIT policies.

1377 ANDROGEN RECEPTOR MRNA IS UPREGULATED BY ESTROGEN IN MOUSE PROSTATE PRIMARY CELL CULTURE.

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Prostate development is dependent upon androgen stimulation of mesenchymal tissue, which in turn directs the proliferation and differentiation of epithelial buds. The androgen-responsiveness of developing prostate tissue is influenced by estrogens. Low, physiological doses of estrogens and high, pharmacological doses of estrogens have opposite effects on prostate development. Small increases in estrogen exposure during development result in permanent increases in prostate size and androgen receptor (AR) mRNA and protein expression, while high doses of estrogens result in a decrease in prostate size. We have developed an *in vitro* model system capable of reproducing the low dose estrogen-induced upregulation of AR mRNA in a primary culture of mesenchyme cells collected from the male fetal mouse urogenital sinus during the initial formation of the prostate gland. Immunofluorescent staining for the mesenchymal cell marker vimentin has confirmed that these primary cultures contain a homogenous population of mesenchymal cells. Androgen receptor mRNA expression in the cells was quantified by real-time RT-PCR. Our results show a dose-dependent increase in AR mRNA expression with estrogen treatment. The estrogen-induced increase in AR mRNA expression is eliminated by concurrent treatment with an antiestrogen. In contrast to *in vivo* results, the dose-response curve for estradiol-induced AR mRNA expression is monotonic over six orders of magnitude. These results suggest that the low-dose effects of estradiol on prostate development are estrogen-receptor-dependent, are largely mediated through local effects on prostate cells, and can be modeled in a primary cell culture system. The high-dose effects of estradiol cannot be modeled in a primary cell culture system, and may be mediated through systemic effects of pharmacological doses of estrogens. Future work will focus on the interactions among nuclear receptors in prostate development and on the effects of environmental estrogens on prostate development.

1378 TRUNCATED ESTROGEN RECEPTOR PRODUCT (TERP-1) EXPRESSION IN THE RAT VAGINA.

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During the estrous cycle changing levels of circulating estrogens play a critical role in vaginal growth, epithelial morphogenesis, cytodifferentiation, and secretory activity. These estrogenic effects require the estrogen receptor alpha (ERalpha). The ERalpha-ligand complex subsequently interacts with estrogen response elements, resulting in enhancement or suppression of gene expression. A tissue-specific ERalpha variant, so called truncated ER product-1 (TERP-1) is functional active and able to modulate the trans-activating activity of ERalpha. TERP-1 expression is hormonally regulated and specifically modulated by estrogens during the estrous cycle. TERP-1 has been suggested not be translated to make significant levels of protein outside the male and female pituitary gland. We, therefore, examined TERP-1 expression in the rat vagina. In addition, we tested the hypothesis whether *in utero* exposure of a xenoestrogen can affect TERP-1 expression in the vagina of the offspring, because in previous studies (a) TERP-1 expression has been regulated by neonatal estrogen treatment and (b) striking morphological changes were observed in the vagina of post-pubertal offspring after exposure with different estrogens. For the first time, we can demonstrate on mRNA- and protein-level prepubertal- and cycle-dependent TERP-1 mRNA- and protein-expression in the rat vagina. We show that TERP-1 expression is increased in control animals from the neonatal to the prepubertal period and during diestrus stage when compared to the estrous. Furthermore, TERP-1 expression is increased during estrus in the vagina of female offspring *in utero* exposed to 0.1 mg bisphenol A (an estrogenic chemical) / kg bodyweight when compared to the control group. The ability of TERP-1 to both enhance and inhibit ERalpha-dependent activity leads to the suggestion that TERP-1 expression may play a physiological role in estrogens feedback of vaginal epithelium.

1379 PHYTOCHEMICALS CAN ALTER CATECHOL-O-METHYLTRANSFERASE (COMT) ACTIVITY IN CYTOSOLIC FRACTIONS FROM HUMAN MAMMARY TISSUES.

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Phytochemicals are natural dietary constituents from fruits and vegetables. Although phytochemicals can exert (weak) estrogenic actions, they are suggested to protect against breast cancer. Some of these phytochemicals are known to affect estrogen metabolizing enzymes. In breast tissue, estradiol is mainly metabolized to 2- and 4-hydroxyestradiol (2-OH-E2 and 4-OH-E2). In several studies, 4-OH-E2, but not 2-OH-E2, acted as a carcinogen. COMT converses the hydroxyestrogens to their inactive methoxy derivatives (2-MeO-E2 and 4-MeO-E2). In this study we investigated the effects of quercetin (Q), catechin (Ca), genistein (G) and chrysin (Ch) on COMT activity in cytosolic fractions of healthy human mammary tissues. The potency of these phytochemicals to affect estrogen metabolism was compared with that of Ro 41-0960, a known COMT inhibitor. Tissues from reduction mammaplasty were homogenized and cytosolic fractions were prepared through ultracentrifugation. Cytosolic protein was incubated with various concentrations of a phytochemical and equimolar concentrations (7.5 μM) of 2-OH-E2 and 4-OH-E2. Methoxyestrogens were extracted with dichloromethane and analysed by GC/MS. The catalytic efficiency of COMT was higher for 2-MeO-E2 formation (Vmax/Km; 3.0/5.0 = 0.6) than for 4-MeO-E2 formation (Vmax/Km; 1.3/9.5 = 0.1). At 7.5 μM 2- and 4-OH-E2, the constitutive activity was 4.2 ± 1.3 and 0.94 ± 0.16 pmol/min/mg protein for 2- and 4-MeO-E2 formation, respectively. Ro 41-0960, Q and Ca concentration-dependently inhibited COMT activity with EC50 values of 0.03, 0.4 and 1.2 μM, respectively, for both 2- and 4-MeO-E2 formation. G and Ch slightly reduced COMT activity at the highest concentration tested (30 μM). These data show that phytochemicals with a catechol structure have the potency to reduce COMT activity in mammary tissues which might reduce the clearance of potentially mutagenic estradiol metabolites.

1380 REGULATION OF METHOXYCHLOR-INDUCED OXIDANT PRODUCTION BY CHOLINERGIC SIGNALING.

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The pesticide methoxychlor, an organochlorine derivative of DDT, has been widely used as a substitute for DTT due to its lower toxicity and diminished environmental persistence. Recently, reports of endocrine-modulating activities induced by methoxychlor have engendered public concern about this widely used agent. Although the association between human exposure to methoxychlor and hormone-disruptive activities mediating disorders of endocrine organs remains unclear, methoxychlor has been found to exert adverse effects on fertility, early pregnancy, and *in utero* development, in wildlife and laboratory animals. Findings indicating that exposure to methoxychlor can induce oxidative stress point to a role for reactive oxygen species (ROS) in the pathological effects of exposure to this agent. Therefore we tested the effects of methoxychlor on oxidant levels in mouse and human endothelial cells. Methoxychlor was found to be a potent inducer of ROS in these cells. ROS production was dependent on the dose of methoxychlor in a range of 0.01 -1 μM, and inhibited by pre-treatment of the cells with N-acetyl-L-cysteine, a stimulator of glutathione synthesis. In experiments designed to investigate the effects of methoxychlor on endothelial function, we found that methoxychlor induced ROS production was enhanced 8-9 fold by the acetylcholine receptor agonist carbachol. Furthermore, in the presence of α-bungarotoxin, a nicotinic acetylcholine receptor antagonist, methoxychlor-induced ROS production was dramatically inhibited. In addition, methoxychlor induced expression of the type II major histocompatability antigen (Ia), an effect that was also facilitated by carbachol and inhibited by α-bungarotoxin. These data indicate that exposure to methoxychlor results in the production of ROS and expression of Ia antigen, and that this effect can be modulated by cholinergic signals. We speculate that interactions between cholinergic signaling and methoxychlor-induced ROS production mediate, in part, the neurendocrine abnormalities induced by methoxychlor in developing animals. Supported by ES06897 and CA93798

1381 STEROID AND THYROID HORMONAL RECEPTOR GENE TRANSCRIPTION ASSAY AND ONE-GENERATION REPRODUCTION STUDY OF BUTYLATED HYDROXYANISOLE.

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Butylated hydroxyanisol (BHA) has been widely used as an antioxidant to preserve and stabilize the freshness of food and feed. It is generally recognized as safe however it is classified as suspect carcinogen and endocrine disrupter. We investigated endocrine disrupting effects of BHA in steroid and thyroid hormone receptor gene transcription assay and in rat one-generation reproduction study. BHA induced weak transcription activity by binding with androgen receptor as induction ratio (IR) compared to testosterone was 1.37 % at 10 mM while it induced high transcription activity by binding with thyroid receptor as IR compared to T3 was 85% at 10 mM. In addition, BHA showed agonistic reaction with T3. In one-generation study, BHA induced increase of relative organ weight of liver, kidney, adrenal gland and thyroid gland and decrease of mating rate, cohabitation period for conception and T4 in serum in parent generation when treated orally for 7 weeks in male rats and for around 10 weeks in female rats. BHA also aroused increase of the relative organ weights of liver, kidney, adrenal gland, brain but decrease of those of vagina, spleen, and prostate gland. In one-generation rats, sex ratio of male was decreased and the anogenital distances were shortened and vaginal patency and preputial seperation were observed later than control group. Also, BHA decreased sperm motility and number and the width and length of sperm head. T4 in serum was lower than control. Conclusively, BHA is disruptor of androgen and thyroid hormonal system *in vitro* and *in vivo* study. NOAEL and ADI of BHA according to the results from one-generation study were estimated as 10 mg/kg B.W. and 0.1 mg/kg B.W./day, respectively.

1382 MOLECULAR CHARACTERIZATION OF THYROID TOXICITY: ANCHORING GLOBAL GENE EXPRESSION PROFILES WITH BIOCHEMICAL AND PATHOLOGICAL ENDPOINTS.

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Thyroid toxicity in rodents caused by a large and diverse set of xenobiotics is commonly observed as a potentially adverse effect. Hypersecretion of thyroid stimulating hormone following disruption of the hypothalamic-pituitary-thyroid axis has been recognized as a common mode of action for the incidence of proliferative lesions in thyroid follicular cells. Organic iodides have been shown to induce thyroid hypertrophy and increase alterations in colloid in rats, although the mechanism involved in this toxicity is unknown. Organic iodides increase hepatic enzymes and release free iodide into the circulating blood. It is unknown whether these and/or some other effect(s) on the thyroid cause toxicity. To evaluate the effect that free iodide has on the thyroid, rats were exposed for 2 weeks by daily gavage to sodium iodide (NaI), phenobarbitol (PB), or propylthiouracil (PTU). PB and PTU were used as positive control thyroid toxicants to compare the effects of compounds with alternative mechanisms (increased thyroid hormone metabolism and decreased thyroid hormone synthesis, respectively). Adult male rats, (~8 weeks of age), were dosed with 0.1, 1, 10, or 100 mg/kg/day NaI; 100 mg/kg/day PB; or 10 mg/kg/day PTU. Thyroid histopathology, liver biochemistry, serum hormones, serum iodide, and thyroid gene expression parameters were analyzed. Mean final body weights were significantly decreased (91% of control) in rats treated with PTU. Relative liver weights were significantly increased in rats treated with NaI at 10 and 100 mg/kg/day and in PB-treated rats (108, 113, and 144% of control, respectively), and were significantly decreased in PTU-treated rats (92% of control). Relative thyroid weights were significantly increased in PTU-treated rats (300% of control) only. Follicular cell hypertrophy and pale-staining colloid was present in PB-treated rats and more severe hypertrophy/colloid changes along with diffuse hyperplasia were present in PTU-treated rats. Differential transcript profiles were detected with rat Affymetrix gene chips.

1383 OCT-1: A KEY PLAYER IN VASCULAR BIOLOGY - IMPLICATIONS FOR CARDIOVASCULAR TOXICITY.

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High levels of oxidized LDL lead to vascular injury, but its mechanism of action is poorly understood. To become available oxLDL particles are internalized *via* the lectin-like oxLDL receptor and intracellular available oxLDL may trigger endothelial toxicity and vascular dysfunction by, as yet, unknown mechanisms. Previously, we demonstrated endothelial dysfunction to be linked to activation of the transcriptional repressor OCT-1 (Thum and Borlak, 2000). Here we test our hypothe-

sis of OCT-1 driven repression of genes upon oxLDL induced injury. We thus treated cultured human endothelial cells with ascending doses of oxidized LDL (10 microgram/ml - 100 microgram/ml) and demonstrate a dose dependent increase in intracellular radical oxygen production. This was accompanied by reduced levels of nitric oxide and repression of endothelial NO synthetase (eNOS) and other major cytochrome P450 monooxygenase activities. Importantly, OCT-1 gene expression was increased in a dose-dependent fashion (up to 5-fold) and OCT-1 binding to the regulatory elements of responsive genes may provide a mechanism of endothelial gene silencing. Electro-mobility shifts are now on the way to ascertain the proposed OCT-1 / DNA interaction. Reference: Thum T, Haverich A, Borlak J. Cellular dedifferentiation of endothelium is linked to activation and silencing of certain nuclear transcription factors: implications for endothelial dysfunction and vascular biology. FASEB J. 2000 Apr;14(5):740-51. This work is supported by Grants from the Lower Saxony Ministry of Science and Culture and the German Ministry of Science and Education (BMBF).

1384 ARTERIAL CARCINOGEN METABOLISM COULD INITIATE THE ACCELERATED ATHEROSCLEROSIS SEEN IN SMOKERS.

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HYPOTHESIS: The accelerated atherosclerosis seen in smokers is initiated by the toxic products formed during the arterial metabolism of the 2 major carcinogens of tobacco smoke, the polycyclic aromatic hydrocarbons (PAH)s and N-nitrosoamines (NNA)s. BACKGROUND: A number of laboratories have reported that arteries from various animals metabolize the PAHs to form the ultimate carcinogens. These reactions are catalyzed by cytochrome P450s, CYP1A1 and 1B1. The 1A1 is present in the aortas of various animal after PAH induction. No studies have reported the presence of the P450s catalyzing NNAs activation, CYP2A6 and 2E1. OBJECTIVES: We sought to determine whether these P450s are present in human arterial walls. METHODS: We prepared microsomes by standard procedures from the arteries of patients undergoing abdominal plasties. The P450s were determined by immunoblotting after Enhanced Chemoluminescence detection. RESULTS: We obtained 125 ug of microsomal protein from each artery. All 4 CYPs were observed at concentrations comparable to those found in hepatic microsomes after PAH induction. SIGNIFICANCE: These data support the hypothesis that the accelerated atherosclerosis seen in smokers is due to the metabolism of the PAHs and NNAs in the arterial wall leading to toxic injury and initiation of the accelerated atherosclerosis seen in smokers. These observations may have broad implications for the prevention of this disease process.

CYP 1A1 1B1 2A6 2E1	1A1	1B1	2A6	2E1
pmol/mg protein	4	14	30	90

1385 REGULATION OF ACTIVATOR PROTEIN-1 (AP-1) BY 8-ISO-PGE2 IN A THROMBOXANE A2 RECEPTOR-DEPENDENT AND -INDEPENDENT MANNER.

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The thromboxane A 2 (TXA2) receptor (TP) is represented by two alternatively spliced isoforms, termed the platelet/placental (TP-P) and endothelial (TP-E) type receptors. Experimental evidence suggests that TP isoforms may be regulated by novel ligands termed the isoprostanes, which paradoxically act as TP agonists in smooth muscle and TP antagonists in platelet preparations. Here we have investigated whether prototypical isoprostanes (8-iso-PGF2alpha and 8-iso-PGE2) regulate the activity of TP isoforms expressed in Chinese Hamster Ovary (CHO) cells using activator protein-1 (AP-1)-luciferase activity as a reporter. AP-1-luciferase activity was increased by a TP agonist (U46619) in CHO cells transfected with the human TP-P and TP-E receptors and this response was fully inhibited by TP antagonists (ISAP, SQ29, 548). AP-1-luciferase activity was potently (nM) increased by 8-iso-PGE2 in CHO TP-P and TP-E cells, and this response was partially inhibited by cotreatment of cells with TP antagonists, while 8-iso-PGF2alpha was without effect. Cyclooxygenase inhibitors did not abolish 8-iso-PGE2 mediated AP-1-luciferase activity, indicating that this response is not dependent on de novo TXA2 biosynthesis. Interestingly, 8-iso-PGE2-mediated AP-1-luciferase activity was near maximal in naive cells between 1-10 nM concentrations, and this response was not inhibited by TP antagonist or reproduced by agonists for TP or EP1/EP3 receptors. These observations (1) support a role for novel ligands in the regulation of TP-dependent signaling, (2) indicate that TP-P and TP-E couple to AP-1, (3) provide further evidence that isoprostanes function as TP agonists in a cell-type specific fashion, and (4) indicate that additional targets regulated by 8-iso-PGE2 couple to AP-1.

1386 HYPERTENSION IN ARYL HYDROCARBON RECEPTOR NULL MICE CORRELATES WITH PLASMA ENDOTHELIN-1, BUT NOT ANGIOTENSIN II.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor known to mediate the toxicity of environmental pollutants, such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. The role of the AhR in development and physiological homeostasis is uncertain; however, functional inactivation as in AhR null mice results in progressive cardiac hypertrophy. Cardiac hypertrophy is often preceded by hypertension and elevated levels of vasoconstrictive peptides, such as endothelin-1 (ET-1) and angiotensin II (Ang II). We have previously reported that congenic AhR null mice exhibit elevated mean arterial blood pressure (MAP) at 3 mo which increases further at 5 mo. In addition, AhR null mice exhibit elevated plasma ET-1 at 5 mo, compared to age-matched C57Bl6 AhR wildtypes. To determine the role of Ang II and ET-1 in AhR null hypertension, AhR null mice were treated orally with ACE-inhibitor, captopril, at 400 mg/kg from 3-to-5 mo. At 5 mo, indwelling catheters were surgically inserted into the femoral artery and MAP was measured 3 days post-surgery. Mice were then sacrificed, blood collected, and plasma ET-1 and Ang II measured by RIA. Captopril treatment of AhR null mice reduced 5 mo MAP to 3 mo levels, compared to untreated AhR null mice (5 mo Rx, 116 ± 2; 5 mo no Rx, 123 ± 2, $p<0.001$; 3 mo no Rx, 114 ± 0.9 mmHg, $p=0.15$), but failed to reduce MAP to wildtype levels (102 ± 2 mmHg, $p<0.001$). Captopril also reduced plasma ET-1 in 5 mo AhR null mice to 3 mo levels (5 mo Rx, 10.3 ± 1.3; 5 mo no Rx, 12.5 ± 1.0, $p<0.02$; 3 mo no Rx, 11.2 ± 0.5 fmol/ml, $p=0.18$), but failed to reduce ET-1 to wildtype levels (6.2 ± 0.7, $p<0.001$). Finally, captopril reduced Ang II by 72 and 80% in AhR null mice compared to 3 and 5 mo untreated AhR null mice, respectively (5 mo Rx, 89.4 ± 16; 5 mo no Rx, 456 ± 42, $p<0.001$; 3 mo no Rx, 356 ± 95 fmol/ml, $p<0.001$). These data show that hypertension in AhR null mice correlates with plasma ET-1, but not Ang II. Future studies will determine whether ET-1 mediates hypertension in AhR null mice. Supported by ES10433 and AHA 0151511Z.

1387 EMBRYONIC CARDIAC HYPERTROPHY AND NEONATAL MACROSOMIA IS DEPENDENT ON AHR MATERNAL GENOTYPE.

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The aryl hydrocarbon receptor (AhR) is a cytoplasmic protein, originally characterized because of its high affinity binding for 2, 3, 7, 8, -tetrachlorodibenzo-p-dioxin (TCDD). AhR is required for most, if not all, of the toxic effects of TCDD; however, studies using AhR null mice have identified new roles for the AhR in normal physiology and development, distinct from its role as an environmental sensor of toxicants. Previous work in our laboratory has shown that adult AhR null male mice develop significant cardiac hypertrophy by 5 mo. In the current study, we investigated the role of the AhR in fetal cardiac development and discovered that AhR null mice showed a significant increase in heart weight, body weight, and heart/body weight ratio at birth. Importantly, a series of mating experiments determined that this phenotype was dependent on the maternal genotype alone, as mice born to AhR null females had increased heart and body weights compared to mice born to wildtype and AhR heterozygous dams, regardless of neonatal genotype. This implicates a maternal effect as the cause of the embryonic phenotype, and led us to explore whether AhR null mice develop gestational diabetes, which is linked to fetal cardiac hypertrophy and neonatal macrosomia. We found that pregnant mice lacking the AhR exhibit altered insulin regulation and responsiveness as seen by decreased fasting plasma insulin ($p<0.05$) and a trend towards insulin resistance ($p=0.08$). However, hyperglycemia and altered glucose tolerance, characteristics of gestational diabetes, were not observed. These results suggest that gestational diabetes is not responsible for the fetal cardiac hypertrophy and neonatal macrosomia in mice born to AhR null females, but alterations in insulin regulation and tissue responsiveness to insulin cannot be eliminated as potential causative factors. Supported by ES10433 to MKW.

1388 QUANTITATIVE GENE EXPRESSION CHANGES IN PERIPHERAL BLOOD LEUKOCYTES (PBL) OF RATS WITH FENOLDOPAM-INDUCED VASCULAR INJURY.

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The identification of changes in mRNAs or proteins in accessible tissues that are predictive of vascular damage by drugs would facilitate testing and prioritization of candidate compounds and may assist with assessments of clinical relevance. In agreement with previous reports on the effects of fenoldopam, the present study demonstrated the induction of mesenteric vascular lesions in rats within 24 hours of dosing. PBL were isolated from rats (6 per group) dosed s.c. with either 60 mpk of fenoldopam, or with saline vehicle. Using qRT-PCR we measured expression changes for cytokines (IL1-IL18) and 33 additional targets. IL-1 beta, IL-7 and IL-10 show 2.8, 2.4 and 2.5-fold induction, respectively. Of the additional targets, significant induction was measured for: diaphorase 4 (3.4-fold), ERBB-2 (2.8-fold), IGF2 (2.9-fold), iNOS (4.2-fold), LP1 (4.2-fold), MET-HGFR (12.8-fold), MIP-1 beta (2.9-fold), and TNF-alpha (3.6-fold). Changes in the expression of product from some of these genes (IL-1 beta, TNF-alpha, iNOS, MET-HGFR) have been previously implicated with the development of vascular lesions. These data imply that certain PBL gene expression alterations may serve to herald the onset of drug-induced vascular injury and further investigations are in progress to assess sensitivity, specificity, and interspecies utility.

1389 IDENTIFICATION OF GENES LINKED TO DOXORUBICIN CARDIOTOXICITY AND TO THE CARDIOPROTECTANT EFFECT OF DEXRAZOXANE IN RATS.

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Doxorubicin (DOX) is a cardiotoxic anti-neoplastic drug that induces diffuse cardiac pathology characterized by myofibrillar loss and cytoplasmic vacuolization. Administration of dexrazoxane (DZR) prior to DOX significantly but only partially protects the heart from injury. Spontaneously hypertensive rats (SHR), which have increased sensitivity to DOX cardiotoxicity, were used to investigate gene expression changes in heart on Affymetrix RGU34A arrays. Adult male SHR were given either 2 or 3 weekly injections of 3 mg/kg DOX with or without pretreatment with 50 mg/kg DZR, or 1 mg/kg/wk DOX for 9 weeks. After 9 weeks of DOX, significant cardiac pathology is observed, and genes involved in tissue injury and remodeling are upregulated. An increase in immune cell markers was also seen, which is consistent with the increases in number of cardiac dendritic cells observed in a previous study. Very few significant changes in gene expression, histopathology or clinical chemistry were observed after 2 injections of DOX. After 3 weekly injections of DOX, alterations in serum levels of cardiac troponin T, cholesterol, triglycerides, and total protein were detected along with minimal myocyte damage. DZR alone had little effect on gene expression, but a subset of genes was identified that had significantly different levels of expression in SHR that received DZR in addition to DOX compared to DOX alone. Some members of this gene set reflect DZR inhibition of hydroxyl radical production by DOX (e.g., lower induction levels of GST and NAD(P)H-diaphorase). DZR pretreatment also modulated DOX-induced decreases in some genes involved in heart function like GLUT4 and the type II ryanodine receptor. A larger set of genes was identified that were significantly changed by DOX treatment +/- DZR. These genes may provide mechanistic clues for the rational design of clinical strategies to further reduce DOX cardiotoxicity.

1390 THE ROLE OF GLUTATHIONE S-TRANSFERASE A4-4 IN ENDOTHELIAL CELL DEFENSE AGAINST CYTOTOXICITY DUE TO OXIDATIVE STRESS.

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A group of immunologically related a-class mammalian glutathione S-tranferases (GSTs) which utilize 4-hydroxynonenal (4-HNE) as the preferred substrate has been proposed to be a major cellular defense system against oxidative injury by products of lipid peroxidation, including aldehydes such as 4-hydroxynonenal (4-HNE) and acrolein. Previous studies have demonstrated that GSTs play an important role in protection of vascular smooth muscle cells against oxidant damage, but the role of GSTs in the endothelial cell, which plays a critical role in atherosclerosis, is not well studied. We tested the hypothesis that overexpression of GST would increase resistance to the, toxic aldehydes such as acrolein, formaldehyde, 4-HNE, and direct oxidative stress from H2O2. Stable transfection of mouse islet endothelial cells (MS1) with mGST A4-4, an isozyme of GST with activity in vascular wall, was established; transfected cells demonstrated enzyme activity three times (toward 1-chloro-2, 4-dinitrobenzene) and four times(toward 4-HNE) higher than wild-type MS1 cells or cells transfected with vector alone. Transfected cells expressed significantly ($p<0.05$) increased resistance to the cytotoxicity of allylamine (which is metabolized to acrolein), acrolein, 4-HNE, and H2O2, but little difference in resistance to formaldehyde. Transfected MS1 cells also showed a significantly higher rate of proliferation compared to wild-type or vector transfected MS1 cells. Intracellular malondialdehyde and 4-HNE levels determined by mass spectrometry were lower in mGST A4-4-transfected cells than in cells transfected with vector alone or MS1 wild type. Our results indicate that expression of mGST A4-4 in endothelial cells plays a key role in protecting the blood vessel wall against oxidative stress and, thus, is likely to be a critical defense mechanism against atherogens.

1391 SYNERGISTIC TOXICITY INDUCED BY DOXORUBICIN AND ANTI-ERBB2 IN RAT NEONATAL CARDIOMYOCYTES.

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Anti-erbB2 (Herceptin -Trastuzumab) is an effective treatment for breast cancers that over-express the Her2/neu oncogene. Unfortunately, cardiomyopathy is an adverse synergistic effect of Herceptin treatment in 28% of patients also exposed to the anthracycline doxorubicin. The mechanism(s) of synergistic cardiac toxicity observed with doxorubicin and anti-erbB2 treatment is not well understood. We have developed an *in vitro* system to study this toxicity using rat neonatal cardiomyocytes and an anti-rat-erbB2 monoclonal antibody (Mab 7.16.4). This antibody has been shown to induce the same biological effects in rat cells expressing erbB2, as Herceptin does in human breast cancer cells, including similar epitope recognition, inhibition of cell growth, reversion of phenotype and reduction in cancer cell growth *in vivo*. The ability of Mab 7.16.4 to bind specifically to erbB2 in rat cardiomyocytes was verified by FACS analysis. When given together, doxorubicin (.05microM) and anti-erbB2 (1microg/ml) induces synergistic toxicity in rat neonatal cardiomyocytes (by 48 hours as determined by MTT assay) compared to doxorubicin, anti-erbB2, saline or isotype control IgG2a treatments alone. Interestingly, cytotoxicity was found to correlate with activation of caspase-3 as assessed by immunofluorescence analysis. Treatment with anti-erbB2 for 10 min induces tyrosine phosphorylation of the erbB2 receptor, known to lead to activation of Akt and ERK/MAPK in response to the natural ligand, Neuregulin. However, activation of these pathways should suppress activation of caspase-3, suggesting that Mab 7.16.4-induced erbB2 phosphorylation may activate distinct signaling pathways that will be explored in future studies. Once the mechanism of toxicity is understood, successful cardiac-specific strategies can be developed for humans to prevent cardiac injury without affecting the anti-neoplastic effect of these two important breast cancer treatment drugs.

1392 *IN VITRO* INVESTIGATION OF DRUG-INDUCED HEPATIC STEATOSIS: BIOCHEMICAL AND GENOMIC APPROACHES.

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The novel pharmaceutical agents #021 and #974 unexpectedly caused lipid accumulation in the liver when administered to rats and dogs. In an attempt to elucidate the molecular mechanisms underlying the hepatosteatosis induced by these compounds, a rat primary hepatocyte culture was employed. Initially, cultures were exposed to both compounds at various concentrations over a 24-hour time course, and cytotoxicity measured. This established for both compounds that 2μM and 20μM were a sub-toxic and toxic concentration respectively over this time course, and these concentrations were used in subsequent studies. Lipid accumulation in response to compound treatment was measured using Nile Red staining. Both compounds induced significant staining over a 18 to 24 hour period when administered at 20μM, as did the known hepatosteatotic agent amiodarone. No staining was observed in control cultures, or in cultures treated with compounds at 2μM. Transcript profiling analysis was also carried out using the Affymetrix GeneChip™ system. Rat hepatocyte cultures were exposed to vehicle controls, #021 and #974

over a time course of 1, 2, 4, 8, and 24 hours. Experiments were performed on two separate hepatocyte preparations, and RNA isolated for the gene array analysis. Following bioinformatic analysis of the data, a number of putatively regulated genes were selected for confirmation by quantitative RT-PCR. Among those genes confirmed as being regulated by the test compounds were some "known" steatosis-associated genes, including CYP2E1 (down-regulated), aldolase B (down-regulated), and FATP (upregulated at early time points). Also, some novel genes changes not previously associated with this type of toxicity were identified, and the significance of these is currently being investigated. In conclusion, rat primary hepatocyte cultures appear useful for studying liver toxicity induced by #021 and #974, since there is good concordance between the cytotoxic, biochemical and genomic endpoints measured.

1393 COMPARATIVE *IN VITRO* TOXICITY STUDIES BETWEEN PRIMARY RAT HEPATOCYTES AND RAT HEPATIC CELL LINES USING GENE EXPRESSION ANALYSIS.

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Cultured liver cells to study chemical hepatotoxicity have become a powerful alternative to *in vivo* toxicological models. Primary hepatocytes are especially useful in screening cytotoxic and genotoxic compounds due to their similarities to liver cells *in vivo*. However, the *in vitro* model that best correlates with liver cells *in vivo* has yet to be determined. In order to select a model cell line, the present study was conducted to compare gene expression profiles of primary rat hepatocytes and rat hepatic cell lines (BRL 3A and Clone 9). Gene expression analysis, using Affymetrix microarrays (Rat Genome U34A), indicated significant changes in gene expression profiles both up and down regulation in rat liver cell lines compared to rat primary hepatocytes. We have also investigated cadmium (Cd) toxicity in primary hepatocytes and hepatic cell lines using LDH leakage, MTT reduction and ATP as end points. The fluorescence probes YO-PRO-1 and propidium iodide were used to detect apoptotic, necrotic and viable cells in Cd exposed liver cell lines. Cd induced apoptosis in liver cell lines at 50 uM while a much lower concentration (1 uM) was required in primary rat hepatocytes. In summary, the results show that primary cells were more sensitive to Cd compared to liver cell lines. The differences in gene expression observed between primary and immortalized hepatoctyes appear to support the increased sensitivity of primary cells to this heavy metal. Further, a detailed analysis of differential gene expression in primary cells and cell lines will assist in selecting a cell line model for toxicity screening of new chemicals.

1394 EVALUATION OF HEPATOCYTE SPHEROIDS AS A MODEL FOR TOXICITY STUDIES.

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The present studies characterized the utility of primary hepatocyte spheroids for toxicological investigations. Current *in vitro* models of hepatotoxicity have been scrutinized for lack comparability to liver. Among the inherent deficits are: resistance to enzyme induction, lack of tertiary liver structure, and low correlation with *in vivo* heptatoxicity. Primary hepatocyte spheroids offer an alternative cell culture environment amenable to formation of a liver-like structure. Thus the present study examined parameters of hepatocyte function in order to assess the value of hepatocyte spheroids as models for toxicology. Hepatocytes were isolated from livers of male Sprague-Dawley rats by collagenase perfusion. Following isolation of hepatocytes, cells were cultured in spinner flasks to facilitate spheroid formation as described (Abu-Absi et al., 2002). The ability of hepatocyte spheroids to demonstrate drug metabolizing enzyme induction (DME) was assessed as an index of hepatocyte functionality. Measurement of individual DMEs was performed by transcript profile analysis (TxP). Spheroids were exposed to classical inducers of rat cytochromes P450 for 6 or 24 hr. Among the compounds tested were: PCN (CYP3A), phenobarbital (CYP2B1/2B2), benzo[a]pyrene (CYP1A1 and 1A2), DEHP, clofibrate, and WY-14643 (CYP4A). Measurement of gene induction was carried out using SYBR green sequence detection. Total RNA was isolated and reverse transcribed into cDNA. The cDNA was used as template for the SYBR green detection assay utilizing primers specific for each CYP450 gene. Expression of the cytochromes was compared to genes whose transcript levels were stable across experimental conditions. Initial histological characterization indicated that hepatocyte spheroids were present as compact aggregates of multiple individual hepatocytes with high viability. Treatment of spheroid culture with enzyme inducers demonstrated responsiveness similar to that reported for each inducer *in vivo*. In addition, treatment of spheroids resulted in differential dose/time response for measured transcripts.

1395 *IN VITRO* HEPATOTOXICITY STUDY OF BARAKOL USING HUMAN HEPATOMA CELL LINE HEP G2.

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Human hepatoma cell line HepG2 was used to assess the hepatotoxic effects of barakol, isolated from young leaves of Cassia siamea. Barakol at concentrations of 0.25, 0.50, 0.75 and 1 mM were added to HepG2 cells for 24, 48, 72 and 96 hours. Cytotoxicity was assessed by a rapid MTT cell viability screening assay. Activities of lactate dehydrogenase enzyme in the culture medium were determined at 24 and 48 hours of barakol incubation. Acetaminophen, the well-known hepatotoxin, at concentrations of 1, 3, 5, 7 and 10 mM were also assessed in parallel in the same manner. Using MTT assay, barakol demonstrated a dose- and time-dependent cytotoxic effects in the same manner as acetaminophen. Barakol exhibited a significant ($p < 0.05$) cytotoxic effect at concentrations of 0.75 mM or greater at 24 hours of exposure. At 48, 72 and 96 hours of exposure, cytotoxicities of barakol were shown at concentrations of 0.50 mM or greater. Fifty percent cytotoxicities (IC50) of barakol on HepG2 cells at 24, 48, 72 and 96 hours of exposure were 5.70, 0.96, 0.77 and 0.68 mM, respectively whereas the corresponding IC50 of acetaminophen were 12.14, 11.13, 1.39 and 1.30 mM, respectively. At the same concentration of 1 mM, barakol was significantly ($p < 0.05$) more cytotoxic to HepG2 cells than acetaminophen at every time point of compound exposures. However, both barakol and acetaminophen caused lactate dehydrogenase leakage in the same extent at 1 mM concentration and 48 hours exposure. A depleted reduced glutathione as well as a decrease of GSH/GSSG ratio following barakol exposure might explain the mechanism by which barakol induced hepatotoxicity.

1396 IDENTIFYING EARLY APOPTOTIC EVENTS IN RAT HEPATOMA (H4IIE) AND KIDNEY EPITHELIAL (NRK-52E) CELLS USING CELLOMICS™ ARRAYSCAN® II.

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The use of multiple endpoint analysis and detailed dose responses early in drug discovery can provide information on a compound's potential to produce apoptosis. Although many compounds induce apoptosis through activation of caspases, this alone may not account for all apoptotic mechanisms. In order to more accurately identify compounds that induce apoptosis, the Cellomics™ Arrayscan® II system has been added to rapidly monitor apoptotic markers other than caspase activation. Utilizing an optical imaging system and multiple fluorescent channels, the Arrayscan® II concurrently analyzes changes in three apoptotic endpoints: Nuclear Fragmentation/ Condensation (NFC), Mitochondrial Mass/ Potential (MMP), and F-Actin Content (FAC). This study compared caspase activity assays to the Arrayscan® II following exposure of H4IIE cells to known inducers of apoptosis. Paclitaxel (0-300 μM) did not reveal any significant increase in caspase activity after 3 and 6 hr exposures. However, the Arrayscan® II detected a substantial increase in MMP at all doses. Increases in caspase activity were observed at 12 and 24 hr, but the Arrayscan® II showed increases in all three apoptotic markers (MMP, NFC, FAC). Staurosporine treatment showed increases in MMP at very low doses (<0.001 μM) and increases in NFC and FAC at higher doses (>0.05 μM) after 3 hr. Caspase activity was only noted at the highest treatment (10 μM). Caspase activity was not observed in either H4IIE or NRK-52E cells exposed to gentamicin (0-10 mM) for 72 hr. However, Arrayscan® II clearly showed significant increases in the NFC and FAC markers in NRK-52E cells, but not in the H4IIE cells. These findings were supported by ELISA results that showed DNA fragmentation in only the NRK-52E cells. These data support *in vivo* studies that showed renal specific toxicity with gentamicin. In summary, the Arrayscan® II screen appears to be a valuable and highly sensitive method for identifying compounds that induce apoptosis.

1397 ASSESSMENT OF HIGH PRECISION RAT LIVER SLICES AS AN *IN VITRO* MODEL FOR UPTAKE AND ACTIVITY OF PHOSPHORODIAMIDATE MOPHOLINO OLIGOMERS.

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The objective of this study was to evaluate the suitability of high precision rat liver slices as a model to predict the antisense activity of phosphorodiamidate Morpholino oligomers (PMOs) in rat liver *in vivo*. PMOs are third generation antisense agents that are efficient inhibitors of translation *via* a non-RNase H, sequence-specific steric-blockade process. Previous studies with PMOs have demonstrated high bioavailability in the liver, as well as sequence-specific and

dose-dependent target gene inhibition following systemic administration in rats. The high precision rat liver slice model offers an extremely efficient method to study gene inhibition by allowing a large sample size while minimizing the number of animals required. The present studies addressed PMO biodistribution in rat liver slices. Uptake of PMO was investigated with respect to both dose and time. Livers were resected from Sprague-Dawley rats and 200 µm thick slices prepared utilizing a Brendel/Vitron tissue slicer. Slices were cultured in glass scintillation vials containing Waymouth media using a dynamic organ culture system in a 95% O_2 and 5% CO_2 environment. Effect of PMO concentration on uptake was studied using incubation with fluorescein-labeled PMO at concentrations of 1, 10, 30, and 100 µM for 18 hrs. Effect of time on PMO uptake was studied by incubation of slices at a concentration of 10 µM for various time points ranging from 10 seconds through 18 hours. Effect of media serum content on PMO activity was studied for serum concentrations ranging from 0 through 25%. The results indicate that the uptake of PMO into cultured rat liver slices was dose-dependent and time-dependent after 1 minute. Fluorescence photomicrography was employed to confirm intracellular distribution of PMO. We conclude that PMOs demonstrate high biodistribution in precision rat liver slices. This technique can serve as an efficient model for predicting the *in vivo* antisense activity of PMOs in rat liver while reducing the number of animals required in the investigation.

1398 AN *IN VITRO* PREDICTIVE TOXICOGENOMICS SCREEN (PTS) FOR HEPATOTOXICITY.

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The application of genomics to toxicology offers the promise of greater sensitivity at much lower cost than traditional diagnoses. We describe an *in vitro* toxicogenomic based high-throughput rat hepatotoxicity assay predictive of 11 different liver pathologies. In Phase I of this project, our research group employed GeneCalling® to identify multivariate markers, or gene expression patterns, associated with these specific toxicities. This database includes greater than 100 toxic and non-toxic compounds administered sub-acutely to male Wistar rats. Data for liver histopathology and gene dysregulation were analyzed using statistical learning techniques to identify toxicity markers and generate a series of 11 predictive models. Phase II created an *in vitro* PTS by assessing gene dysregulation in primary hepatocyte cultures after 24 hour exposure to the same compounds used in Phase I. *In vitro* samples were assigned the same toxicity annotation as observed *in vivo* on a per compound basis. Although the identity of toxicity marker genes differ between *in vitro* and *in vivo*, the two sets do overlap in terms of toxicologically relevant pathway dysregulation. The genes in this marker set have been transferred to customized microarrays that are part of our industrialized toxicogenomics assay. Preliminary results indicate we are able to rank compounds based on the likelihood they will produce toxicity. Furthermore we have an accuracy of greater than 90% for approximately 80-85% of all samples on average. We envision this assay being integrated into the drug development pipeline to assess rat liver toxicity at either an early point during hit identification or later during lead optimization.

1400 USE OF HIGH THROUGHPUT METHOD FOR SCREENING THE MEIC GROUP IN THE HUMAN LIVER C3A CELL LINE TO EVALUATE THE RELEVANCE AND RELIABILITY OF THE ACTIVTOX SYSTEM FOR PREDICTING ACUTE SYSTEMIC TOXICITY.

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Rationale: A perennial question surrounding the use of *in vitro* ADMET systems is whether they, in fact, represent the *in vivo* situation. In an attempt to answer this question, the Scandinavian Society for Cell Toxicology organized the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) study. As part of the study, MEIC chose fifty chemicals where reliable acute toxicity information from humans was available from death records. We have analyzed a subset of these chemicals using the ACTIVTox system as a means of examining the same question: does *in vitro* reflect *in vivo*? **Methods:** All assays were performed using Amphioxus Cell Technologies' ACTIVTox human C3A hepatocyte cell line. Toxicity was evaluated by use of cellular proliferation or membrane integrity assays, both of which utilized the conversion of resazurin into a fluorescent resorufin product as an end-point. **Results:** The IC50 values determined in the two assays were compared to the MEIC database where the *in vivo* toxicity information was derived from a LD50 with human acute lethal doses. For many of the compounds, the IC50 determined in both assays was comparable. However, for several of the test compounds, there was a disparity in the results obtained between the two assays. In the latter cases, whenever there was a difference in the two assays, we chose the lowest IC50. In this way, no toxicity would be underestimated. **Conclusion:** There was a remarkable coincidence observed in the data. No compounds were dramatically underestimated thus eliminating the fear of missing toxic compounds, and three compounds were significantly overestimated. These compounds are copper sulfate, hexachlorophene and thioridazine, all three known hepatotoxins. This study lends support to the use of *in vitro* ADMET systems as reliable models for predictive *in vivo* toxicity.

1401 COMPARATIVE DOSE-RELATED EFFECTS OF INHALED CARBON BLACK PARTICLES IN THE LUNGS OF RATS, MICE, AND HAMSTERS.

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After inhalation of high concentrations of carbon black (CB), rats, but not mice or hamsters, develop lung tumors that are associated with particle overload in the lungs. Since this response is not related to any direct genotoxicity of the particles, it is hypothesized that it is due to species-specific particle clearance kinetics and the processes of inflammation and cell proliferation. In this study, we compared particle-induced inflammation in response to inhaled CB (1, 7, 50 mg/m^3; 6 hr/day, 5 days/week for 13 weeks) in female F-344 rats, B6C3F1 mice, and F1B hamsters at several post-exposure time points (1 day; 3 and 11 months post-exposure). Aerosol particle sizes ranged from 1.2 to 1.6 µm (mass median aerodynamic diameter) with geometric standard deviations of 2.2-2.8. At the end of exposure, mice had retained the highest amount of CB in terms of particle surface area per gram of lung; hamsters had the lowest retained amount. However, rats demonstrated the slowest clearance such that the CB burden in the high dose group was higher than in mice or hamsters 11 months after exposure. For the low and mid dose groups, cellular and biochemical indices of inflammation, proinflammatory cytokines, and antioxidant levels were similar for all three species. At the highest dose, however, the responses in rats were greatest in magnitude and were more persistent. The results suggest that rats are the most susceptible of the three species to lung particle overload effects; hamsters are the least sensitive to the effects of inhaled CB. The results further suggest that an exposure dose of 1 mg/m^3 can be considered the NOAEL in this study for female rats, mice, and hamsters. This work was funded by the International Carbon Black Association.

1402 INFLAMMATORY RESPONSES IN THE MOUSE EXPOSED INTRATRACHEALLY TO CARBON BLACK, POLYSTYRENE PARTICULATE, AND DIFFERENT SIZE OF COAL FLY ASH.

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Inhaled particulate matter causes inflammation in the lung. Magnitude of the response differs with the size, exposure dose, and chemical constituent of the particulate. This study is conducted to compare changes in the lung inflammation due to different size and physicochemical nature of the particulates. We fractionated coal fly ash (CFA) to fine, mean diameter=1.26µm (F-CFA), and to coarse, 5.75µm (C-CFA), particulates. Carbon black (CB, 0.2-0.01µm) was chosen as a surrogate of diesel exhaust particulate and polystyrene particulate (PSP, 0.208µm) was used as a non-metal reference. Two doses, 0.1 (low) and 0.5 (high) mg/mouse, of CFAs and 0.5mg/mouse of CB and PSP, as well as solvent, were intratracheally instilled to groups (n=6 to 8) of 6 weeks old ICR male mice under halothane anesthetization. Mice were sacrificed to obtain bronchoalveolar lavage fluid (BALF) and lung free cells. Another set of identically treated mice was sacrificed for histopathological examination. The number of lung free cells in all exposed groups increased from day 1 to day 3, then it became comparable to control group at day 7. However, the difference between C-CFA and F-CFA group was not evident nor the difference between high and low dose group. Amounts of albumin and protein and lactate dehydrogenase activity in the BALF of all exposed groups showed statistically insignificant elevation throughout 7 days. Again, dose dependency in these endpoints was not pronounced. Histopathological inflammation scoring indicated that the score in high dose group of F-CFA, as well as CB and PSP, were augmented and remained higher for 7 days than that of control group and those of matched exposed groups in low dose. Results revealed that time course and magnitude of the lung responses were similar regardless of instilled particulates, CFA size difference between 1.26 and 5.75µm did not evoke apparent change, and dose-response between 0.1 and 0.5mg/mouse did not exist except for histopathological evaluation. (This study is sponsored by Dr. Yasunobu Aoki, NIES, Japan).

1403 RATS, BUT NOT HAMSTERS, HAVE PERSISTENT ALVEOLITIS AND TYPE II CELL PROLIFERATION AFTER CHRONIC INHALATION OF CARBON BLACK PARTICLES.

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Rats, but not hamsters, chronically exposed to high levels of airborne carbon black particles (Cb) or other low toxicity particles develop lung tumors in association with alveolar inflammation and epithelial proliferation. The purpose of the our study was to determine the postexposure (PE) persistence of alveolitis and type II cell proliferation in the lungs of rats and hamsters similarly exposed to Cb. Female F344 rats and 212 F1B Syrian golden hamsters were exposed by inhalation to 3 concentrations of Cb (1, 7, 50 mg/m3) for 6 h/day, 5 days/wk for 13 wk. Rodents were sacrificed one day or 13 wk PE. The left lung lobe of each animal was processed for light microscopy. Numeric cell densities of alveolar type II cells and neutrophils were determined using standard morphometric techniques. Compared to air-exposed controls (0 mg/m3 Cb), rats exposed to 7 and 50, but not 1, mg/m3 Cb had increased numeric cell densities of neutrophils (alveolitis) and alveolar type II cells (hyperplasia). At 1 day PE, rats exposed to 7 and 50 mg/m3 Cb had 75% and 128% more type II cells, respectively, than air-exposed controls. Type II cell proliferation persisted throughout 13 wk PE only in the lungs of rats that were exposed to 50 mg/m3 Cb (158% more than controls). In contrast, no alveolitis was evident in any of the Cb-exposed hamsters at one day or 13 wk postexposure. A mild and transient increase in the number of alveolar type II cells (37% more than controls) was evident only in hamsters exposed to 50 mg/m3 Cb and sacrificed one day PE. No type II cell hyperplasia was evident in the lungs of Cb-exposed hamsters sacrificed 13 wk PE. These results indicate that rats, but not hamsters, chronically exposed to high airborne concentrations of Cb develop severe and persistent pulmonary inflammation and alveolar epithelial proliferation. (Research sponsored by the International Carbon Black Association)

1404 AMBIENT AIR PARTICLES OF DIFFERENT SIZE FRACTIONS CAUSES RELEASE OF INFLAMMATORY CYTOKINES, CELL TOXICITY AND APOPTOSIS IN EPITHELIAL LUNG CELLS.

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A number of epidemiological studies have provided indications that smaller size may be more potent than larger to induce health effects. Nonetheless, the relative significance of various size fractions of ambient particulate matter (PM) to induce inflammatory and toxic effects in lung cells has not extensively been investigated. We explored the potency of different size fractions of urban ambient air particles to induce release of inflammatory cytokines in the human alveolar cell line A549 and primary rat type 2 cells. A mineral-rich PM10 fraction of mechanically-derived ambient air particles was also included. The coarse fraction of ambient air particles demonstrated a similar or higher potency to induce release of the proinflammatory cytokines IL-8/MIP-2 and IL-6 compared to the fine and ultrafine fractions. The coarse fraction was also the most toxic. In contrast to the A549 cells, no induction of cytokine release was induced by the ultrafine particles in the primary type 2 cells. The mineral-rich road PM10 was equally or more potent than the various size fractions of the ambient air particles to induce cytokines in both cell types. In conclusion, the induction of inflammatory and toxic effects in lung cells can be caused by both the coarse and fine fraction of ambient particulate matter.

1405 INDUCTION OF TUMOR NECROSIS FACTOR α (TNFα) SIGNALING GENES IN ALVEOLAR MACROPHAGES (Mϕ)AFTER EXPOSURE TO ULTRAFINE PARTICLES (UFP).

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It is known that phagocytosis of UFP by Mϕ stimulates the release of proinflammatory cytokines such as TNFα. However, the subsequent effects of these cytokines on gene expression are less understood. TNFα is associated with the activation of Mϕ, production of cytokines and reactive oxygen species, proliferation and differentiation of monocytes, as well as recruiting other inflammatory cells to the site of injury. Using microarray analyses, we had previously identified genes induced by TNFα in Mϕ. Since exposure to UFP stimulates the expression and secretion of TNFα, we conducted experiments to determine whether genes directly stimulated by TNFα were also induced by UFP. Among the genes induced by direct exposure to TNFα were TNFα itself, as well as several genes involved in regulating TNFα signaling; TNFα receptor II (TNFRII), silencer of death domain (SODD), and zinc finger protein 36 (*zfp36*). The expression profile of these genes paralleled one another; they were induced after 1h, reached maximal expression after approximately 4h and then declined to near basal levels by 24h after TNFα challenge. Exposure to UFP also caused induction of TNFα, SODD, TNFRII, but the kinetics of induction differed from that observed with exogenous TNFα challenge. For these genes, the induction was gradual and sustained for up to 24h. Furthermore, unlike TNFα treatment, exposure to UFP did not induce *zfp36*, a gene whose product is reported to promote the degradation of TNFα mRNA. Thus, the differences in kinetics of TNFα regulated gene expression after TNFα or UFP challenge may, in part be due to differences in *zfp36* regulation. Taken together, our study demonstrates that direct challenge of Mϕ with either TNFα or UFP led to a different temporal expression profile of genes involved in regulating TNFα signaling

1406 EXPOSURE OF RAT LUNG MACROPHAGES TO JP-8 JET FUEL.

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A variety of adverse health effects have been reported in military and civilian fuel handlers which have been attributed to exposure to JP-8 vapors and aerosols. Many of the reported effects are similar to those produced by endotoxin (LPS). Both commercial and military fuel stores have been shown to be contaminated by LPS in addition to other microbial products. The purpose of this investigation was to determine the effect of LPS contamination in jet fuel on pulmonary macrophage cytokine expression. We exposed cultured rat lung macrophages to samples of JP-8, samples of JP-8 that had been filtered to remove LPS, and filtered samples of JP-8 spiked with a known quantity of LPS. We looked at the cytokine expression of IL-1β, IL-6 and TNF-α in the spent media. We observed increases in IL-6 and TNF-α in the jet fuel exposed groups, as compared to the controls. Filtering LPS attenuated these findings, however the change was not statistically significant. Our results suggest that LPS in jet fuel may be partly responsible for the elevation of cytokine expression associated with JP-8 jet fuel exposures.

1407 FINE DUST PARTICULATE MATTER INDUCES POTENT CYTOKINE RELEASE THROUGH TRPV1 ACTIVATION IN LUNG CELLS.

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Fine particulate air pollution exposure is associated with a significantly increased risk of lung cancer, cardiopulmonary disease and mortality. Despite the fact that epidemiological evidence linking particulate matter (PM) pollution and disease has existed for over half a century, a comprehensive mechanistic understanding of PM toxicology does not yet exist. It is known that inflammation is an important initial event in PM lung toxicity that may mediate the subsequent development of cardiovascular health problems. In this work, fine particulate samples <2.5 micromolar (PM 2.5) were examined for production of interleukin-6 (IL-6) in the human epithelial lung cell line BEAS-2B. Unexpectedly, a Utah desert dust sample induced significantly greater IL-6 production than a coal fly ash particulate sample of anthropogenic origin. Particulate matter may mediate lung cell toxicity through interactions with the vanilloid receptor TRPV1. To further test this supposition, cytokine production by PM 2.5 in a BEAS-2B cell line engineered to over-express TRPV1 was measured. This engineered cell line exhibited a significantly greater PM 2.5-induced IL-6 response, corroborating the importance of TRPV1 in particulate matter-lung cell interactions. In addition, PM 2.5-exposed BEAS-2B cells treated with capsazepine (CPZ), an antagonist of TRPV1, exhibited a marked decrease in IL-6 production. In order to examine potential cell-signaling pathways responsive to PM 2.5-induced TRPV1 activation, expression of the MAP kinase ERK was examined in cells treated with PM 2.5 and CPZ. ERK expression was found to increase in a dose-dependent manner with PM 2.5 concentration and was reduced in cells treated with CPZ. These data demonstrate that desert dust particles believed to be benign are in fact surprisingly pro-inflammatory, and that particulate matter likely elicits lung cell toxicity through interactions with TRPV1 and subsequent activation of an ERK-mediated cell-signaling pathway. This work was supported by NIST Contract #60NANBOD0006.

1408 OVERLAPPING MOLECULAR TACHYKININERGIC EFFECTS IN F344 RATS FOLLOWING SIDESTREAM CIGARETTE SMOKE EXPOSURE.

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Environmental tobacco smoke induces many adverse health effects in the lung directly involving bronchopulmonary C-fiber afferents, but the underlying molecular tachykininergic mechanisms are not completely clear. Young female Fischer 344 rats were randomly divided in two groups (24/group): capcaicin and its vehicle pretreatment, respectively. Half of rats/each group were exposed to air (control) or 0.4 mg/m3 total particulate matter of sidestream cigarette smoke (SSCS) for 4 hours/day, respectively, for 7 continuous days through a nose-only exposure chamber. SSCS resulted in typical neurogenic inflammation in the lung as indicated by elevation of plasma extravasation and proinflammatory cytokines, such as interleukin (IL)-1β and IL-12. SSCS depleted the tachykinin peptide substance P (SP), but induced an upregulation of the transcript level of β- preprotachykinin-I (PPT-I) encoding SP. SSCS also induced overexpression of neurokinin-1 receptor (NK-1R) or reduction of its mRNA level in the lungs. Moreover, neutral endopeptidase (NEP) was substantially inactivated by SSCS exposure. Taken together, our findings suggest that overlapping tachykinergic mechanisms may involve in the initiation of SSCS-associated neurogenic inflammation. All of these changes may be regarded as important factors that switch neurogenic pulmonary responses from their physiological and protective functions to a detrimental role.

1409 LACK OF ROLE OF VR1 IN RESPIRATORY RESPONSES TO IRRITANTS.

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The receptors through which irritants stimulate sensory nerves are not known. The vanilloid receptor (VR) is expressed in respiratory sensory nerves and *in vitro* studies suggest that it may be activated by electrophiles or by protons. The goal of the current study was to examine the respiratory responses to inspired irritants in spontaneously breathing wild type (C57Bl/6J) and VR1 knockout [B6.129S4-Vr1(tm1Jul)]mice. Three irritants were studied: capsaicin (a known VR agonist), acrolein (a prototypical electophilic irritant) and acetic acid (a prototypical acidic irritant). Capsaicin induced sensory irritation (slowed breathing rate due to a pause during expiration) and also airway obstruction (increased in SRaw) in wild type but not VR1 knockout mice. Acetic acid also induced sensory irritation and airway obstructive responses. Surprisingly these responses were identical in both wild type and knockout mice. Similar to acetic acid, the sensory irritation and airway obstructive responses to acrolein were also identical in both strains. Thus, although stimulation of the VR1 can result in airway obstruction and sensory irritation, this receptor does not appear to play a role in induction of these responses by the irritants acrolein or acetic acid. (Supported by the University of Connecticut Pulmonary Research Consortium and ES08765.)

1410 TACHYKININ SUBSTANCE P SIGNALING INVOLVES IN DIESEL EXHAUST (DE)-INDUCED BRONCHOPULMONARY NEUROGENIC INFLAMMATION IN RATS.

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This study characterized the molecular neurotoxicity of diesel exhaust (DE) on the tachykinin substance P (SP) signaling system in the lungs. A total of 96 female Fischer 344 (F344)/NH rats (~175g) were randomly assigned to 8 groups in a 2 X 3 factorial design [Capsaicin vs. non-capsaicin (vehicle) pretreatment and filtered room air vs. 2 exposure levels of DE] with diesel engine room control. The rats were nose-only exposed to air or low and high levels of DE, respectively, directly from the diesel engine for 4 h/day, 5 days/week, for 3 weeks. The findings demonstrated that exposure to DE induced dose-dependently bronchopulmonary neurogenic inflammation, especially in the capsaicin-pretreated groups, as measured by plasma extravasation, edema, and inflammatory cells. Inhalation of DE affected SP signaling processes, including stored SP depletion and the gene/protein overexpression for neurokinin-1 receptor (NK-1R). DE also significantly decreased the activities of neutral endopeptidase (NEP), which is a main degradation enzyme for SP. Consequently, these changes may be regarded as critical factors that switched neurogenic pulmonary responses from their protective functions to a detrimental role that perpetuates lung inflammation. It suggests that the tachykinergic mechanism may be involved in the initiation of DE-associated lung inflammation and these changes may possibly associated with the mass concentration of DE particles due to their physical-chemical characterizes.

1411 13-WEEK INHALATION TOXICITY STUDY OF BITUMEN FUMES IN WISTAR(WU) RATS.

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A 90-day toxicity inhalation study was conducted in Wistar WU rats (Crl:WU) with bitumen fumes generated from a sample of bitumen fume condensate collected from a storage tank. The composition of the fume was aimed to be similar to an exposure atmosphere during road paving. The objective of this study was to determine the concentration levels and maximally tolerated dose for a future carcinogenicity study. 16 male and 16 female rats per group, about 10 weeks old, were exposed to clean air, or to target concentrations of 4, 20, and 100 mg/m^3 THC (total hydrocarbon). The exposure atmosphere comprised a mixture of aerosol particles and hydrocarbon vapour. The duration of the exposure was 6 hrs/day, 5 days/week for 14 weeks. In males (100 mg/m^3) differences in body weight were observed, which increased during the course of the study resulting in a markedly lower mean body weight (- 10%) compared to the controls. Milder effects on body weight (-5%) were noted in all female groups exposed to bitumen fumes. A statistically significant lower food consumption was noted in the male high dose group. Statistically significant test substance-related histopathological changes were only observed in the nasal and paranasal cavities in the bitumen high dose group. Eosinophilic cytoplasmic inclusions (hyalinosis) were observed exclusively in nasal epithelial cells of the high dose group. Associated with degenerative hyalinosis was focal/multifocal basal cell hyperplasia which could be observed in the olfactory/respiratory transition areas of high-dosed males. Another significant treatment-related change was multifocal mucous (goblet) cell hyperplasia. Multifocal mucosal inflammatory cell infiltration was a further exposure-related effect which could be observed exclusively in the bitumen high dose group. The NOAEL of Bitumen fumes under the above described conditions in Wistar (WU) rats is 20 mg/m^3 THC. Concentration levels of 4, 20, and 100 mg/m^3 THC are proposed for the planned carcinogenicity study. The study was sponsored by ARBIT, Germany.

1412 FOUR-WEEK INHALATION TOXICITY OF N-VINYL CARBAZOLE IN RATS, FURTHER STUDIES.

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A previous 4-week inhalation study with n-vinyl carbazole (nVC) showed nasal and laryngeal effects in rats exposed at 2, 10, or 44 mg/m^3. To determine a no adverse effect level, groups of 15 male Crl:CD®(SD)IGS BR rats were exposed nose-only to nVC at design concentrations of 0.02 (vapor), 0.2 (vapor) or 2 mg/ m^3 (combined aerosol vapor concentrations). Exposures were 6 hours/day, 5 days/week, over a 4-week period. Five rats/group were sacrificed for respiratory tract evaluation at the end of the 4-week exposure period and at the end of 1 month and 3 month recovery periods. No lung weight, body weight, or clinical signs of toxicity were seen at any concentration. At the end of the exposure period, minimal to mild histopathologic effects were observed in the upper respiratory tract at concentrations of 0.2 and 2 mg/ m^3. Rats exposed at 2 mg/ m^3 showed mucoid depletion of goblet cells in the anterior section of the nose along with hypertrophy of basilar epithelial cells in the respiratory mucosa lining the nasal septum. In addition, the 2 mg/ m^3 rats showed squamous metaplasia in the laryngeal epithelium. The incidences of the nasal lesions in the 2 mg/ m^3 rats had decreased but repair was not complete by the end of the 3 month recovery period. The laryngeal lesions in these rats were not present after 1 or 3 month recovery periods. The only effect observed in the 0.2 mg/ m^3 rats was mucoid depletion of goblet cells in the anterior section of the nose. This lesion was not observed in the recovery rats at this concentration. No histopathologic effects were observed in the rats exposed to nVC at 0.02 mg/m^3.

1413 VP 14637: TWO-WEEK INHALATION TOXICITY STUDY IN NEONATAL DOGS.

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VP 14637 (phenol, 2, 2-[(4-hydroxyphenyl) methylene]bis[4-[[(5-methyl-1H-tetrazol-1-yl)imino]methyl]]) is an inhibitor of respiratory syncytial virus (RSV) fusion protein. VP 14637 is under investigation for treatment of RSV infection in

humans. This study was conducted to characterize the potential toxicity, and the toxicokinetics of VP 14637 in male and female neonatal beagle dogs. Beginning at 10 days of age, three groups of neonatal dogs were given inhalation exposures (oronasal by nebulizer) to aerosolized VP 14637 (mean deposited dosages of 31, 99, or 262 mg/kg/day). Based upon exposure duration, and measured minute volumes and aerosol parameters, the dosage of 262 mg/kg/day was the highest possible dosage. Measured aerosol particle size data (MMAD = 2.6-2.8 mm) allowed for respiratory deposition fractions of 50-70%. As control, two other groups were exposed only to air, or to the vehicle (ethanol/propylene glycol/water) for VP 14637. After 2 weeks of dosing, main study animals were necropsied. A sub-group, exposed to vehicle or 262 mg/kg/day of VP 14637 for 2 weeks, continued in a 1-month treatment-free observation period and then necropsied. After the 14th daily exposure, mean concentrations of VP 14637 in plasma increased with increasing dosages, but were <4 ng/mL indicating that systemic exposure to inhaled VP 14637 was low. At the end of the dosing and observation periods, there were no findings (parameters assessed: clinical status, respiratory function, ophthalmology, electrocardiography, clinical and gross pathology, organ weights, and histology) related to treatment vehicle or VP 14637 at dosages up to 262 mg/kg/day. Specifically, there was no evidence of respiratory impairment, and no histologic changes in the respiratory tract attributed to treatment with vehicle or VP 14637. In conclusion, inhalation of the highest possible dosage of VP 14637 for 2 weeks, given the constraints of studying neonatal dogs, had no observed effects.

1414 *IN VIVO* AND *IN VITRO* RESPIRATORY TRACT INHIBITION OF RAT CYP450 FOLLOWING EXPOSURE TO *m*-XYLENE AND METABOLITES.

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Xylenes are used as a solvent in paints, cleaning agents and gasoline. Exposure occurs primarily by inhalation. The volatility and lipophilicity of the xylenes make the lung and nasal mucosa the primary target organs. m-Xylene (m-XYL) is metabolized *via* CYP450 isozymes to 3-methybenzyl alcohol (3-MBA) and subsequently to m-tolualdehyde (m-ALD). The purpose of this work was to determine if pulmonary and nasal mucosa CYP isozymes involved in the metabolism of the xylenes are inhibited in a dose dependent manner following inhalation of m-XYL: 100 and 300 ppm; 3-MBA: 50 and 100 ppm; or m-ALD: 50 and 100 ppm, and to investigate the type of inhibition caused by these compounds using *in vitro* kinetics. A single 6 hour inhalation exposure to m-XYL (100 ppm and 300 ppm) inhibited pulmonary CYPs 2B1, 2E1, and 4B1 in a dose dependent manner. Inhalation of 3-MBA (50 ppm and 100 ppm) inhibited pulmonary CYPs 2B1 and 4B1 in a dose dependent manner. m-ALD (50 ppm and 100 ppm) inhibited pulmonary CYPs 2B1 and 2E1 in a dose dependent manner, while 4B1 activity was increased dose dependently. Nasal mucosa CYP 2B1 and 2E1 activity was inhibited following exposure to m-XYL (100 ppm and 300 ppm) dose dependently. 3-MBA (50 ppm and 100 ppm) inhibited nasal mucosa CYPs 2E1 and 4B1 dose dependently. CYPs 2B1, 2E1 and 4B1 were inhibited in a dose dependent fashion following inhalation of m-ALD 50 ppm and 100 ppm. *In vitro*, IC50 values for m-XYL, 3-MBA and m-ALD for pulmonary CYP 2B1 were 0.1, 1, 0.5 mM and nasal mucosa were 0.1, 1, 0.1 mM; pulmonary CYP2E1 were 1, 8, 0.01 mM and nasal mucosa were 0.05, 10, 0.1 mM; pulmonary CYP 4B1 were 3, 6, 0.3 mM and nasal mucosa were 5, 1, 0.1 mM. IC50 values were used to investigate the type of inhibition caused by these compounds. Eadie-Hofstee plots for CYPs 2B1, 2E1 and 4B1 for lung and nasal mucosa show non-competitive inhibition by m-XYL, 3-MBA or m-ALD. Alterations of cytochrome P450 activity caused by m-XYL may result in changes of metabolic profiles of xenobiotics in coexposure scenarios in increased or decreased toxicity in an organ-specific manner.

1415 EVALUATION OF ACUTE RESPIRATORY EFFECTS IN HEALTHY ADULTS FOLLOWING CONTROLLED ENVIRONMENTAL EXPOSURES TO FRAGRANCED INCENSE STICKS.

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Recently, attention to the potential for indoor air quality impact from burning incense has increased with focus on incense smoke as source of particulate exposure in indoor air. This study evaluated acute pulmonary responses and symptoms in healthy adults following burning of incense formulations, differing only in fragrance. Airborne particulates were monitored using a TSI aerodynamic particle sizer (APS) following a controlled burning paradigm. Mean aerodynamic diameters of airborne particles for all exposures were 0.55µm–0.63µm. Particle mass concentrations were similar across all treatments with an average range of 2.0 µg/m^3–6.6 µg/m^3. Twenty-seven (27) healthy adult subjects were exposed for 5 minutes to each of twelve separate incense burns, randomized over a 12–hour period. Ten different incenses were evaluated with one treatment repeated (3x) to evaluate reproducibility of response over time. Irritation was assessed by comparing pre vs. post exposure responses (20 total symptoms grouped as upper respiratory, lower respiratory and nonspecific) *via* questionnaire and pulmonary function *via* FEV_1 measurement. Sensory questionnaires allowed comparison of fragrance strength/aesthetics to reported subjective symptom responses and pulmonary function. No statistically significant differences (Wilcoxon Signed Rank Test; 5% level) were found between pre vs. post exposure for any treatments under any clinical symptom group. Analysis using paired student t-test of FEV_1 data revealed small but statistical differences ($p>0.001$) between pre vs. post exposures for all treatments, the greatest change in any exposure group was a decline in FEV_1 of 0.28 which is not considered clinically significant. No cumulative effect on FEV_1 for particulate exposure in this design was noted and no relationship was apparent between fragrance strength/perception to symptoms or pulmonary function.

1416 EXPOSURE CHARACTERIZATION FROM A FRAGRANCED PLUG-IN AIR FRESHENER.

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Consumer exposure to inhaled fragrance materials is not well characterized, thus raising concerns about asthma, lung disease, and neurological effects. A plug-in air freshener was developed to simulate a product that gives a continuous low level of scent over time. The surrogate formulation contained 20% dipropylene glycol mono-methyl ether (DPM) as a solvent and 8.89% each of benzyl acetate (BA), eugenol, hexyl cinnamaldehyde (HCA), 1, 3, 4, 6, 7, 8-hexahydro-4, 6, 6, 7, 8, 8-hexamethylcyclopenta-γ-benzopyran (HHCB), hydroxycitronellal, β-ionone, d-limonene, linalool, and methyl dihydrojasmonate (MDJ). Representative fragrance materials were chosen based on volatility, chemical structure, toxicity, and volume of use. The product was plugged into a standard wall outlet (110 volts) and was heated to 60-70oC to control emissions for a minimal change in the hedonics of the formulation. Samples of the reservoir were taken at 1, 3, and 6 hours on the first day and twice weekly thereafter. Amount remaining was quantitated using gas chromatography and a nonpolar column, following calibration with neat materials to determine retention times. Current gravimetric analysis showed the greatest losses were seen from Days 1 to 7. The data are presented in the table below. Emission was calculated from absolute and percent weight loss of each material from the reservoir. Losses decreased thereafter and approached constancy. The concentration of total test formulation averaged 0.144 g/d. In a 41 m^3 room, the concentration converts to approximately 0.0035 ppm formulation/d. These data show relative constancy of emission at low levels of exposure and will be used in the design of future clinical studies.

day	0	1-7	8-14	15-20	21-25	26-28
g wt loss/day	0	0.22	0.15	0.13	0.11	0.11

1417 SIMULTANEOUS ANALYSIS OF ACETONE CONCENTRATION IN THE NASOPHARYNGEAL AND EXHALED BREATH OF HUMAN VOLUNTEERS.

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A controlled human inhalation study was conducted to quantitate the nasal clearance of acetone using real-time ion-trap mass spectrometry (MS/MS). Previous investigations in human volunteers have been difficult due to methodological limitations. New methods were used to evaluate nasal wash-in and wash-out during cyclic-breathing to measure nasal clearance in two human volunteers exposed to uniformly labeled 13C-acetone (~1 ppm) for 30 minutes under different breathing maneuvers (inhale nose/exhale nose; inhale nose/exhale mouth; inhale mouth/exhale nose). An air-sampling probe inserted in the nasopharyngeal cavity of the volunteer was connected to an MS/MS system capable of sampling every 0.8 sec. A second MS/MS system simultaneously sampled from the exhaled breath stream *via* a breath-inlet interface. The breathing cycle was monitored throughout the exposure using a plethysmograph. Graphical overlays of the plethysmography and MS/MS results show clear differences in 13C-acetone levels in the nasopharyngeal

region as a function of breathing maneuver. In both volunteers steady-state 13C-acetone concentrations were rapidly obtained; 40 to 75% of the compound was absorbed, depending on the breathing maneuver. For one volunteer the overall nasopharyngeal clearance followed the order of mouth inhale/nose exhale > nose inhale/nose exhale >/= nose inhale/mouth exhale. Whereas, clearance profiles in the other volunteer were comparable regardless of breathing strategy. The simultaneous acquisition of both nasopharyngeal and exhaled breath indicated that exhaled 13C-acetone exceeded the nasopharyngeal concentration by as much as a factor of 2, which is consistent with a wash-in and wash-out of acetone during cyclic-breathing. These results demonstrate the utility of real-time MS/MS analysis to quantitate nasal clearance of vapors in humans and should prove useful for refining current default risk assessment assumptions. (Supported by EPA Contract 68-C-00-122).

1418 DEVELOPMENT OF A MODEL SYSTEM TO EVALUATE VINYL ACETATE-INDUCED INTRACELLULAR ACIDIFICATION IN NASAL EPITHELIUM.

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Vinyl acetate is a synthetic organic ester that has been shown to be carcinogenic to rats after inhalation exposure. Vinyl acetate is metabolized by carboxylesterase to form acetic acid and acetaldeyde, which is a key step in the pathogenesis of non-neoplastic and neoplastic responses. A pharmacokinetic and pharmacodynamic model for the effects of vinyl acetate predicts that three protons should be produced per mole of vinyl acetate causing acidification of exposed tissues. Isolated rat hepatocytes have demonstrated an intracellular drop in pH upon exposure to vinyl acetate using ratiometric fluorescent imaging. The objective of this study is to develop a model system using ratiometric confocal microscopy to monitor intracellular pH changes and to test the hypothesis that vinyl acetate metabolism in nasal epithelium leads to a reduction in intracellular pH. Respiratory and olfactory turbinate explants were removed from male CrlCD:BR rats. Cells were isolated from the turbinates and plated onto culture dishes for imaging. Cells were loaded with the pH sensitive dye SNARF-1 for 30 min. Using standard confocal laser excitation (488nm), the ratio of SNARF-1 emission at 620-680nm to emission at 550-600nm was measured for various conditions. Exposing the cells to pH standard solutions of pH 7.5, 7.0, and 6.5 containing nigericin generated a pH calibration curve. Cells were exposed to up to 1mM vinyl acetate or 30mM NH_4Cl solution as a positive control. After exposure, cells were washed to show that the responses were reversible. Vinyl acetate exposures produced a decrease in intracellular pH. Conversely, exposure to 30mM NH_4Cl causes a significant increase in intracellular pH for isolated cells. Both effects were reversible. These results show that vinyl acetate can lower pH in nasal epithelium and the that intracellular pH can be measured using a confocal imaging system. (Supported by the Vinyl Acetate Toxicity Group).

1419 INHIBITION OF HEAT SHOCK PROTEIN INDUCTION IN MOUSE OLFACTORY EPITHELIUM BY *IN VIVO* ADMINISTRATION OF PURINERGIC RECEPTOR ANTAGONISTS.

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Heat shock proteins (HSPs) accumulate in cells exposed to a variety of physiological and environmental factors such as heat shock, oxidative stress, toxicants and odorants. Ischemic, stressed, and injured cells release ATP in large amounts; moreover, a recent toxicology study showed that intracellular ATP significantly decreased when the olfactory epithelium (OE) was damaged by noxious fumes, presumably through release by injured cells[1]. Our hypothesis is that noxious stimulation (in this case, strong odor) evokes the release of extracellular ATP in the OE. Extracellular ATP, a signal of cellular stress, induces the expression of HSPs *via* purinergic receptors. As previously shown in rats[2], *in vivo* odorant exposure (0.02% heptanal, 3 hours) led to transient selective induction of HSP25 and HSP72 in sustentacular support cells in Swiss Webster mouse OE. We investigated whether administration of purinergic receptor antagonists suramin and pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid (PPADS) can block the induction of HSP expression. Mice were injected with 100 μmoles/kg suramin + PPADS and exposed to 0.02% heptanal for 3 hours. Purinergic receptor antagonists blocked the expression of HSP25 and HSP72 in sustentacular support cells. The olfactory neuroepithelium is constantly exposed to airborne pollutants and microbes, consequently, it continuously regenerates. ATP released by acutely injured cells could act as an early signal of cell and tissue damage, causing HSP expression and stimulating regeneration due to the mitogenic and growth-promoting effects of purinergic receptor activation. This research was supported by NIH NIDCD DC04953. 1. Kilgour JD, Simpson SA, Alexander DJ, & Reed CJ. (2000). A rat nasal epithelial model for predicting upper respiratory tract toxicity: *in vivo*-in vitro correlations. Toxicology 145, 39-49. 2. Carr V, Menco BP, Yankova MP, Morimoto RI, & Farbman AI. (2001). Odorants as cell-type specific activators of a heat shock response in the rat olfactory mucosa. J.Comp.Neurol. 432, 425-439.

1420 SENSORY IRRITATION AND ODOR FROM BRIEF EXPOSURES TO GLUTARALDEHYDE VAPOR.

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Glutaraldehyde (GA) vapor occurs in various occupational settings, most notably in healthcare where glutaraldehyde-based products serve to disinfect instruments. The potency of GA to evoke odor, sensory irritation of the eyes, and nasal pungency in brief exposures (ca. 3 sec for the nose and 25 sec for the eyes), with analytical confirmation of concentration, merits attention. A psychophysical study of 53 females, aged 18 to 35 years and screened for normal health of the upper airways and eyes, led to psychometric functions for odor detection (n=43), detection of ocular irritation (n=41), and nasal pungency (n=40). The methodology entailed performance in forced-choice tasks and repetitive verification of delivered concentrations by the OSHA 64 method during testing. The subjects in each task yielded their own individual functions. Subjects began to detect odor above chance at concentrations below 0.1 ppb and reached 50% detection at 0.3 ppb. The subjects began to detect the chemesthetic properties of ocular irritation and nasal pungency above chance at concentrations of about 200 ppb and reached 50% detection in the zone 400-500 ppb. The functions for chemesthesis rose particularly sharply, from near-chance performance to near-perfect detection, over a span of concentration of 4:1. For subjects who achieved 50% chemesthetic detection by the highest test concentration of 800 ppb, the geometric coefficients of variation equaled 1.6 and 1.5 for ocular irritation (n=34) and nasal pungency (n=25), respectively. For these brief exposures, no subjects gave evidence a chemesthetic threshold as low as the STEL of 50 ppb. Supported by the Dow Chemical Company and BASF.

1421 MUCOUS CELL METAPLASIA IN RAT NASAL EPITHELIUM AFTER A 13-WEEK EXPOSURE TO CARBON BLACK PARTICLES.

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Rats chronically exposed to high levels of carbon black particles (Cb) develop numerous and persistent lung lesions including chronic alveolitis, alveolar septal fibrosis, and type II cell hyperplasia. The purpose of the present study was to determine if long-term inhalation of Cb would also cause chronic lesions in the nasal airways of rats. Female F344 rats were exposed to three concentrations (1, 7, 50 mg/m3) of high surface area Cb (HSCb) or one concentration (50 mg/m3) of low surface area Cb (LSCb) for 6 h/day, 5 days/wk for 13 wk. Rats were sacrificed one day or 13 wk post-exposure (PE). The nasal airways of each rat were processed for light microscopic analysis. Chronic rhinitis and mucous cell metaplasia (MCM) with increased numbers of mucous goblet cells and increased amounts of intraepithelial mucosubstances (IM) in transitional and respiratory epithelium were present in rats exposed to HSCb. Persistant HSCb-induced nasal lesions were evident only in rats exposed to 7 or 50 mg/m3. Standard morphometric techniques were used to determine the amounts IM in the surface epithelium lining the nasal airways. The magnitude of these nasal lesions was both dose- and time-dependent. Rats exposed to 50 mg/m3 HSCb and sacrificed at one day and 13 weeks PE had approximately 750x and 8x more IM, respectively, in the maxilloturbinates than that of control rats exposed only to filtered air (0 mg/m3 Cb) and sacrificed at similar times PE. Interestingly, rats exposed to 50 mg/m3 LSCb had no nasal lesions. These results indicate that Cb-induced nasal lesions in rats were dependent on dose, particle surface area, and time PE. The persistent nature of the HSCb-induced MCM in rats suggests that Cb exposure may have the potential to induce similar long-lasting alterations in the nasal airways of humans (Research sponsored by the International Carbon Black Association).

1422 QUANTIFICATION OF A 3-METHYLINDOLE MERCAPTURATE ADDUCT IN THE URINE OF CIGARETTE SMOKERS.

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3-Methylindole (3MI) is a degradation product of the amino acid tryptophan, and a pyrolysis product present in cigarette smoke. The pnuemotoxicity of 3MI is well established, and its toxicity is dependent on activation by cytochrome P450 en-

zymes. The most reactive of the intermediates formed *via* dehydrogenation of 3MI is 3-methyleneindolenine. This highly reactive electrophile is trapped endogenously with glutathione and the mercapturate adduct can be detected and quantified in urine by HPLC. Urine samples were obtained from cigarette smokers participating in a smoking cessation study. Samples were obtained before and after cessation. The quantity of mercapturate present in urine increased with the number of cigarettes to a maximal value of 558 nmols mg creatintine-1 ml-1 at 15 cigarettes smoked per day. Interestingly, at numbers higher than 20 cigarettes/day the detectable amount of adduct begins to decrease and ultimately is reduced to a low value of 20 nmols mg creatintine-1 ml-1 at 35 cigarettes per day. Adduct levels dropped by an average of 147% after 30 days without smoking. The non- linearity of adduct concentration vs. number of cigarettes consumed can be interpreted in at least two ways. Previous studies in our laboratory indicated that 3-methyleneindolenine inhibits the P450 enzyme (CYP2F1) required for its formation. Therefore, at higher substrate concentrations, the enzyme is inactivated and less detectable product is formed. Alternatively, cytochrome P450 expression in lung is highly cell-type specific, and another interpretation of these data is that the cells responsible for the majority of the metabolic activity are killed at higher cigarette consumption levels. (Supported by NIH Grant HL13645)

1423 INHALATION OF CADMIUM AT A CONCENTRATION ASSOCIATED WITH SIDESTREAM CIGARETTE SMOKE ALTERS ANTIMICROBIAL HOST DEFENSE.

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Cadmium (Cd), a potent immunotoxicant and human carcinogen present in cigarettes, is released into indoor environments in sidestream smoke (SS). Depending on the cigarette type and tobacco source, smoking a pack of cigarettes introduces 8 - 13 ug Cd into the general indoor environment. Inasmuch as children in homes where members smoke have a greater frequency of lower respiratory tract infections (LRTI) than those in non-smoker homes, a laboratory study was undertaken to determine the role of a single smoke component as a concomitant stressor in suppressing lung immune defense mechanisms. Five- and 3-wk-old rats were exposed by nose-only inhalation to 80 ng CdO for 1 or 3 wk (5 hr/d), respectively. At different timepoints post-exposure, rats were either: intratracheally-instilled with *S. pneumoniae*and bacterial burdens subsequently assessed; sacrificed and their lungs fixed for histopathologic analyses or lavaged for cytokine and immune functional measurements; or, sacrificed and their spleens used to assess lymphoproliferation. Peripheral blood was used to evaluate blood cell profiles. Rats exposed for 1 wk to CdO and then challenged 1 or 2 d post-exposure with *S. pneumoniae* evidenced a 50% increase in bacterial lung burdens 4 hr post-infection compared to infected air-exposed controls; by 7 d, bacterial numbers reached control levels. Although inhalation of CdO for 1 or 3 wk had no effect on lung histology, lavage cell differentials, circulating blood cell profiles, or lymphoproliferation, macrophage oxyradical production was depressed for up to 7 d post-exposure in rats from both regimens. Results of this study not only provide information necessary for defining health risks of low-dose exposure to inhaled Cd particulates, but offer a possible mechanism underlying the relationship between SS and decreased resistance to LRTI in infants. Philip Morris Foundation.

1424 TOBACCO SMOKE EXPOSURE AND BONE STRENGTH IN MICE.

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Smoking has been implicated in early onset of menopause and the development of osteoporosis (bone fragility) in women smokers. In the present study, we have measured various biomechanical properties of femurs (cortical bone) obtained from control and smoke-exposed mice to determine if experimental exposure to cigarette smoke influences bone strength characteristics. Female C57BL mice were exposed to sidestream cigarette smoke in a whole-body exposure chamber, set at 30mg smoke particulates/m^3 for 4hrs/d and 5d/wk for 12 consecutive weeks. Femurs were collected and frozen (-20^{0}C) in saline for subsequent biomechanical testing. Mid-shaft femurs were mechanically tested in 3-point bending (Instron 5543, MA) at a rate of 3mm/minute and then analyzed for various structural (ultimate & yield load, and stiffness) and material (ultimate & yield stress and flexural modulus) strengh parameters. Elevated levels of blood carboxyhemoglobin and pulmonary CYP1A1 protein in smoke-exposed mice confirmed their effective exposure to gaseous and particulate constituents of smoke. The data (Table) showed that flexural modulus, a material strength parameter, was significantly decreased ($p<0.05$), and stiffness, a structural parameter, was marginally reduced ($p<0.1$) in femurs obtained from smoke-exposed mice when compared to control group. We conclude that exposure to tobacco smoke adversely affects some biomechanical strength properties of cortical bone in mice.

FEMORAL BONE STRENGTH (mean±sd)

	Control (n=16)	Smoke-exposed (n=16)
Ultimate load (N)	16.9±2.4	17.8±1.98
Yield load (N)	14.2±2.5	14.1±2.76
Stiffness (N/mm)	109±22	100±19[a]
Ultimate stress (N/mm^2)	115±20	111±20
Yield stress (N/mm^2)	97±20	89±23
Flexural Modulus (N/mm^2)	2601±527	2165±580[b]

[a]$p<0.1$; [b]$p<0.05$

1425 PYRIDINES THAT INHIBIT DIVERSE BIOLOGICAL PROCESSES ARE MORE CONCENTRATED IN SIDESTREAM THAN MAINSTREAM SMOKE SOLUTIONS FROM COMMERCIAL BRAND CIGARETTES.

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Several pyridine derivatives that have an adverse effect on both oviductal functioning and chick chorioallantoic membrane growth at picomolar doses have been identified in cigarette smoke. The purpose of this study was to determine the concentrations of 2-methylpyridine, 2-ethylpyridine, and 3-ethylpyridine in mainstream and sidestream smoke solutions of various cigarettes. The cigarettes selected included regular and light US filtered cigarettes (Marlboro and Camel), several new US brands that claim to have reduced carcinogens (Omni and Advance), and University of Kentucky research 2R1 and 1R4F cigarettes. The relative absorbance of each smoke solution was first measured using a spectrophotometer (λ=300 nm). Sidestream smoke solutions showed a 6- to 10-fold higher absorbance than mainstream. The Omni sidestream smoke solution had the highest absorbance values compared to the other commercial brands. Next, the smoke solutions were extracted and 2-methylpyridine, 2-ethylpyridine, and 3-ethylpyridine were identified and quantified by gas chromatography-mass spectrometry. The concentrations of all three pyridine compounds were higher in sidestream than in mainstream smoke solutions. The sidestream smoke solutions from Advance cigarettes had the highest concentration of 3-ethylpyridine; from Omni cigarettes had the highest concentration of 2-methylpyridine; and from 1R4F cigarettes had the highest concentration of 2-ethylpyridine. This is the first time that this particular group of toxicants has been shown to be in higher concentrations in sidestream smoke solutions. These findings suggest that there may be additional risks associated with exposure to sidestream smoke.

1426 EFFECTS OF THE ADDITION OF BENZYL ALCOHOL TO TOBACCO ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF CIGARETTE SMOKE.

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Benzyl alcohol is a commonly used tobacco flavoring material in cigarettes. An evaluation of the potential effects of benzyl alcohol on the chemical composition and biological activity of mainstream smoke was conducted. Purge and trap analysis showed benzyl alcohol as the major distillation product at 100°C, suggesting that it will volatilize prior to the burning tobacco and not be available for pyrolysis. Benzyl alcohol was incorporated into American-blend cigarette tobacco at a target level of 0.05% and at multiple target levels of 0.5% (10X) and 2.1% (42X). A cigarette without benzyl alcohol served as the control. Mainstream smoke constituents analyzed were selected from those proposed by the US CPSC or classified as human or animal carcinogens by the IARC. Results obtained from genotoxicity (Salmonella Reverse Mutation Assay of the particulate matter), cytotoxicity (Neutral Red Uptake Assay of particulate matter and gas vapor phase), and inhalation toxicity

(90-d, rat nose-only whole smoke inhalation) were comparable to those obtained with the control cigarette. Smoke constituent changes occurring in benzyl alcohol treated cigarettes were generally within 20% variation of controls and considered unrelated to benzyl alcohol addition. This assessment suggests that benzyl alcohol did not extensively alter the normal chemical balance nor biological activity of the cigarette smoke, even at the exaggerated levels tested.

1427 CHARACTERIZATION AND VALIDATION OF A RODENT NOSE-ONLY EXPOSURE SYSTEM FOR ACUTE TOXICITY EVALUATION OF POLYMER AND TOBACCO COMBUSTION PRODUCTS.

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An apparatus consisting of a Deutsches Institut für Normung (DIN) 53-436 moving furnace, connecting glasswares, and 24-port nose-only inhalation unit, was constructed and validated to evaluate irritant effects of tobacco/polymer combustion products (CPs). To approximate cigarette burning conditions a furnace temperature (FT) of 750°C and DIN wet total particulate matter (DWTPM) of 150 µg/L were selected. Male SW mice (12/run) were exposed once for 30 min to 100% tobacco blend (TB) or 70/30 (wt/wt%) TB-Nylon 12 (N12) CPs. CO/CO_2, NO/NOx, DWTPM, hydrogen cyanide (HCN), acetaldehyde, formaldehyde, acrolein, and nicotine were measured in the test atmosphere. Respiratory function (Buxco®) during exposure, postexposure blood cyanide (CN^-) levels, respiratory tract histopathology (1 & 7 days postexposure) and alveolar macrophages (AM), LDH and protein changes in BAL fluid (Day 1) were evaluated. Validation demonstrated steady DWTPM and spatial uniformity (<10%). Nose port temperature (22-26°C), RH (45-75%) and FT (750±20°C) were acceptable on runs conducted over 3 separate days. Variability for most analytes was within 10%. Replacement of tobacco mass with N12 resulted in nicotine, and NO/NOx decreases, and increases in DWTPM, HCN, aldehydes and CO relative to the TB run. Reductions in respiratory rate and minute volume were slightly greater in the N12-group than TB-group. Blood CN^- levels were below LOD for both groups. Day-1 postexposure lesions in nasoturbinate, lateral wall, and maxilloturbinate were minimal to moderate and limited to nose level 1. Vacuolization and necrosis were more severe in the N12-group compared to TB-group, but these lesions resolved by day 7. No changes in BAL parameters were noted, except a decrease on the ratio of resting/activated AM in the N12 and TB-groups compared to naïve control. This validation and pilot study have shown that the system may be useful in the acute toxicity assessment of CPs.

1428 COMPARATIVE ANALYSIS OF BRONCHOALVEOLAR LAVAGE (BAL) FLUID BIOMARKERS IN MICE FOLLOWING ACUTE CIGARETTE SMOKE EXPOSURE.

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Although potential biomarkers of effect (e.g. inflammation) have been used clinically to evaluate cigarette smoke (CS), their link to chronic obstructive pulmonary disease is not clear. The use of animal models can help clarify the association between biomarkers and disease. Recent studies suggest that C57/BL/6 (C57) mice develop emphysema following chronic CS exposure, while ICR mice do not. Based on this information we investigated acute mainstream cigarette smoke-induced biomarker responses in these two mouse strains. Young male C57 and ICR mice received 2-hr nose-only inhalation exposures to CS from 1R4F reference cigarettes (600µgTPM/L) or filtered air for 1 or 7 consecutive days. BAL fluid samples (n=3-6) were collected at 0, 4, 12, and 24 hours post-exposure and analyzed for potential biomarkers of effect {LDH, protein, cell differentials, KC (IL-8), IL-13 TNFα, IFNγ, IL-5, IL-10, IL-6, IL-1β, GM-CSF, GSH/GSSG, 8-isoprostane, collagenase/elastase and desmosine}. At 0-hr blood samples were analyzed for biomarkers of exposure (HbCO, nicotine and cotinine). Exposure biomarkers were slightly greater in the C57 relative to the ICR. After 1-day exposure ICR KC levels were significantly increased 2.8-fold over C57 at 4 hrs post-exposure. Following 7-days exposure the peak response shifted from 4 to 12 hrs post-exposure and increased 19 and 28 fold in C57 and ICR respectively. Also at 12 hrs, ICR BAL fluid KC and GM-CSF levels were significantly increased 4.3 and 2.2 fold respectively over C57. Neutrophils (3.5 folds) were greater in exposed ICR relative to C57 at each time point. In summary, ICR mice had a greater acute CS-induced biomarker response when compared to C57 mice. This suggests that while rapid changes in certain biomarkers may provide relevant information about early CS-induced biological effects, more studies are needed to determine if they are predictive of disease.

1429 VALIDATION OF A NEW LUNG FUNCTION METHOD IN ORALLY INTUBATED MICE USING A MODEL FOR RESPIRATORY ALLERGENICITY.

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Rationale: The aim of the study was to compare a novel system for invasive lung function measurements in mice with standard lung function measurements in conscious mice. Therefore, airway response was measured in a model of fungal asthma. Methods: Female BALB/c mice were sensitized with Aspergillus fumigatus extract (AF) on day 0, boosted on day 14 and challenged intratracheally or inhalationally on day 21 (AF groups). Negative controls (NEG) received the vehicle alone. Lung function was measured during inhalational allergen challenge to assess early airway response (EAR) and 48 h after challenge to assess airway hyperresponsiveness (AHR) by provocation with increasing doses of methacholine (MCh) aerosol. Two groups (AF, NEG) were anesthetized, intubated orally and placed into a novel whole-body plethysmograph in supine position to measure lung resistance, dynamic compliance and midexpiratory flow (EF50). For comparison, two additional groups (AF, NEG) were placed consciously in a head-out plethysmograph to measure EF50 to assess AHR. Results: The conscious AF animals showed a marked hyperresponsiveness: the MCh dose needed to induce a 50% decrease in EF50 was 3.3 times lower compared to NEG. In comparison, the intubated AF animals showed also a marked hyperresponsiveness: the MCh dose needed to induce a 50% decrease of EF50 was 3.1 times lower, and to induce a 100% increase in resistance was 3.2 times lower than compared to NEG. In addition, a marked early airway response was found in this model of allergic asthma: lung resistance was increased 266 % above baseline value (NEG: 32 %). Conclusion: The new technique is a valid method to measure allergic airway responses precisely without injuring the animals in a model for respiratory allergenicity.

1430 ASSESSMENT OF A SINGLE CONTROLLED VENTILATION INHALATION EXPOSURE TO A THERAPEUTIC PEPTIDE IN CYNOMOLGUS MONKEYS.

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Delivery of therapeutic peptide molecules to the circulatory system *via* pulmonary inhalation is of great interest to the pharmaceutical industry. Using a controlled ventilation inhalation procedure and a target tidal volume of 75% vital capacity, an aerosol of DX-88, a recombinant kallikrein inhibitor peptide, co-mixed with the radioactive tracer Technetium-99m was delivered into the lungs of six anesthetized female Cynomolgus monkeys. Immediately following dosing, whole-body gamma scintigraphy images were collected to assess the lung deposition pattern of the aerosol and quantitatively determine the mass of DX-88 deposited within the lungs. Blood was collected post-exposure at predetermined time points over 24 hours and serum was analyzed for DX-88 concentration. Pharmacokinetics of DX-88 was evaluated by non-compartmental methods. Toxicity, based on clinical observations, body weights, food consumption, electrocardiology, heart rate, blood pressure and clinical, macroscopic and microscopic pathology, was evaluated through 14 days post-exposure. Scintigraphy analysis of the lung deposition patterns indicated aerosol deposition occurred primarily in the central lung with some deposition also in the trachea. The mean estimated inhaled dose of DX-88 was approximately 3 mg/kg with a measured deposited dose of 2 mg/kg. Pharmacokinetic analysis indicated a peak DX-88 serum concentration (9.93 ug/mL) at approximately 30 minutes post-exposure with a systemic clearance of approximately 1.5 mL/min/kg and a terminal half-life of 130 minutes. There were no remarkable clinical findings related to DX-88 over the conduct of the study. This study demonstrated that a single inhaled dose of DX-88 could be effectively delivered to the circulatory system with no adverse toxicity.

1431 EXPOSING THE ISOLATED PERFUSED RAT LUNG TO RESPIRABLE AEROSOLS OF DRUGS AND POLLUTANTS.

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The isolated perfused lung (IPL) of rodents is a valuable model for studying absorption and local metabolism of pollutants and drugs in the lungs. However, there have been relatively few options for exposing the IPL to respirable aerosols of dry powders. We have connected a previously developed aerosol generator for bolus

type exposures to the IPL of the rat. The generator uses compressed air to create a short burst of a highly concentrated aerosol in a 300 mL holding chamber. The particle size typically ranges from 1-3 μm. We exposed the rat IPL to tritium-labelled diesel soot at an air concentration of ~3 mg/L and a flow rate of 300 mL/min in a minute-long exposure immediately after aerosol generation. The lungs were ventilated at 60 breaths/min and the tidal volume was 0.7 mL. At the end of exposure the lungs were removed for determination of soot deposition in major airways and lung lobes. Results show that at the present flow rate, ~3% of the soot in the air passed over the lungs was deposited. Based on the amount inhaled, the deposition was ~20%. More than 90% of the deposited soot was located from the level of the lobar bronchi and below, indicating a highly respirable aerosol. We will use the system for studying mechanisms of absorption and metabolism of toxicants and drugs in the lungs. (Research supported by the Swedish Agency for Innovation Systems, Vinnova, and the Swedish Council for Working Life and Social Research, FAS)

1432 THE ASSESSMENT OF PULMONARY INFLAMMATION IN RODENTS USING EXHALED BREATH.

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Identification of individuals exposed to biological warfare (BW) agents, prior to the onset of clinical symptoms, is of extreme importance in the resolution of situations where a BW attack is suspected. The pulmonary system is a major portal for the introduction of BW agents and the early inflammatory response of the lung may be useful for the identification of the BW organism and the evaluation of exposed individuals. Exhaled breath has been used to assess the physiological state of humans by examining the levels of recoverable volatile compounds. More recently, there has been an effort to recover non-volatile compounds, such as proteins, with some success in humans and cattle. The work described here focuses on the capture, in the exhaled breath of laboratory rodents, of proteins elaborated due to pulmonary inflammation. Fifty mice were placed in a nose-only Canon exposure tower and the exhaled breath was condensed in dry ice-acetone-cooled collection vessels. The direction of airflow through the tower was reversed to minimize the loss of condensate on the stainless-steel surfaces. The recovery of water as condensate was approximately 80% of the maximum calculated volume of water in the exhaled breath. Bronchoalveolar lavage (BAL) was performed on ten animals to determine the inflammatory status of the lung. Protein concentrations in the exhaled breath condensate were at or near the limit of detection (1 μg/ml) of the assay and were approximately equivalent to 0.001% of the protein recovered by BAL. Pulmonary inflammation elicited by endotoxin treatment was readily detectable in BAL fluid as increased concentrations of protein, tumor necrosis factor, and interferon (IFNg). Very low concentrations of IFNg were detectable in concentrated exhaled breath condensate and corresponded to approximately 0.001% of the levels measured in the BAL fluid. The apparent capture of a protein indicative of inflammation was demonstrated. Optimization of system parameters will include the use of other species, such as the laboratory rat.

1433 ARGININE METABOLISM IN LUNG TISSUE: IMPLICATIONS FOR MEASUREMENT OF NOS ACTIVITY.

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Nitric oxide synthase(NOS), converts L-arginine to nitric oxide (NO) and citrulline. Synthesis of NO requires NADPH and other co-factors. Within the lung, NO plays a key role in regulation of pulmonary artery pressure, cytotoxicity, and inflammation. Thus, inactivation of NOS by inhaled toxicants can have serious health effects. We are investigating the effects of *in vivo* tobacco smoke exposure on NOS activity assayed by the conversion of 3H L-arginine to 3H L-citrulline. We examined whether this technique is subject to interference by other pathways of arginine metabolism when it is used on lung homogenates. Frozen mouse lungs were homogenized and centrifuged at 100, 000 g for 1 hr. The cell membrane (pellet) and cytosol (supernatant) fractions were added to buffer containing 3H labeled L-arginine, unlabeled arginine, NADPH, BH4, calmodulin, and calcium. The reaction solution was incubated for 40 min at 37 C. Ion exchange columns were used to retain 3H L-arginine and 3H L-citrulline was collected from the filtrate. Both pellet and supernatant fractions produced 3H L-citrulline but the rate of production was higher in the absence of NADPH, indicating the presence of a non-NADPH dependent pathway of arginine metabolism. When N-hydroxy-nor-L-arginine, an arginase inhibitor, was added to the reaction mixture, all of the citrulline production by the lung pellet and supernatant fractions was NADPH dependent (ie due to NOS). Arginase converts arginine to ornithine. Ornithine can be metabolized to citrulline in tissues with an intact urea cycle. Although the lung lacks the enzyme to convert ornithine to citrulline, addition of 3H ornithine to lung homogenates under conditions used to measure NOS activity resulted in radiolabeled material that passed through the ion exchange column. This result indicates that measurement of NOS activity in lung homogenates can give inaccurate results due to interference by arginase. Also competition between arginase and NOS for arginine is another mechanism that can potentially affect NO production in the lung. Supported by a grant from Phillip Morris

1434 IDENTIFYING POTENTIAL RESPIRATORY SENSITIZERS: PROTEINS.

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Exposure to respirable xenobiotic proteins has been linked to the development of type I respiratory allergy. Application of strict exposure guidelines can minimize occupational exposure, but testing guidelines to distinguish between allergenic and antigenic proteins are not available. In this study male BDF1 mice were intranasally instilled (50μl/instillation) with saline or saline containing 0.05, 0.5, or 5.0 μg of an allergenic bacterial protease (Subtilisin Carlsberg; SC), or 0.5, 5.0, or 25 μg of a potent protein antigen (Keyhole limpet hemocyanin; KLH) on days 1, 3, 10, 17, and 24. Exposure-related differences in airway hyperreactivity of mice exposed to 5.0 μg doses of SC or KLH were assessed, on day 25, by methacholine challenge in whole-body plethysmography chambers. Mice were sacrificed on days 11 and 15 (3 instillations) or days 25 and 29 (5 instillations). Serum levels of total IgE and protein-specific IgG1/IgG2a were determined by ELISA. Right lung lobes were lavaged to determine total and differential inflammatory cell counts. Left lung lobes were analyzed to determine the amount of stored mucosubstances in epithelium lining large (G5) and small (G11) diameter main (axial) pulmonary airways. Both SC and KLH induced similar dose and time-dependent increases in stored mucosubstances and influx of eosinophils into bronchoalveolar airspaces. Both proteins induced dose and time-dependent increases in total serum IgE levels and modest increases in IgG2a, but, only SC also induced elevated levels of IgG1. Only SC-exposed mice had increased airway hyperreactivity (increased sensitivity to methacholine challenge) compared to saline-instilled control mice. These data suggest that elevated antigen-specific IgG1, not total IgE, levels are most predictive of a neoantigen's potential to induce airway hyperreactivity. We are testing additional proteins and propose that both total IgE levels and antigen-specific IgG1/IgG2a ratios must be used to assess the potential of xenobiotic proteins to induce allergic respiratory sensitization.

1435 OBSERVATIONS AND RECOMMENDATIONS REGARDING THE BUXCO AEROSOL DELIVERY/UNRESTRAINED PLETHYSMOGRAPH SYSTEMS.

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The BUXCO rodent plethysmograph exposure system is an aerosol delivery device used primarily for conducting methacholine airway challenge tests in rodents. Operation and configuration of the exposure system will affect the aerosol characteristics and therefore the methacholine challenge response. For nebulizers, different volumes of the same concentration of solution placed in the nebulizer reservoir will yield significantly different aerosol output (~factor of 2 for 2 x volume of same solution concentration). However, different volumes of solution did not affect the size distribution of the aerosol, with observed methacholine aerosol size distributions consistently in the 2-micron size range (mass median diameter). The caliber and length of aerosol delivery lines may affect background environmental noise levels as measured within the animal chambers, and may also create moderate but observable distortion in the respiratory signals, which has potential impact on PenH computations. Tuving of various calibers (internal diameters) have been investigated to asses the degree of these effects. Airway challenges are typically conducted in sequence. When challenges are conducted sequentially, it was observed that both methacholine concentration and humidity have a lag time for clearance from the chamber. It is important that the system is thoroughly flushed during sequential use, or residual methacholine and humidity will build up in the chamber, potentially confounding concentration-response relationships potentially causing water condensation that could plug aerosol transit lines. Part of this work supported by the National Environmental Respiratory Center with support from multiple government and industry sponsors, including the USEPA. This abstract does not represent the views of any sponsor.

1436 EVALUATION OF THE EFFECTS OF INTERMITTENT EXPOSURE TO ELEVATED INHALED CO_2 CONCENTRATIONS UPON BLOOD GAS AND HEMATOLOGIC PARAMETERS IN THE CANINE.

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Evaluation of inhaled therapeutics in repeat-dose non-clinical studies is a critical step in approval of inhaled therapeutics for use in man. Deep lung deposition is the preferred site of deposition for many inhaled therapeutics. Since tidal breathing animals use only a small portion of their total lung volume, improved penetration into and deposition of aerosols in the lung could be achieved through increased tidal volume. Increases in inspired CO_2 will produce increases in minute ventilation as the body attempts to maintain acid-base balance. The increase in minute ventilation will be produced primarily through increases in tidal volume. Six beagle dogs (3/sex) were exposed to an approximately 4% CO_2 atmosphere using a head-dome exposure system daily for 1 hour for 7 consecutive days. Respiratory parameters were monitored before and during exposure. Venous blood gases (pO_2 and pCO_2) and pH were monitored before and after each exposure. Blood samples were collected before and after the 7-day exposure period for hematological evaluations. Increased concentration of CO_2 in the test atmosphere resulted in increases (2.1 to 2.5 fold relative to pre-exposure values) in tidal volume, minute volume and peak inspiratory flow. Small (1.1 to 1.2 fold) increases in frequency and inspiratory time were observed. Generally, blood levels of pO_2 and pCO_2 increased and pH decreased post exposure relative to pre exposure values. Evaluations of erythroid parameters indicated a decrease in red blood cell count, hematocrit, hemoglobin and percent reticulocytes after seven days of exposure when compared to pre-study baseline. There were little or no changes in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration or mean corpuscular volume. While increased respiratory pattern control and increased inhaled volumes would be a useful tool in non-clinical repeat dose inhalation toxicity studies, the use of increased concentration of CO_2 in the test atmosphere in the non-anesthetized animal model would require further studies.

1437 GENERATION OF LIFE-LIKE RODENT NASAL MODELS.

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Transport of gases and particles in the upper respiratory tract can be simulated and have been used to predict airflow and gas uptake patterns in the three dimensionally reconstructed nasal passages of F344 rats (Kimbell et al., 1997, Toxicol. Appl. Pharmacology 145, 388-398; Kimbell et al., 2001, Toxicol. Sciences. 64, 100-110; Moulin et al., 2002, Toxicol. Sciences. 66, 7-15). The model used in the previous studies was made from step sections through a fixed plastic embedded specimen. One potential source of error with this type of specimen is decreased surface area to nasal airway volume ratio for a dead, fixed specimen compared with those visually evaluated from MRI scans of live rodent nasal airways (Wiethoff et al., 2001, Toxicol. Appl. Pharmacology 175, 68-75). To obtain more life-like specimens for modeling, rats were killed with CO2 and not exsanguinated. This helped to maintain the natural size and shape of the highly vascularized nasal turbinates and septum. The specimens were fixed very briefly in 10% neutral buffered formalin. The nasal airways of the specimens were filled with cryo-embedding medium, which had been saturated with Xerox copy toner to enhance the contrast of the airway with the tissue structures and provide support during sectioning. The specimens were frozen and step-sectioned. Photographs of the block surface were taken and airway perimeter, area and x, y coordinate files were generated using image analysis software. Evaluation of images indicated that the structures of the rat nasal turbinates are much larger in the frozen specimen than they appeared in previous paraffin or plastic embedded slide sets confirming what was seen by Wiethoff et al. in mouse MRI scans. These data show airway volume to be decreased with much of the airway space seen in fixed paraffin or plastic sections taken up by the enlarged turbinates. Computational meshes can be constructed from this data set and used for simulations of airflow, gas uptake and/or particle deposition. This study provides a next step in model refinement by providing more life-like dimensions for the rat nasal turbinates and airways.

1438 AN AUTOMATED AEROSOL SYSTEM THAT USES REAL-TIME DOSIMETRY FOR INHALATION EXPOSURES WITH PRIMATES.

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Well-characterized inhalation exposure systems are critical for preclinical testing and pathogenesis studies involving nonhuman primates (NHP). Estimation of dose in inhalation studies has been traditionally determined through the use of predictive measures such as whole-body plethysmography (WBP) or estimated from historical data. A dynamic aerosol system has been developed that integrates complete data acquisition and control for all exposure parameters during inhalation. Among the functions is real-time respiratory measurement, which allows the length of exposure to be based on respiration during exposure rather than reliance upon predictive measures. This function automatically compensates for fluctuations in respiration during exposure. Anesthetized rhesus macaques (n=15) were sham-exposed to test the dosimetry function of the newly-developed system. WBP was performed immediately before dosing with the automated system. A cumulative tidal volume (10 liters) was used to demonstrate the dosimetry function. Time to reach the requisite cumulative tidal volume ranged from approximately 1 to 7 minutes and was weakly associated with animal weight ($r^2 = 0.26$). Analysis of the variance between methods was significantly different ($p<0.01$); similarly, there were intra-animal differences in breath frequency, tidal volume, and minute volume measurements in 10/15 (67%; $p<0.01$) of the NHPs under study. A post hoc calibration of the WBP unit with a mechanical ventilator indicated a 5-10% reduction of tidal volume measurement as breath frequency or supplied tidal volume increased; the automated system showed minimal differences within the same range. Results indicate that the real-time dosimetry function returns accurate respiratory measurement and closed-loop computer controlled feedback improves real-time data collection for the entire inhalation procedure. These preliminary data demonstrate that real-time dosimetry can be successfully integrated as a control mechanism into a complete system for aerosol dosing of NHPs with superior accuracy and precision.

1439 1, 3-BUTADIENE SOOT (BDS) AS A STANDARD REFERENCE MATERIAL FOR INHALATION TOXICOLOGY STUDIES.

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Incomplete combustion of 1, 3-butadiene gives rise to BDS containing a broad spectrum of PAHs (128-2000 amu and beyond) in high amounts (1000-5000 cigarette equivalents of the benzopyrene isomer series). The BDS particles also contain other mutagenic PAHs, stable and transient free radicals and strong oxidants. Under simple constant burn conditions, 94 % (w/w) of the particles reproducibly are <4 um. Human lung epithelial cells (NHBE and BEAS-2B) treated with BDS extracts, or BDS alone, in serum-free culture exhibited genotoxicity, autofluorescence, morphological alterations and conspicuous cell blebbing after one treatment and incubation times of 4 - 72 h. Direct exposure of the cells to BDS a) in the plume and b) by addition of collected particles on the surface of the culture medium yielded the same effects more rapidly when the DMSO vehicle was absent, indicating that DMSO was unnecessary for BDS bioassays. Indeed, BDS may have interacted with the DMSO, thus attenuating the observed BDS toxicity. As indicated by SEM and dynamic light scattering experiments, microparticles (<0.5 um) moving through the medium to the cells were sufficient to elicit autoflourescence and other endpoints. Potentiometry and voltammetry data supported these observations: neat BDS added directly to the surface of the culture medium produced rapid changes in solution redox potential. These data and those from elemental and organic chemical analyses indicate that BDS particles have highly reproducible size frequency and compositional features that favor its use as a standard material for inhalation toxicology studies. As the mixture is almost completely PAH in nature, the effects of polar species, trace metals, and inorganic compounds on observed endpoints can be discounted. Further, BDS can be applied directly (no vehicles) to putative target cells in culture, mimicking *in vivo* exposures. This allows for alignment and intercomparison of dosimetry and observed effects in cell culture assays with those in inhalation studies *in vivo.*

1440 OXYGEN TENSION AFFECTS PHENOTYPE IN CULTURED BONE MARROW-DERIVED MACROPHAGES.

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The unique role of alveolar macrophages (AM) in clearance without inflammation, while maintaining pro-inflammatory capabilities, requires complex regulation. A better understanding of normal lung immune homeostasis could lead to vast improvements in our ability to address chronic inflammation following environmental exposures. This study tested the hypothesis that AM are functionally adapted to the relatively high oxygen partial pressure (PO2) of the lung, and that this induces them to be predominantly immune suppressive through modification of redox sensitive transcription factor pathways. Balb/c mouse bone marrow derived macrophages (BMC) were differentiated under two different PO2, and then compared functionally to AM and peritoneal macrophages (PM) using flow cytometry, and microtiter assays for phagocytosis and antigen presentation. BMC differentiated in normoxia (PO2 140 mmHg, BMC140) are similar to AM in having low ex-

pression of surface markers of activation, and showing relatively poor phagocytic and antigen presenting cell (APC) activities. However, BMC grown in low oxygen tension like that found in other tissues (<40 mmHg, BMC40), express more activation markers and are better phagocytes and APCs, making them similar to peritoneal macrophages (PM). We have also shown that BMC140 are more oxidative intracellularly than BMC40, based on oxidation of the fluorescent dye, dichlorofluorescein (DCF), and produce more glutathione (GSH), possibly to compensate. Finally, NF-kB translocation in response to lipopolysaccharide (LPS), measured by laser scanning cytometry and immunofluorescence, was reduced in BMC140 as well as in AM, compared to BMC40 and PM, respectively. These data suggest that the regulation of the immune suppressive AM phenotype may occur, at least in part, *via* inhibition of the NF-kB pathway due to the unique redox environment. Alteration of that redox control following toxic exposures may lead to more active phenotypes that may be responsible for chronic inflammation. Supported by EPA Center Grant and NRSA fellowship, ES11249.

1441 ELIMINATION OF CARBON MONOXIDE IN AWAKE RATS.

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Rats are often used for studying carbon monoxide (CO) poisoning. However the characteristics of CO elimination in rats have not been fully defined. This study examined CO elimination and respiratory patterns in rats exposed briefly (5 min) to high concentration CO (1000, 3000, 6000, and 12000 ppm). Awake male Sprague-Dawley rats (250-380g) were placed into tube restrainers attached to a dynamic exposure chamber. Respiratory measurements were made using partial body flow plethysmographs. Rats were allowed to breathe USP air immediately before and after the CO exposures. All animals survived the exposures, through a 4-hr post-exposure sampling period. Prior to exposure mixed-blood COHb saturation in all rats was essentially zero. At the end of the 5-min exposure average COHb was found to increase to 17+/-4, 30+/-6, 46+/-10, and 59+/-12% in the 1000, 3000, 6000, and 12000 ppm CO groups, respectively. One hr after exposure, COHb decreased to 9+/-2, 14+/-4, 16+/-4 and 22+/-6% in the four CO groups. At 2 hrs COHb decreased to 7+/-2, 7+/-3, 8+/-3, and 10+/-3%, and at 4 hrs COHb further returned towards pre-exposure control (3+/-1, 3+/-2, 4+/-2, and 3+/-1%) in above groups, respectively. Elimination half time for COHb was approximately 147+/-12, 93+/-7, 57+/-4, and 49+/-3 min in above groups, respectively. During the first hr after exposure average minute ventilation volumes increased 20, 50, 100, and 160% above respective controls (pre-exposure) in the above groups, respectively. In the second hr these values decreased to 10, 20, 50, and 60% above controls, and further decreased to 10, 10, 20 and 20% above controls by the fourth hr post exposure. These results suggest that COHb elimination in rats appears to follow a first-order rate. Furthermore, COHb elimination in rats is extremely rapid (compared to reported results from large animal species and humans), and hyperventilation is an important mechanism for regulating the recovery from CO poisoning in this species.

1442 INHALATION NOSE-ONLY EXPOSURE OF NEONATAL AND JUVENILE RATS.

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Due to the recent focus on non-clinical testing of pharmaceutical products used for pediatric populations, the feasibility of inhalation nose-only exposure of neonatal and juvenile rats was assessed. Five litters of 4 male and 4 female rat pups obtained from timed-mated CD females were used for the study. Exposed animals, 2 pups/sex/litter, were acclimated to the restraint tubes for increasing periods of time (1, 2 and 4 hours) from Day 7 to 9 post partum. Animals were exposed to an air atmosphere (generated by a PARI LC PLUS nebulizer) on a nose-only flow-through chamber for 4 hours daily from Day 10 up to Day 34 post partum. A cage-control group comprised of 2 pups/sex/litter was maintained under normal laboratory conditions from Day 7 up to Day 34 post partum for comparison to the animals exposed to inhalation procedures. Detailed examinations were performed at least once weekly and body weights were monitored at intervals throughout the study. On Day 21 post partum, all pups were weaned from their dams. One pup/sex/litter from the exposed and cage-control groups was euthanized on Days 21 and 35. All these pups underwent a complete macroscopic examination. There were no clinical signs considered related to the inhalation procedures. There were lower body weight gains in inhalation exposed animals when compared to the cage-controls. However, individual body weights remained within the expected range. There were no macroscopic observations considered related to the inhalation restraint procedure. Nose-only exposure of neonatal and juvenile rats was considered to be a feasible method. Therefore, neonatal and juvenile rats can be used for toxicity assessment of drugs intended for pediatric populations *via* the inhalation route.

1443 CYTOTOXICITY AND CYTOKINE PRODUCTION IN LUNG EPITHELIAL CELLS *IN VITRO* FOLLOWING CHROMIUM AND MANGANESE EXPOSURE.

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Condensed vapors of heavy metals are a significant hazard of welding activities, associated with an increased risk for developing respiratory disease. Chromium, nickel and manganese are predominant metals in welding fumes, and were therefore the focus of the present study. Molecular and cellular effects of heavy metal exposure to lung epithelial cells *in vitro* were investigated using cytotoxicity assays, immunoblot analysis of protein phosphorylation and immunochemical detection of the inflammatory cytokines IL-6, IL-8 and TNF-alpha. We show that chromium(VI) and manganese, but not nickel, are cytotoxic to normal human lung epithelial cells *in vitro* (SAEC and BEAS-2B), at concentration ranges correlated to concentrations of these metals found in welding fumes. Chromium(VI) and manganese (0.2 - 200 uM) caused cytotoxic effects within one hour post-treatment, with a maximal effect of 64% and 60% loss of cell viability, respectively following 24 hrs exposure. The toxic effect was associated with increased levels of intracellular phosphoprotein, a measure of intracellular signalling responses, and subsequent release of inflammatory cytokines IL-6 and IL-8, while no release of TNF-alpha was observed. Changes in cellular phosphoprotein levels and cytokine release were apparent within 1 hour following heavy metal treatment. IL-6 and IL-8 are released from lung epithelium to recruit cells of the immune system to sites of tissue damage and to initiate tissue repair mechanisms. Therefore, the observed effects of chromium(VI) and manganese in lung epithelial cells demonstrate a mechanism through which cytotoxicity of these metals in the lung can result in inflammatory responses and tissue restructuring, both of which are hallmarks of various respiratory diseases.

1444 EFFECTS OF EXPOSURE TO DIESEL EXHAUST PARTICLES (DEP) ON PULMONARY ACTIVATION OF MUTAGENIC AGENTS.

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DEP have been shown to induce a transient increase in CYP1A1 that returns to basal level at 7 days post-exposure, but to cause a sustained decrease in CYP2B1 in rat lung, whereas carbon black (CB) results only in a sustained decrease of CYP2B1. The present study examined the effects of DEP-altered pulmonary cytochrome P450 on the metabolization of mutagenic agents. Sprague-Dawley rats were intratracheally instilled with saline or a single dose of DEP or CB at 35 mg/kg body weight. At 1, 3, or 7 days post-exposure, S9, microsomes, and cytosol were isolated from lung tissue. The mutagenicity of 2-aminoanthracene (2-AA) and 2-aminofluorene (2-AF) were monitored using the Ames test with *S. typhimurium* YG1024. The results show that S9 or microsomes, but not the cytosolic enzymes, activated the mutagenicity of 2-AA and 2-AF in a dose-dependent manner. The CB-exposed S9, with down-regulated CYP2B1, significantly reduced 2-AA mutagenicity compared to the control S9 at all time points. In contrast, the induction of 2-AA mutagenicity by DEP-exposed S9, with down-regulated CYP2B1 but induced CYP1A1 at 1 and 3 days post-exposure, decreased with increasing exposure time. At 1 day post-exposure, the DEP-S9 had a similar effect to that of control S9, but induced higher mutagenicity than the CB-S9. This DEP-derived S9 activated 2-AA mutagenicity decreased at 3 days post-exposure and further declined to the level of CB-S9 at 7 days post-exposure. Heat-inactivated S9 or addition of a CYP1A1 inhibitor, alpha-naphthoflavone, blocked 2-AA activation. These results suggest that both CYP1A1 and CYP2B1 activate 2-AA mutagenicity. At 3 days post exposure, DEP-S9 decreased, while CB-S9 increased 2-AF mutagenicity compared to control S9, suggesting that both CYP1A1 and CYP2B1 decrease 2-AF mutagenicity. These results show that mutagenic activation is substrate-specific, depending on competing metabolic pathways. DEP exposure, which alters the cytochrome P450 system, may have a profound effect on pulmonary handling of mutagenic agents.

1445 EXPOSURE OF CULTURED MYOCYTES TO ZINC RESULTS IN ALTERED BEAT RATE AND INTERCELLULAR COMMUNICATION.

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Exposure to ambient air pollution particulate matter (PM) is associated with increased morbidity and mortality. Recent toxicological studies have reported PM-induced changes in a number of cardiac parameters, including heart rate variability, arrhythmias, repolarization, and internal defibrillator discharges. The purpose of

this study is to identify cellular processes that may contribute to cardiac dysfunction following PM exposure. Isolated neonatal rat ventricular myocytes were cultured for 11 days and then exposed to different concentrations of Zinc (Zn), a soluble component found in many PM samples. Zn significantly decreased spontaneous myocyte beat rate by 15% 2 hrs following exposure and 17% at 4 hrs compared to baseline. Since beat rate is influenced by intercellular communications, fluorescent recovery after photobleaching (FRAP) was then measured. FRAP rate, a physiological measure of cell-to-cell communication and gap junction permeability, was significantly decreased by 76% 4 hrs after exposure but not at 2 hrs. Changes were measured in gene expression of gap junction proteins which mediate communication between myocytes. Exposure of myocytes to Zn for 24 hrs resulted in a 76% increase in mRNA coding for connexin 43. These data suggest that soluble metals found in air pollution particles can affect the ability of cardiac myocytes to spontaneously beat, possibly by disruption of cell-to-cell communication. These effects potentially reflect disturbances in repolarization and impulse propagation which could contribute to PM-associated cardiac morbidity and mortality. This abstract does not necessarily reflect EPA policy.

1446 IRON-MEDIATED AMIODARONE RADICAL FORMATION.

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Numerous adverse effects of amiodarone (AM) may be mediated by free radical species. The current study investigated AM radical formation in microsomal and microsome-free systems. Hamster liver microsomes were incubated with AM, NADPH, and the spin trap α-phenyl-N-t-butylnitrone (PBN). Analysis by electron spin resonance (ESR) spectroscopy revealed a PBN-trapped radical with a triplet of doublets pattern and splitting constant values consistent with those of a carbon-centred radical. Incubation in the absence of NADPH, with microsomes exposed to carbon monoxide, with boiled microsomes, or under anaerobic conditions, did not alter the signal intensity or splitting constant values of the PBN-trapped radical, indicating that cytochrome P450 activity was not required for its formation. However, signal intensity was decreased by addition of the reducing agent sodium dithionite, suggesting a role for iron redox reactions in radical signal generation. Microsome-free incubations with AM and Fe^{2+} sulphate generated a signal pattern with intensity and splitting constant values similar to those in the microsomal incubations, whereas Fe^{3+} sulphate did not generate a measurable signal. Furthermore, the magnitude of the Fe^{2+}-mediated signal was decreased by sodium dithionite or the iron chelator deferoxamine, indicating that Fe^{2+} oxidation to Fe^{3+} is involved in radical generation. Thus, AM radical formation occurs in microsomal and microsome-free systems, and Fe^{2+} appears to play an important role in its generation. (Supported by Canadian Institutes of Health Research grant number MT-13257).

1447 EFFECTS OF SOLUBLE AND INSOLUBLE FRACTIONS OF A STAINLESS STEEL MANUAL METAL ARC WELDING FUME ON FREE RADICAL PRODUCTION AND LUNG INJURY AND INFLAMMATION.

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The goals of this study were to examine the roles of the soluble (Sol) and insoluble (Insol) fractions of a stainless steel welding fume (Tot) collected during manual metal arc welding in the development of rat lung damage and inflammation and correlate those effects with free radical production and metal composition. Welding fume fractions were separated by incubation in saline followed by centrifugation and filtration. Metal composition analysis by energy dispersive spectroscopy revealed both Cr and Mn in the Sol fraction. Using electron spin resonance, the generation of radicals from Cr(VI) was observed, with the highest signal from Tot, an intermediate signal from Sol, and a low signal from Insol. To examine the effects of the fumes on lung damage and inflammation, male Sprague-Dawley rats were intratracheally instilled with Tot at 2 mg/rat, or the equivalent volume of Sol, Insol, or the vehicle (saline). On days 1, 3, and 6, the right lung was assayed for lipid peroxidation (LPO) products while the left lobes were subjected to bronchoalveolar lavage (BAL). LPO was elevated in the lungs following Tot treatment, with most of the effect attributed to the Insol fraction. All fractions caused increases in BAL cell number, including elevated macrophage recovery. However, the Tot and Insol fractions caused increased PMN numbers at days 1 and 3, while the Tot and Sol fractions led to increased eosinophils at day 1. The changes in left lung weight were additive (Sol + Insol = Tot) on day 6, while albumin, total protein, and LDH activity in the first BAL fraction were all additive at day 3. These findings indicate that the ability of the fume to produce free radicals and much of the damage observed in the lungs of rats after Tot treatment depends on both the Sol and Insol fractions of the fume. However, PMN recruitment and LPO were due mainly to the insoluble particulates while eosinophil recruitment was due to the soluble components, most likely soluble metals.

1448 PULMONARY TOXICITY STUDIES WITH TiO2 PARTICLES CONTAINING VARIOUS COMMERCIAL COATINGS.

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Most of the pigment-grade TiO2 samples that have been tested in inhalation toxicity tests have been of the generic variety -(i.e., generally uncoated TiO2 samples or 99% TiO2 and 1% Al2O3). However, questions have been raised over the past twenty years regarding the potential pulmonary toxic effects, if any, of the commercially coated TiO2 materials, generally which contain higher concentrations of aluminum oxide and/or amorphous silica on their surfaces. The aims of this study were to assess in rats, using a well-developed short-term pulmonary bioassay, 1) the acute pulmonary toxicity of several intratracheally instilled commercial coatings on pigment grade TiO2 particles relative to uncoated, TiO2 particle control samples and 2) to bridge the results of these instillation studies with data previously generated from inhalation studies with uncoated TiO2 particles, using the uncoated TiO2 particles as the inhalation/instillation bridge material. Groups of rats were intratracheally instilled with 2 or 10 mg/kg of TiO2 particles containing coatings ranging from 0-7% Al2O3 and/or 0 - 10.5% amorphous silica. Saline-instilled rats served as controls. The lungs of sham and exposed rats were evaluated by bronchoalveolar lavage at 24 hr, 1 week, 1 month and 3 months postexposure (pe). The results demonstrated that the high dose (10 mg/kg) pigment grade TiO2 particles with the greatest Al and amorphous silica coatings produced the most sustained and intense lung inflammatory response, as evidenced by lung cell differentials and BAL fluid levels of LDH and protein, but not alkaline phosphatase. This effect was measured through 1 month but not a 3-month postexposure period. Our bioassay results indicate that the coatings containing the largest concentrations of amorphous silica and aluminum oxide produced the most significant lung inflammatory response but it is important to note that these effects were transient and not sustained. Studies are ongoing to assess the lung tissue responses of rats exposed to the various commercial coatings on pigment grade TiO2 particles.

1449 DEVELOPMENT OF A RAT MODEL OF INHALATION FUME FEVER.

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Occupational fume fevers represent a transient flu-like syndrome in humans associated with the inhalation of freshly formed metal or polymer ultrafine UFP (<100 nm) or fine-sized particles (< 2 mm), notably zinc oxide or PTFE/FEP. Presently, the key physical/chemical features of particulates associated with fume fever are unknown. Some preliminary studies have demonstrated that rats exposed to fine aerosols of ZnO particles or aged, combusted FEP UFP develop a transient lung inflammatory response which seems to mimic fume fever in humans. The development of animal models of fume fever, may provide clues to the mechanism of inflammatory injury occurring in workers following exposures. In preliminary, range-finding studies, rats were exposed to aerosols of zinc oxide (ZnO) particles for a single period of 1 or 3 hours (either 25 or 50 mg/m3), and the lungs of ZnO and sham-exposed animals were evaluated at 24, 72, or 168 hrs (1 week) postexposure using bronchoalveolar lavage fluid (BALF) analyses. Three hour exposures to ZnO at 25 mg/m3 produced a moderate and transient pulmonary inflammatory response (~22% pmns) at 24 hours postexposure, which was reduced at 72 hrs (~10% pmns) and resolved by 1 week postexposure. BAL fluid parameters reflected this minor inflammatory response, as the BAL fluid LDH, protein, and alkaline phosphatase values from the 3 hr ZnO rats were approximately 2X relative to sham controls at 24 hrs pe. Rats exposed to ZnO for 1 hr demonstrated very little response at any time postexposure. Three hour exposures to ZnO at 50 mg/m3 produced a similar pattern, but of greater intensity - i.e., (~45% pmns) at 24 hours postexposure, which was reduced at 72 hrs (~15% pmns) and resolved by 1 week postexposure. BAL fluid LDH, protein, and alkaline phosphatase values in the 3 hr ZnO rats were > 3X relative to sham control values. Studies are ongoing to determine the mechanism of this transient pulmonary inflammatory response, as well as the effects of ultrafine ZnO and PTFE and other metals.

1450 RECOVERY OF MANUAL METAL ARC-STAINLESS STEEL WELDING FUME EXPOSURE INDUCED LUNG FIBROSIS IN SPRAGUE-DAWLEY RATS.

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Welders with radiographic abnormalities of pneumoconiosis have shown a gradual clearing of the x-ray identified effects following removal from exposure. In some cases, pulmonary fibrosis associated with welding fume would appear to a more se-

vere form of fibrosis in welders. To investigate the disease and recovery process of pneumoconiosis induced by welding-fume exposure, rats were exposed to welding fumes with concentrations of 63.6 ±4.1 mg/m^3 (low dose) and 107.1 ±6.3 mg/m^3 (high dose) total suspended particulates for 2 h per day in an inhalation chamber for 2 h, 15, 30, 60 and 90 days, thereafter the rats were removed from the exposure, and allowed to recover from the welding fume induced lung fibrosis for 90 days. When compared to the control group, the lung weights increased significantly both in the low and high-dose group from day 15 to day 90. The histopatholgical examination combined with fibrosis specific staining indicated that the lungs in the low-dose group did not exhibit any significant progressive fibrotic changes. Whereas, the lungs in the high dose group exhibited early delicate fibrosis from day 15, which progressed in to the preivascular and peribronchiolar regions by day 30. Interstitial fibrosis appeared at day 60 and became prominent by day 90, along with additional appearance of pleural fibrosis. The recovery, evaluated by body and lung weight, and histopathological examination, was noted from the rats both in the high and low dose group that had been exposed up to 30 days. Although the rats exposed for 60-90 days at the low dose recovered from the fibrosis, the rats exposed for 60-90 days at the high dose could not recover fully from the fibrosis. Taken together, the pneumoconiosis induced by welding-fume exposure could be recovered if the degree of exposure was short-term and moderate.

1451 MECHANISMS OF NICKEL INDUCED LUNG CANCER—NICKEL SUBSULFIDE *vs* NICKEL SULFATE.

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Early epidemiological and rodent bioassay data indicate that the carcinogenic potential of nickel compounds depend on their chemical form. The purpose of this study was to determine why the nickel sulfate ($NiSO_4$) is not carcinogenic, while nickel subsulfide (Ni_3S_2) is. Male F344/N rats were exposed by inhalation to 0, 0.03, 0.11, or 0.22 mg Ni/m^3 as $NiSO_4$ or to 0, 0.04, 0.11 or 0.44 mg Ni/m^3 as Ni_3S_2, 6 hr/day, 5 days/wk for up to 13 wks. Groups of rats were sacrificed after 3 and 13 wks of exposure and after a 13 week hold period. Endpoints included evaluations of pulmonary inflammation, epithelial and nonepithelial cell proliferation, DNA strand breaks and global DNA methylation. Histologic findings were qualitatively similar for the two compounds and included pulmonary inflammation, (Ni_3S_2 > $NiSO_4$), bronchiolar epithelial degeneration ($NiSO_4$> Ni_3S_2), type II cell hyperplasia (Ni_3S_2 > $NiSO_4$), and bronchiolar epithelial apoptosis ($NiSO_4$ > Ni_3S_2). Small increases in labeling index were observed in Ni_3S_2 exposed rats at 3 and 13 wks. $NiSO_4$ inhalation did not result in significant changes in labeling index at 13 wks. DNA damage in isolated lung cells (epithelial cells and macrophages) was greater among Ni_3S_2 than $NiSO_4$ exposed rats at 3 wks. In contrast, the extent of DNA induced strand breaks was greater among $NiSO_4$-exposed than Ni_3S_2 exposed rats when assays were performed on DNA isolated directly from lung tissue. The extent of damage increased with time and dose. Both compounds increased the extent of global DNA methylation at 13 ws, but the effect of cytotoxicity on the ability to detect methylation is not clear. While histologic lesions persisted throughout the hold period, alterations in labeling index, DNA damage, and methylation did not. Results obtained show few differentiators between $NiSO_4$and Ni_3S_2 that explain the observed differences in their carcinogenic potential. (Research supported by Nickel Producers' Environmental Research Association, Inc.).

1452 EFFECTS OF ARSENITE ON INFLAMMATORY RESPONSES OF HUMAN BRONCHIAL EPITHELIAL CELLS.

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The majority of the health concerns related to arsenic stem from its potential presence in water, but it has been documented as a trace pollutant in airborne particulate matter (PM). Arsenic may play a role in lung carcinogenesis as well as in PM related morbidity and mortality increases. In the present study we examined the cytotoxicity of arsenic on human bronchial epithelial cells (BEAS-2B) and its potential to induce the inflammatory mediator interleukin-8 (IL-8). BEAS-2B cultures were exposed for 24 hours to sodium arsenite (0.05-500 μM) after which the median toxic dose was found to be ~100 ± 9.6 μM as determined by lactate dehydrogenase release and ~48 ± 0.4 μM by neutral red uptake. IL-8 release peaked at an arsenite concentration of 50 μM (836 pg/ml IL-8 vs. 31.2 pg/ml for the control) with no significant increases detected at other concentrations (n=4). This data suggests that arsenite can induce an inflammatory response in BEAS-2B cells similar to that observed with other air pollutants such as metal-laden PM. Because oxidative stress might play a role in arsenic toxicity we examined the cell media for the presence of aldehydes by HPLC/MS and intracellular oxidation of 2', 7'-dichlorofluorescin. We found no evidence of increased oxidant formation after up to 24 h. exposures. The fact that we did not find evidence of oxidative stress with our system does not imply that it is not a contributing factor for the observed effects. Further analyses into the possible reasons for the IL-8 increase as well as other evidence for arsenite induced oxidative stress are underway. [This is an abstract of a proposed presentation and does not necessarily reflect official EPA policy. Supported by NIEHS T32-ES07126-20 and EPA/UNC CT827206].

1453 VANADIUM CONCENTRATIONS IN LUNG, LIVER, KIDNEY, TESTES AND BRAIN AFTER THE INHALATION OF 0.02M OF V2O5. AN EXPERIMENTAL MODEL IN MICE.

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Our interest for Vanadium (V) is related with its increase in the atmosphere as a consequence of the combustion of Mexican Maya oil and fuel, which are rich in this element. With this antecedent we decided to structure an inhalation model in mice which inhaled 0.02M V2O5 2 hours /twice a week for 4 weeks. Animals were sacrificed at the end of each week and samples from: lung, liver, kidney, testes and brain were processed to determine V concentration by Atomic Absorption Spectrometry . Results showed that V concentrations highly increased after the first week of inhalation in all the organs evaluated, and remained almost the same during the 4 weeks. The highest concentrations were found in the liver followed by the kidney; the organ with the lowest concentration was the testes. In the kidney, however, at week four, a decrease in V concentration was observed. Conclusions: Although the lung is the first organ reached by vanadium, we found that the liver and the kidney had the highest V concentrations. The differences between organs may be due to the different mechanisms of handle the element in each organ or as a consequence of the organ susceptibility. So, it is important to evaluate a correlation with tissue morphologic variations and V concentration to establish the time course modifications that triggers those changes. Supported in part by DGAPA 210301 and P.O.S Institute.

1454 DIFFERENTIAL *IN VITRO* IMMUNOLOGICAL RESPONSES TO ZINC (ZN), AN ACTIVE COMPONENT OF URBAN PARTICULATE MATTER (PM).

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Epidemiological evidence suggests that human morbidity and mortality are associated with exposure to ambient air particulate matter of size 10μm or less (PM10). Although urban PM is composed of a complex mixture of trace metals, elemental carbon, and organic compounds, results of numerous studies suggest that component metals, such as Zn, are important mediators of the pulmonary inflammatory response to PM exposure. To examine the mechanism by which this occurs, we hypothesized that Zn stimulates airway cells to release cytokines and chemokines that are important in the inflammatory response. In *in vitro* studies, two cell types (human alveolar type II, A549 cells and RAW 264.7 mouse macrophage-like cells) were exposed to NIST 1648 urban PM and $ZnCl_2$ for 24 hrs. In response to NIST 1648 exposure (200 μg/ml), RAW 264.7 cells released greater amounts of IL-6 and TNF-α compared to culture media controls, and A549 cells responded by increased MCP-1 response but no change in the release of IL-8. RAW 264.7 macrophage-like cells did not respond to Zn exposure; however Zn concentrations ranging from 25-400 μM did stimulate A549 cells to release IL-8 (a chemoattractant for neutrophils). Also, interestingly, increasing concentrations of $ZnCl_2$ led to decreasing MCP-1 (a chemoattractant for monocytes/macrophage) release by A549 cells without a significant decrease in cell viability. These data support the hypothesis that Zn, as a component of ambient air PM, plays a critical role in modulating the immune response to PM through its effects on alveolar epithelial cell chemokine release, which could lead to a preferential influx of neutrophils into the lung following exposure. *Supported by the Baltimore Supersite Program grant R82806301 and NIEHS Training Grant T32ES07263*

1455 A CASE OF DRUG-INDUCED PURE RED CELL APLASIA IN RATS.

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As part of the early preclinical development of a new antipsychotic compound, Wistar rats (6-week old; 3/sex/group) were dosed orally (15-150 mg/kg/d) for up to 7 days. There were expected changes in appearance and behavior, and dose-related

decrease in body weight gain and feed intake, but also a fluid and pale bone marrow in rats given high doses. Erythrocyte, leukocyte and platelet counts were normal as were clinical chemical values. Bone marrow sections showed a mild to severe bone marrow hypoplasia with reduced numbers of erythroid elements. Reticulocyte counts were decreased as was the proportion of the erythroid series on bone marrow smears (increased M:E ratio) leading to the hypothesis of a central and preferential effect of the compound on the erythroid lineage. In a mechanistic experiment, rats (9-week old; 6/sex/group) were given 0, 5, 15, 45 or 90 (close to MTD) mg/kg/d for 9 days and phlebotomized (13 ml/kg, ie 20% blood loss) on the 7th day of exposure to stimulate erythroid regeneration. Red blood cell mass, reticulocyte counts and erythropoietin (EPO) levels were monitored before and 4, 24 and 48 hr after bleeding. Plasma EPO measured by ELISA using mouse monoclonal antibodies against huEPO, known to cross-react with rEPO, was found elevated in all groups 24 and 48 hr after stimulation. Reticulocytes increased 24 hr after bleeding in controls with a peak at 48 hr, while compound-exposed rats had low reticulocyte counts suggesting an impairment of the proliferative response to EPO, confirmed by hypoplastic erythroid bone marrow and reduced splenic extramedullary erythropoiesis. Rat bone marrow cultured with the compound and EPO or GM-CSF indicated that the erythroid colony-forming unit (CFU-E) is the primary target for toxicity of this compound.

1456 TOXIC, ALLERGIC OR IDIOSYNCRATIC TOXICITY OF KAVA PYRONES - RELATION TO REGULATORY DECISIONS.

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Severe hepatotoxicity has been linked in recent years to intake of Kava pyrones. The assumed toxicity has led to withdraw these preparations from the German market; decisions in other countries are pending (September 2002). We reassess Kava extract toxicity with regard to known mechanisms of hepatic injury. Hepatotoxicity is known since 1990 but has attracted major attention only since 1999; spontaneous reports indicate the potential severity. The increase in spontaneous reports in Europe since 1999 may be due to raised awareness, mistaken association and/or aggravation of preexisting liver disease; multiple reporting at different agencies has also occured. Kava preparations do rarely cause serious liver toxicity. In one case, an idiosyncratic reaction has been supported by reexposure, in another case, peripheral lymphocyte proliferation was increased. In all other cases, including two lethal cases, no attempt has been made to elucidate possible mechanisms of toxicity. Known *in vitro* activities do not correlate to the observed clinical symptoms. The long delay between starting Kava intake and appearance of adverse effects, and the lack of a dose response curve suggest that direct toxicity is not relevant in therapeutic concentrations. The few data available from animal experiments suggest direct toxicity to appear only at much higher doses not relevant for anxiolysis. Current therapeutic alternatives are benzodiazepines and antidepressants also known to exert drug toxicity including hepatotoxicity. Despite the wave of reports Kava pyrones still appear to be a reasonable alternative for long term treatment of anxiety; suitable precaucions should be taken to minimize patient risks.

1457 CORRELATION OF PCB BURDEN MEASURED IN AIR OR BLOOD.

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Banned since the 1970th, PCB still persists in the environment and causes continuous exposure. Present exposure stems mainly from food contamination and airborne pollution. To estimate the body burden, the six typical congeners 28, 52, 101, 138, 153 and 180 (sentinel congeners) are determined. After correction for the relative amount of these congeners in a PCB mixture the total body burden from all 209 congeners is derived empirically. For PCB congeners present in food - i.e. mainly congeners 138, 153 and 180 - the amounts present in body fat and serum roughly coincide with food levels. For the more volatile congeners 28 and 52 mainly liberated from industrial products and found in inhalative exposure, even high air levels do not result in elevated serum levels. In most cases, no PCB 28 or 52 are detected in serum from exposed persons even with air concentrations exceeding 1 000 ng/m3. To understand the discrepancy between detectable exposure and undetectable body burden we calculate PCB expected serum concentrations from published pharmacokinetic parameters and exposure levels. Surprisingly, even at 1 000 ng/m3, no PCB 28 and 101 should be detectable assuming a limit of detection of 100 pg/ml serum. PCB 52 having a much larger serum half life, is calculated at twice the limit of detection. These calculations are in good agreement with the lack of detection in serum from long time exposed persons. The calculated serum concentrations for steady state conditions naturally are mainly determined by the excretion half times, which have been published for rats and humans. In children and young adults the total amount of fat is still increasing, and even lower serum concentrations are to be expected; in starving, dieting, old or sick individuals, when body fat is lost, stored PCB may be liberated in much greater amounts and serum concentrations greater than expected from steady state. The results from our model calculations can explain the lack of detectable serum PCB 28 and 52 even at rather high exposure concentrations.

1458 PRECLINICAL SAFETY OF BG00001, A REPLICATION DEFECTIVE ADENOVIRAL VECTOR EXPRESSING THE HUMAN INTERFERON-β (HIFNβ) GENE, FOLLOWING INTRAPROSTATIC DOSING IN RHESUS MONKEYS.

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In orthotopic models of human prostate cancer BG00001 treatment led to complete tumor regression of pre-established tumors. To support initiation of clinical trials, male rhesus monkeys (5/group) were administered BG00001 at doses of 0, 0.5, 5 and 500 x 10^9 viral particles (vp) *via* intraprostatic injection during laparotomy. Subsets of animals were necropsied at 6, 22, and 61 days postdose. There were no BG00001-related changes in clinical signs, or other postdose observations. Postmortem findings were related to surgery and/or injection procedures. Microscopic evidence of inflammatory changes in the prostate interstitium, seminal vesicles, and urinary bladder were present in all dose groups and resolved over time. Real time PCR detected BG00001 DNA in plasma of high-dose animals at Day 3 but vector was undetectable at subsequent timepoints. Changes in serum IFN-β activity followed a similar time course, indicating the therapeutic hIFN-β transgene was expressed resulting in systemic exposure. Vector DNA was also found in liver of both high-dose animals and one low-dose animal necropsied on Day 6 but not at later time points. BG00001 was detected in prostate tissue of all groups administered BG00001. Vector persistence in the prostate was noted thru Day 22 in the low-dose group and until Day 61 in the mid- and high-dose groups. BG00001 was also found in seminal vesicles and the ureter in most high-dose animals. No BG00001 was found in testes suggesting male germ cells were not inadvertently transduced by vector. There were no BG00001-related histopathology findings in testes. No effects on sperm motility, concentration and sperm morphology in semen were noted. Intraprostatic administration of BG00001 was well tolerated by male rhesus monkeys at all administered dose levels. The results of this study suggest that unlike current therapies for prostate cancer, BG00001 treatment may not cause sterility.

1459 MULTIPARAMETER HYPOTHESIS FOR IDIOSYNCRATIC DRUG TOXICITY.

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Idiosyncratic drug toxicity is a serious problem in drug development. This often-fatal event occurs at low incidence (<1/5000) and therefore cannot be effectively detected in routine clinical trials. Its occurrence is usually discovered *via* postmarketing survey after the drug in question is exposed to a large number of individuals, leading to the necessity of withdrawal of the drug from the market or severely limiting its applications. I propose here that idiosyncratic drug toxicity requires the co-occurrence of multiple independent events. The final incidence is a result of the product of the incidence of each independent events. I further propose that the critical events are functions of several critical parameters which are: chemical properties (chem); dose of exposure (exp); drug metabolizing enzyme activities (met); and genetic predisposition to sensitivity of drug toxicity (gene). The equation of the probability for idiosyncratic drug toxicity (Pidt) is as follows: P(idt)=P(chem)xP(exp)xP(met)xP(gene). While different drug will be represented by different probability functions for each critical parameter, I propose that idiosyncratci drug toxicity can be eliminated by critically examining the chemical properties during drug discovery stage; exposure levels during drug development stage; metabolic properties, including drug, food, and evironmental interactions during drug development; and pharmacogenomics during clinical trials. The most practical approach is to reduce P(chem) *via* the discovery of chemical properties that would lead to idiosyncratic drug toxicity, and the elimination of drug candidates with these undesirable qualities. Toxicogenomics may be the most useful approach for the discovery of key chemical properties related to idiosyncratic drug toxicity.

1460 EVALUATION OF THE TOXICITY OF BROMOCHLOROACETIC ACID ADMINISTERED FOR 26 WEEKS IN DRINKING WATER TO B6C3F1 MICE AND F344 RATS .

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Bromochloroacetic acid (BCA), a chlorine disinfection by-product, is found at ppb levels in finished drinking water. Male and female B6C3F1 mice and F344/N rats were exposed to 250, 500, and 1000 mg/L BCA in the drinking water for 26 weeks.

At necropsy the colon, small intestine, kidney, liver, stomach and urinary bladder were excised and prepared for a histopathological examination. Mouse: Dose-dependent decreases in body weights were observed for both sexes (16-20% maximum). A dose-dependent increase in relative liver weights was observed for male (50% maximum) and female (70% maximum) mice. Kidney weights were not affected. BCA enhanced the incidence and severity of vacuolization in the cytoplasm of periportal hepatocytes which appeared more prominent in the livers of female mice compared to male mice. BCA treatment increased mean numbers of lymphoid nodules, subunits of Peyer's patches, in the middle and distal colon segments of male mice, but not female mice. No BCA-associated pathology was seen in any of the other organs examined in the control and high dosed groups. Rat: Alterations in the body, liver and kidney weights of male and female rats exposed to BCA in the drinking water were not observed (all <10%) when compared to the control values. Altered vacuolization in the cytoplasm of periportal hepatocytes was less severe than that observed for the mouse. BCA treatment increased the number of extended (3-4 days estrous or 4 days diestrous) and abnormal (> 4 days estrous or 4 days diestrous) cycles in the female rat. A normal cycle persists for 4 or 5 days with either 1 or 2 day estrous and 2 or 3 days diestrous. The extended and abnormal cycles were paralleled by increased serum estradiol concentrations. BCA treatments did not alter serum progesterone or corticosterone levels over the control values. (This is an abstract of a proposed presentation and does not necessarily reflect the views of the EPA).

1461 SAFETY, BIODISTRIBUTION AND PERSISTENCE EVALUATION OF EP HIV-1090 DNA VACCINE IN RABBITS.

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Vaccinations using cloned DNA vectors containing antigen-specific gene fragment(s) have been shown to be useful in eliciting immune responses against the infectious agents, EP HIV-1090 plasmid DNA vaccine was constructed to encode multiple T-lymphocyte epitopes and designed to induce immune responses against the Human Immunodeficiency Virus Type 1. The objective of these studies was to determine the safety, biodistribution and persistence of this vaccine. For the safety study, male and female rabbits were immunized with either 0, 1, or 6 mg of the vaccine on Days 1, 11, 22, 33, and 44. While some slight transitory injection site irritation was observed, the severity and duration of effects were similar for both the control and high dose groups. There were no test article-related effects observed on the animals; therefore, the no-observed-adverse-effect level is considered to be at least 6 mg/immunization. In order to address the concern of potential integration of vaccine sequences into the host genome, kinetics of clearance of the vaccine from tissues was evaluated using a quantitative PCR-based technique. Positive plasmid signals were found in a number of tissues up to 7 days after treatment, but vaccine was cleared from most of these tissues by 56 days after treatment. Plasmid signals persisted at the injection sites (skin & muscle) for up to 16 weeks; however, no plasmid signals were seen in the muscle after the genomic DNA fraction was subjected to one round of gel purification. Small residual amounts of vaccine were present in the skin of 1 out of 10 animals after 3 gel purifications. When two additional sequence segments of plasmid were evaluated in skin DNA, no plasmid DNA was detected in any of the skin genomic DNA after 2 rounds of gel purification, indicating that DNA vaccine was only loosely associated with genomic DNA and not integrated into the genome of the animal. Work was conducted under NIAID contract N01-AI-95375.

1462 SUBCHRONIC, DEVELOPMENTAL, AND REPRODUCTIVE TOXICITY OF A FLUOROALKYLETHYL PHOSPHATE SURFACTANT.

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A fluoroalkylethyl phosphate surfactant was evaluated in rats in 90-day, one-generation reproduction, and developmental toxicity studies. In the 90-day study, the test substance was suspended in isopropanol (IPA) at oral doses of 0, 10, 60 or 300 mg/kg/day (35% ai) and 60 mg/kg/day IPA. There were no compound effects on mortality, clinical signs or neurobehavior. Effects on body weights, food parameters, red cell mass, and hepatic enzymes were seen at the highest two doses, and increases in thyroid weight and cellular hypertrophy were observed at the high dose. Adverse microscopic changes in the liver (focal hepatocellular necrosis) occurred at all dose levels in males and at only the high dose in females. These effects were not seen with IPA dosed alone. Non-adverse physiological changes included increased liver and kidney weights, hepatocellular hypertrophy, and an increased rate of peroxisome proliferation. The NOEL for female rats in this study was 10 mg/kg/day. In the reproductive study, where surfactant doses were 0, 75, 500 and 3500 mg/kg/day (20% ai in water, no IPA), parental body weights, weight gains and food parameters were reduced at the high dose, with males more affected than females. There were no effects on reproduction parameters at any dose level, except for F1 pup weights, which were progressively reduced from birth to weaning (92% and 76% of control, respectively) at the high dose. In these rats dosed with the active in water, thyroid hypertrophy and liver necrosis were not observed. The NOEL for reproductive parameters was 500 mg/kg/day. In the developmental study, doses were 625, 1250 or 2500 mg/kg/day (20% ai in water). At 2500 mg/kg/day, effects on maternal weight, food consumption, and fetal weight occurred. At 1250 mg/kg/day, there were maternal body weight reductions. No effects were seen at 625 mg/kg/day. Overall, the most sensitive target of toxicity was the liver, but the test substance was not considered a reproductive or developmental toxin.

1463 A DERMAL SAFETY EVALUATION OF *p-(t*-BUTYL)-α-METHYLHYDROCINNAMIC ALDEHYDE (BMHCA).

J. Cocchiara and A. Api. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

The Research Institute for Fragrance Materials, Inc. (RIFM) has a long history of testing fragrance ingredients for sensitization potential as a means of primary prevention of sensitization in the normal general population. Over the last several years, primary prevention of sensitization has evolved from human maximization testing to sensitization safety assessments. An example of a recently completed sensitization safety assessment on BMHCA (CAS Number 80-54-6) will be presented. BMHCA is a colorless to pale yellow liquid with a powerful, floral-fresh odor. The Human Repeated Insult Patch Test (HRIPT) data generated by RIFM, showed that a 25% solution in an ethanol/DEP combination caused one reaction in a total of 225 subjects. The animal data are equivocal. Magnusson-Kligman Maximization studies show both sensitization and no effects at the same challenge concentrations. The clinical evidence is weak since only one study (Larsen et al., 1996) shows >1% reactivity (2/167 reactions or 1.2% reactivity). The Larsen study also showed that 3/167 (1.8%) exhibited irritant reactions. The weight of evidence shows that the no observed effect level (NOEL) for BMHCA is in the region of 25% (or 29, 527 μg/cm2). The NOEL of 25% would support a use level of 2.5% on the skin.

1464 EVALUATION OF POTENTIAL EXPOSURE TO VOLATILE ORGANIC COMPOUNDS EMITTED FROM A SPRAY GRADE CONTACT CEMENT.

C. W. Jarand, C. A. Robbins and B. J. Kelman. *GlobalTox, Redmond, WA.*

A commercially available contact cement, HHR Solvent Type Spray Grade Contact Cement, was analyzed for VOC emissions under differing application conditions and drying periods. A laboratory model was designed to evaluate VOC emissions for a typical application. Analysis was performed following the EPA compendium method TO-17 (Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes). Two different types of samples were prepared which simulated an open or sandwiched sample. A PTFE impinger was used as sampling chamber with VOCs captured on thermal desorption tubes. Desorption tubes were exchanged every 3 hours of sampling over a 12 hour period with an air sampling flow rate of 20 ml/min. Desorption tubes were analyzed by GC-MS. The percentage of toluene emitted relative to the total mass of applied adhesive was determined over the time course of the experiment and was found to follow an exponential decay for both the open and sandwiched samples. A number of other components were identified at reduced concentrations relative to toluene. These components included 2-methyl-2, 3-pentanediol, methylcyclohexane, a cyclopentane or a mix of cyclopentanes at lower concentrations than toluene. Several other aliphatic hydrocarbons, including n-hexane, and aromatic hydrocarbons were identified at significantly reduced concentrations relative to toluene. The relative concentration of toluene to other components was also found to be substantially different between the open and sandwiched samples. The laboratory results were applied to a box model which allowed calculation of concentrations based on time of air exchange and total drying time. Under recommended application conditions and 3 hours of air exchange, the maximum potential toluene concentration was 42 ppm, well below the OSHA PEL Ceiling exposure limit of 300 ppm. All other volatile constituents were calculated to be at significantly decreased concentrations relative to toluene, with none exceeding the OSHA PEL.

1465 RODENT RESPIRATORY SAFETY PHARMACOLOGY STUDIES: A BREATH-SIMULATED MODEL AND ADDITIONAL *IN VIVO* VALIDATION.

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Respiratory safety pharmacology at this laboratory has historically been performed using the Buxco Electronics LS-20 system with Non-Invasive Airway Mechanics (NAM) for determination of respiratory parameters in multiple species. To increase

flexibility and scope for these studies, the Buxco BioSystem XA v.2.5 with flow-derived parameter (FDP) analysis software for assessment of respiratory physiology was validated using a purpose built reciprocating piston-type pump. A variable speed motor, gear and interchangeable pistons varied the volume of air (TV) delivered with each stroke to mimic breathing patterns of different animal species and altering motor speed controlled frequency (f). Reference methods allowed a direct comparison of pump volume output and frequency with the original BioSystem XA derived TV and f values. As this method was considered reliable for use when assessing TV, f and minute volume (MV), a further qualification was performed using Sprague-Dawley and Wistar rats, previously acclimated to head-out plethysmograph chambers. Animals were administered 20 mg/kg morphine or 10 mg/kg theophylline intravenously into the tail vein *via* a port in the chamber to access the dose site. Respiration was monitored for 15 minutes prior to dosing, continuously for 3 hours post dose and again for 15 minutes at 6 hours post dose. Decreases in MV elicited by reduced f were noted for morphine treated animals, with corresponding increases in these parameters for rats dosed with theophylline. Pre-dose values were compared with historical data at this laboratory and were within similar ranges. Respiratory data following dosing were similar to those in published literature. As such, qualification using an independent mechanical pump, with further validation using accepted animal models, is considered to have demonstrated the accuracy of the system to detect both normal and abnormal respiration. The Buxco BioSystem XA is therefore considered acceptable for use on rodent respiratory safety pharmacology studies at this laboratory.

1466 SAFETY PHARMACOLOGY STUDIES FOR THE ASSESSMENT OF COMPOUND-RELATED EFFECTS ON THE GASTROINTESTINAL SYSTEM.

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As stipulated in the ICH S7A guidelines, effects of a test substance on the gastrointestinal system should be assessed where the core battery of studies or the repeat-dose toxicity studies do not address potential adverse pharmacodynamic effects on the functions of this specific organ system. A GLP-compliant series of studies to assess effects on the gastrointestinal (GI) system was validated at CTBR using the Sprague-Dawley rat. These include GI injury potential following single or repeat dose administration, transit time in-vivo, and measurement of gastric secretion. Reference compounds with well-documented effects, were used to demonstrate the effectiveness and acceptability of the assays. Six or 7 week old male Sprague-Dawley rats were used for all tests. Dose levels were based on those in published literature and on experience with studies of a similar type in this laboratory. Gastric and intestinal ulceration indices reflected a dose-related increasing trend for gastrointestinal damage following single and repeat-dose administration. Phenol red (gastric motility indicator) concentration in the stomach, measured colorimetrically, was higher for animals administered atropine and significantly lower for animals administered metoclopramide when compared to control animals. Charcoal propulsion in the intestine was significantly decreased for animals administered atropine and apomorphine when compared to control animals. When administered metoclopramide twice the number of treated animals than control rats were observed to have distal movement equal to or in excess of 80% total small intestine length. Gastric secretion measurements (pH and free and total acid concentration) following administration of omeprazole and ranitidine reflected the expected increase in pH and decrease in free and total acid concentration when compared to control animals. All responses obtained were characteristic of those anticipated following administration of the reference compounds and, as such, these studies are considered validated in the rat CTBR.

1467 A DERMAL SAFETY EVALUATION OF CINNAMIC ALCOHOL.

A. Api and J. Cocchiara. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

The Research Institute for Fragrance Materials, Inc. (RIFM) has a long history of testing fragrance ingredients for sensitization potential as a means of primary prevention of sensitization in the normal general population. Over the last several years, primary prevention of sensitization has evolved from human maximization testing to sensitization safety assessments. An example of a recently completed sensitization safety assessment on cinnamic alcohol (CAS Number 104-54-1) will be presented. Cinnamic alcohol is a white to slightly yellow solid with a warm, balsamic, floral, sweet odor. The Human Repeated Insult Patch Test (HRIPT) data generated by RIFM showed that a 4% solution in an ethanol/DEP combination caused two reactions in a total of 109 subjects. Upon rechallenge using a 24-hour occluded patch, a 24-hour semi-occluded patch and 5-day repeated open application test, reactivity was observed only under occlusive patch conditions at readings. No skin reactivity was observed under semi-occlusive patch conditions or under open exaggerated rub-in conditions. Cinnamic alcohol was evaluated for sensitization in 23 studies in guinea pigs using various test methods such as the Magnusson-Kligman maximization test, Buehler Delayed Hypersensitivity test, Modified Draize Test, Freund's Complete Adjuvant Test, Closed Epicutaneous test, or the Open Epicutaneous Test, at concentrations ranging from 1% up to 100%. Sensitization was observed at all dose levels over 1%. Clinical data show that cinnamic alcohol elicits sensitization in dermatitic patients. The weight of evidence shows that the NOEL for cinnamic alcohol is in the region of 4% (or 4724 ug/cm2). The NOEL of 4% would support a use level of 0.4% on the skin.

1468 PATHWAYS OF TOXICITY:AN EARLY SCREENING APPROACH.

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The objective of this study was to develop screens for early safety evaluation of developmental compounds. Acetaminophen, thioacetamide, tacrine, camptothecin, staurosporine, chloramphenicol, nitrofurantoin, ferric nitrilotriacetate and cyclosporine were selected as model compounds affecting key toxicity pathways which could be monitored at the cellular and gene expression levels. Rat primary hepatocytes were treated for up to 24 hours with the model compound. Apoptosis, oxidative stress, and cytotoxicity were assessed by measuring caspase 3/7 activity, cell-death, reactive oxygen species formation, lactate dehydrogenase level, ATP content and GSH/GSSG ratio. Alterations in the expression of genes involved in these pathways (Bcl-2, p21, Bax, SAG, CDK4, PCNA, Mn-superoxide dismutase, Grp78, heme monooxygenase-1, glutathione peroxidase, glutathione reductase, and gamma-glutamylcysteine synthetase) were measured using Q-RT-PCR. Staurosporine caused a significant dose-related increase in caspase 3/7 activity with a concomitant increase in cytotoxicity parameters. In parallel, Bcl-2, BAX, p21, SAG and MnSOD gene expression increased with time and dose, indicative of apoptosis. Treatment with nitrofurantoin caused an increase in reactive oxygen species formation and caspase activity. In parallel, nitrofurantoin caused an increase in Bax, HOX, glutathione reductase, and gamma glutamylcysteine synthetase gene expression. These results were indicative of oxidative stress as the primary effect of nitrofurantoin with apoptosis as a secondary effect. Similar correlation between functional assays and gene expression changes were observed for the other model compounds. Preliminary results from these studies indicate that these assays could be used for the assessment of key biochemical functions noted above. Gene expression changes corroborated the biochemical findings and allowed for early identification of the toxicity pathways. Thus, this study indicates that such assays can be used as a battery to screen chemical series in early development to rank compounds based on their mechanism-based toxicity potential.

1469 INHIBITION OF VITAMIN K1 2, 3-EPOXIDE REDUCTASE BY PHARMACEUTICALS AND XENOBIOTICS.

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Vitamin K-responsive coagulopathies have been recognized in patients taking phenytoin or sulfinpyrazone, and in mammals exposed to the xenobiotics aflatoxin B1 and the soy isoflavone biochanin A. Deficiencies in vitamin K-dependent proteins, which result in increased prothrombin and activated partial thromboplastin times, PIVKA, and undercarboxylated osteocalcin, have been associated with these compounds. These deficiencies are similar to those caused by anticoagulants such as warfarin (Coumadin), used therapeutically in humans, or in anticoagulant rodenticides. The pharmacological site of the anticoagulant action of warfarin is through inhibition vitamin K1 2, 3-epoxide reductase (VKOR). To help explain the vitamin K-dependent coagulopathies associated with aflatoxin B1, biochanin A, phenytoin, and sulfinpyrazone, inhibition of VKOR activity was investigated in murine hepatic microsomes *in vitro*. Microsomal incubations contained 1 ml of microsomes (2 mg protein/ml), the substrate VKO (12 mM), and varying concentrations of inhibitors (in DMSO). After a 1 minute preincubation, 2 mM dithiothreitol (reducing agent) was added. Using HPLC/PDA, concentrations of vitamin K1 formed by VKOR at each concentration of inhibitor were compared to the vitamin K1 formed by DMSO controls. Coumarin (negative control) at concentrations greater than 36.4 mM did not inhibit VKOR activity. Diphacinone and warfarin (positive controls) at concentrations of 0.006 uM and 0.27 uM, respectively, inhibited VKOR activity by 50%. Biochanin A, phenytoin, and sulfinpyrazone at concentrations of 0.19 mM, 0.49 mM, and 1.9 mM, respectively, inhibited VKOR activity by 50%. Aflatoxin B1 inhibited enzyme activity by 30% at 0.85 mM. These results suggest that inhibition of VKOR by aflatoxin B1, biochanin A, phenytoin, and sulfinpyrazone may help to explain the vitamin K-dependent coagulopathies observed with these compounds.

1470 ANTIMICROBIAL RESISTANCE AND TRICLOSAN.

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Over the last 30 years, triclosan (2, 4, 4′-trichloro-2′-hydroxy-diphenylether) has been safely used in various personal care products, such as dentifrices, bar soaps, liquid hand and body soaps, and deodorants (personal care products constitute roughly 97% of triclosan use). Presently, triclosan is permitted for use in cosmetics and toiletries in Europe at a concentration of up to 0.3% as a preservative, and without specific concentration limits as an antimicrobial ingredient. As part of the safety evaluation process of such an antimicrobial agent, nonclinical and clinical investigations into the potential for resistance to develop or be transmitted were conducted. The *in vitro* studies for triclosan showed that bacterial resistance to triclosan was either intrinsic (*e.g.*, effective cell wall pumps, cell wall effects) or acquired (*e.g.*, decreased cell wall permeability, efflux pump mechanisms). However, despite these *in vitro* findings, clinical studies of 6-12 months duration in oral care and skin care applications have yielded no evidence of the development of resistance. As well, in a separate 5-year clinical trial using triclosan-containing toothpaste, no clinical or microbiological signs of bacterial overgrowth were observed. In addition, the potential for cross-resistance to antibiotics was also evaluated. Nonclinical and clinical data have demonstrated that: (1) although triclosan and isoniazid target the same protein, enoyl-acyl carrier protein reductase, it is at different sites; (2) clinically important antibiotic-resistant strains remain sensitive to triclosan; and, (3) bacterial strains with increased minimum inhibitory concentration (MIC) values to triclosan do not exhibit an increased resistance to antibiotics. The totality of these *in vivo* and *in vitro* studies, conducted from the molecular level up to the human use level, suggest that under the conditions of use, there is no evidence that triclosan poses any risk to humans by reducing or transmitting antibacterial resistance.

1471 A SINGLE-DOSAGE OCULAR AND CUTANEOUS PHOTOTOXICITY SCREEN FOR ORAL DRUGS.

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Many published studies involve safety studies in albino rodents. This report provides a model for evaluating test articles orally administered to rats that have melanin pigment in both cutaneous and ocular tissues. In four studies, male Crl:(LE)BR (Long-Evans) pigmented rats were randomly assigned to dosage groups (five rats per group), and administered (by gavage, 10 mL/kg) vehicle or a suspension of 8-methoxypsoralen (8-MOP, 50 mg/kg) in corn oil. Hair was removed from the backs of rats before formulation administration and one lightly and one darkly pigmented skin site and the eyes of each rat were exposed to UVR one hour after formulation administration. All rats were lightly anesthetized and restrained for UVR exposure. An instrumental exposure dose equivalent to a Standard Erythema Dose was delivered to each rat (SED; defined by Commission Internationale de L' Eclairage Committee, CIES 007/E-1998). Clinical observations were recorded daily; ophthalmologic examinations were performed by a veterinary ophthalmologist before and three days after UVR exposure. Ocular tissues harvested at necropsy and examined histopathologically were: cornea, lens, bulbar conjunctiva, vitreous and aqueous chambers, optic nerve, retina, sclera, iris, ciliary body and choroids. The UVR dose was below the skin and ocular response threshold in the rats administered the vehicle. Skin reactions indicative of phototoxicity occurred in lightly and darkly pigmented skin sites in rats orally administered 8-MOP and exposed to UVR (erythema grades 1 and 2, edema grades 1 and 2). Ocular responses included swelling of peri-orbital skin and diffuse corneal edema after UVR exposure in rats administered 8-MOP. Histopathological evaluation confirmed ocular phototoxicity in the examined tissues. Based on these findings, pigmented rats are useful in assessing the potential for cutaneous and ocular phototoxicity of test articles.

1472 TOXICITY COMPARISON OF LIPOSOMES COMPRISED OF DIOLEOYLTRIMETHYLAMMONIUM PROPANE:DIOLEOYLPHOSPHATIDYLETHANOLAMINE (DOTAP:DOPE) OR DIMETHYLDIOCTADECYLAMMONIUM BROMIDE:DIOLEOYLPHOSPHATIDYLETHANOLAMIN E (DDAB:DOPE) FOLLOWING MULTIPLE IV INJECTIONS IN FISCHER 344 RATS.

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Liposomes serve as carriers for various pharmaceutical agents. Cationically charged liposomes interact with cell membranes and increase the cellular uptake of drugs *in vivo*. Although liposomes composed of varying lipid combinations have been utilized experimentally, only a few of these have been approved for clinical use. The safety testing, quality control and manufacturing requirements present a hurdle in getting new liposomes into the clinic. The toxicity of two different liposomes was compared to determine if one would be more suitable for development. DOTAP:DOPE (1:1 umoles) and DDAB:DOPE (1:1 umoles) were intravenously administered to male Fischer 344 rats every 3 days from day 1 through day 22, either as a single slow injection or 3 slow injections (3 hr intervals) per day. Liposomes were prepared at two concentrations, 1.25 and 2.08 umoles/mL in 5% dextrose, USP. Eight animals per group received doses of 10 or 27 umoles of liposome. Toxicity endpoints included clinical observation, body weight, clinical and gross pathology. Clinical signs included sporadic diarrhea, hunched posture and discolored, swollen tail at the site of injections. Frequency and severity of signs were similar for the two liposomes at the same dose, however, more frequent dosing produced more severe tail lesions such that the 3 injections/day could not be continued through the duration of the study. No treatment-related effects were seen in hematology, clinical chemistry or gross examination of tissues (except the tails). The results of this study indicate no measurable differences in toxicity between DOTAP:DOPE and DDAB:DOPE and consequently no advantage in using one liposome preparation over the other. Conducted under contract N01-CM-87028, Division of Cancer Treatment of the National Cancer Institute.

1473 COMPARISON OF HUMAN *VERSUS* CYNOMOLGUS MONKEY PLATELET AGGREGATION INDUCED BY FIVE DIFFERENT AGENTS.

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We investigated human and cynomolgus monkey platelet aggregation induced by 5 different aggregation agents: adenosine diphosphate (ADP), collagen (COL), arachidonic acid (AA), platelet activation factor (PAF) and epinephrine (EPI). Platelet aggregation in platelet-rich plasma was monitored after adding one of 5 agents using PAKS-4 platelet aggremometer (Helena). Several notable differences were found between human and monkey platelet aggregation after exposure to ADP, PAF or EPI. 1) At low ADP concentration (5 μM), secondary aggregation was observed in human platelets but not in monkey platelets. However, human and monkey platelets showed similar aggregation patterns (primary and secondary) at higher ADP concentrations (10 and 20 μM). 2) Monkey platelets showed no or significantly lower aggregation at up to 30 μg/mL of PAF, whereas even lower concentration (1.0 μg/mL) PAF induced strong aggregation in human platelets. 3) EPI did not induce aggregation of monkey platelets at up to 300 μM, whereas it did induce human platelet aggregation at down to 1 μM. We further investigated the effect of EPI on monkey platelet aggregation subsequently induced by the other aggregation agents. Monkey platelets were first stimulated with EPI (300 μM), and then the maximum no effect dose (MNED) of ADP, COL, AA or PAF was added. Although MNED of ADP, COL, AA or PAF did not induce monkey platelet aggregation alone, pre-treatment with EPI potentiated the induction of aggregation. Any combination of the other 4 agents had no aggregating effect at the MNED. The potentiating effect of EPI was observed even at 1000-fold lower concentration (0.3 μM) but completely inhibited by α2-adrenergic receptor antagonist, yohimbine. We conclude that EPI is able to stimulate monkey platelets and potentiate aggregation when trace amounts of other aggregation agents are added. Therefore, an α2-adrenergic receptor pathway appears to be involved in the enhanced susceptibility of monkey platelet aggregation by inducing agents.

1474 APPLICATION OF EXPERIMENTAL CARDIAC SENSITIZATION RESULTS IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS.

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An increased sensitivity of the heart to intravenous administration of epinephrine has long been recognized as a risk during exposure to hydrocarbons, principally halogenated hydrocarbons. This increased sensitivity of the heart is known as cardiac sensitization, and requires a certain critical blood level of the sensitizing agent and epinephrine. The original research and methods to assess cardiac sensitization potential in animals occurred about 30 years ago, although in the last 15 years others methods have been developed in response to replacement of the chlorofluorocarbons. The cardiac sensitization methodology has been used for semi-quantitative risk evaluations for occupational exposures but is now being used more quantitatively for regulatory purposes. While the risks associated with cardiac sensitization are low based on endogenous adrenaline experiments conducted in the 1970s, the incidence of abusive inhalation of CFCs has led to deaths. However, with the advent of physiologically-based pharmacokinetic (PBPK) modeling, greater emphasis on quantitative risk assessment can be used. In this work, we have examined the various methodologies used for detection of cardiac sensitization to determine the

limitations and advantages. In addition, using the PBPK models developed by others, we examined the potential concerns/opportunities associated with the use of the models based on the variations associated with the cardiac sensitization methodology.

1475 ACUTE TOXICITY OF POLYACRYLAMIDE AND SOL-GEL NANOPARTICLES IN RATS.

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Dynamic NanoPlatforms (DNPs) are nanoparticles (20 - 200 nm) developed for real-time intracellular measurement, with applications for analysis of drug, toxin, and environmental effects on cell function. The flexibility of these nanoparticles has further led to the development of particles for imaging of tumors and the potential simultaneous *in situ* treatment of cancer through photodynamic therapy. DNP matrices are composed of cross-linked polyacrylamide (PAA) or sol-gel silica. This study establishes the biocompatibility of the particles *in vivo* after exposure to PAA or sol-gel nanoparticles. Polyacrylamide (5-500mg/kg) and sol-gel (10mg/kg) suspensions were administered as a single i.v. dose in the tail vein of a conscious rat. Toxicity was assessed at 1, 3, 7, and 28 days by determination of serum levels of enzymes indicating hepatic, renal, pulmonary/cardiovascular and musculoskeletal damage, and histopathology. No significant change in serum levels of enzymes or histopathology was noted with either particle matrix at doses up to 50mg/kg (PAA) or 10mg/kg (sol-gel) for 28 days. However, a single dose of 500mg/ kg PAA-DNPs resulted in mortality of 11/16 (69%) treated rats in less than 24hr. Increases in circulating enzyme indicators of damage suggest the potential for liver, kidney and/or cardiac toxicity. Histopathological evaluation supports these findings. In conclusion, rats exposed to concentrations up to 50mg/ kg (PAA) or 10mg/kg (sol-gel) were not consistently different from controls. The data suggest that the composition of the particle and total body particle loads influence the degree of toxicity observed. Supported by NIH-ES 08846 & NCI-NO1-CO-07013.

1476 A SIX-WEEK INHALATION NEUROTOXICITY STUDY OF METHYL BROMIDE IN DOGS.

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Previous studies of inhaled methyl bromide have produced inconsistent results regarding functional deficits at low concentrations. A National Academy of Sciences report (Methyl Bromide Risk Characterization in California, 2000) recognized a deficiency in neurotoxic assessment in the dog. Therefore, this study was designed to provide well-defined behavioral and neurological endpoints, as well as a comprehensive histopathological evaluation. Exposures were selected to bracket possible human exposure scenarios, while avoiding unnecessary toxicity (for humanitarian reasons). Beagles (4/sex/ group) were exposed by whole-body inhalation to methyl bromide at concentrations of 0, 5, 10 and 20 ppm for 7 hours per day, 5 days per week for 6 consecutive weeks. Clinical examinations were performed daily and detailed physical examinations were conducted weekly. Individual body weights and food consumption were recorded weekly. A functional observational battery (FOB), as well as an automated measure of locomotor activity were conducted twice prior to the initiation of exposures and during study weeks 2, 4 and 6. The laboratory had previously validated both the FOB, which consists of a comprehensive checklist of behaviors in the home cage, on a platform and in an open-field, and the locomotor activity procedure, using a custom-built system for dogs. Following in-situ perfusion after the last inhalation exposure and a macroscopic examination, tissues from the brain, spinal cord and peripheral nerves were examined microscopically. Clinical observations, body weights, food consumption, body temperatures and FOB and locomotor activity parameters were unaffected by methyl bromide. Furthermore, no macroscopic or microscopic changes were observed. Based on the results of study, no evidence of neurotoxicity was observed in dogs following methyl bromide inhalation in concentrations up to 20 ppm.

1477 SEX DIFFERENCES IN EXPRESSION OF PERIPHERAL BENZODIAZEPINE RECEPTOR (PBR) BINDING SITES IN RAT BRAIN AFTER THE ADMINISTRATION OF HEPTACHLOR AND HEPTACHLOR EPOXIDE DURING DEVELOPMENT.

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The peripheral benzodiazepine receptor (PBR) has been found in steroid producing tissues where it functions to mediate the entry of cholesterol into mitochondria. The PBR has also been identified in brain where it is limited to glia. Others have shown that increases in PBRs occur after neuronal damage. In previous work we have shown that acute exposure to heptachlor, a GABA-A antagonist that does not cause neuronal damage, in adult rats results in a rapid increase in PBR binding sites in brain; this was similar to increases found 4 days after trimethyltin neuronal damage. In this study we evaluated the effects of acute exposure of heptachlor on PBR numbers in the developing rat brain. Rats ranging in age from birth to 45 days were exposed to a single dose *per os* (PO) of heptachlor (60 mg/kg in oil). A clear sex difference in PBR numbers after exposure to heptachlor is seen at all ages between 10 and 20 days. During this period, heptachlor increased PBR numbers 2-fold in females but did not affect PBR numbers in males. Increases in PBR numbers in males were not produced by heptachlor until 30 and 45 days of age. The effects of maximal electroshock (MES) were compared with the effects of heptachlor at day 12 and 20. Both males and females exhibited increases in PBR numbers following MES, demonstrating that males are capable of showing increases in PBR numbers during development. Heptachlor epoxide, a more persistent and toxic metabolite of heptachlor was also tested during developmental. In this experiment PBR numbers were significantly increased in males but not in females- just the opposite of the effect of heptachlor. Our findings indicate that there may be an interaction between PBRs and the action of heptachlor in brain. *Supported by: Hawaii Heptachlor Research and Education Foundation.*

1478 EFFECTS OF CHRONIC DERMAL EXPOSURE TO NONLETHAL DOSES OF METHYL PARATHION ON BRAIN REGIONAL ACETYLCHOLINESTERASE (ACHE) AND MUSCARINIC CHOLINERGIC RECEPTORS IN FEMALE RATS.

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The *in vivo* and *in vitro* effects of methyl parathion, a phosphorothionate insecticide, on cholinergic neurotransmitter systems in the brain of rats were investigated. Three groups of adult female rats received 0, 0.1, or 1.0 mg/kg methyl parathion *via* dermal exposure for 95 days. Exposure to 0.1 mg/kg methyl parathion produced inhibition of AChE in the caudate-putamen and thalamic nuclei, whereas 1.0 mg/kg resulted in inhibition of AChE in most brain regions. The same doses of methyl parathion had no effect on [3H]QNB binding to muscarinic receptors in the brain regions examined. The *in vitro* study demonstrated that methyl parathion causes preferential inhibition of AChE and [3H]QNB binding in specific brain regions. As an inhibitor of AChE, methyl paraoxon was 1000-fold more potent than was methyl parathion. Similarly, methyl paraoxon showed brain region-specific inhibition of the enzyme. Generally, the brain stem was highly sensitive to organophosphate-induced inhibition of AChE activity and [3H]QNB binding. Because central respiratory neurons gather in the brain stem, preferential effects there and in other brain regions may underlie lethal toxicity of methyl parathion and other organophosphates. (Supported by CDC grant RO6/CCR419466)

1479 MODULATION OF MUSCARINIC RECEPTORS IN THE RAT BRAIN DURING THE DEVELOPMENT OF TOLERANCE TO METHYL PARATHION.

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Methyl parathion is a potent and irreversible acetylcholinesterase (AChE) inhibitor, which produces peripheral and central cholinergic over-stimulation. Tolerance can be induced by repeated exposure to sublethal doses of methyl parathion. Because extensive enzyme inhibition often induces compensatory changes in cholinergic receptor population, we compared the effect of methyl parathion on brain muscarinic receptors at different time points during the development of tolerance to methyl parathion. Adult male rats were treated with either vehicle or methyl parathion (3 mg/kg/day, s.c.) and observed for the signs of toxicity. Animals were sacrificed 7 or 21 days after the daily treatment for measurement of acetylcholinesterase activity and binding to muscarinic radioligands, [3H] QNB (nonselective), [3H] pirenzepine (M1-selective), and [3H] AFDX-384 (M2-selective). After 21 days of treatment, methyl parathion caused 80% AChE inhibition and substantial reductions in [3H] QNB binding (14.3 ~ 32.7%), [3H] pirenzepine binding (around 21%), [3H] AFDX-384 binding (21.7 ~ 37.7%) in the striatum, hippocampus formation, and cortex. After 7 days of treatment, the inhibition of AChE in brain regions was from 39% to 72%, whereas receptor densities were only marginally affected in a few regions. The timing of the changes in receptor population correlates well with our previous finding in changes of behaviors during the development of tolerance. Our results strongly suggest that down-regulation of muscarinic receptor plays a role in the development of tolerance to organophosphates. (R06/CCR419466)

1480 BRAIN ESTERASE ACTIVITIES IN RATS GIVEN MULTIPLE DOSES OF ORGANOPHOSPHORUS (OP) COMPOUNDS OVER 63 DAYS WITH 30 DAYS RECOVERY.

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Acetylcholinesterase (AChE) and neurotoxic esterase (NTE) activities in the hippocampus (H), cerebral cortex (CC), basal forebrain (BP) and caudate putamen (CP) were examined in adult male Long-Evans rats given OP compounds during a 63-day dosing period with 30 days recovery. Corticosterone 400 mcg/ml in drinking water was given throughout the 90-day test period. The insecticide chlorpyrifos (60 mg/kg sc) was given on Days 7 and 42. Tri-ortho-tolyl phosphate (TOTP), 300 mg/kg PO, was given 7 times between Days 14 & 28 and again between Days 49 & 63. At 63 days, AChE activities in H, CC, BP and CP of rats given only chlorpyrifos were 40%+/-7 (mean+/-SEM, n=6), 32%+/-4, 35%+/-6, and 30%+/-3 of control, respectively. AChE activities after chlorpyrifos + TOTP were <15% of control. Rats drinking corticosterone-treated water and given chlorpyrifos + TOTP also had AChE activities <15% of control. On Day 91, AChE activities were 62%+/-3, 74%+/-2, 61%+/-9 and 62%+/-9, respectively, in the H, CC, BF and CP of rats given only chlorpyrifos. The activities of AChE in rats given TOTP only or given chlorpyrifos + TOTP ranged from 41-75% of control. Corticosterone treatment did not appear to affect AChE activities alone or in OP-treated rats. NTE activities in the H, CC, BF and CP were only affected by TOTP, with activities at 43%+/-5, 40%+/-5, 33%+/-4, and 45%+/-8 of control in these brain regions, respectively, at 63 days. NTE activities in all brain regions were at control values on Day 91. These results demonstrate that the OP treatments had great effects on AChE activity in all brain regions examined, and that recovery was not complete even 30 days after cessation of treatment with TOTP or 47 days after treatment with chlorpyrifos. The 30-day time period was, however, sufficient for recovery of NTE activity in rats given TOTP in multiple doses. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1481 NEUROPATHOLOGICAL STUDY OF THE INTERACTIONS OF STRESS AND TWO NEUROTOXIC ORGANOPHOSPHATES IN RATS.

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Interest exists concerning toxic actions of chemical mixtures and the role of stress in modulating such events. We previously reported data from a study designed to assess effects of stress and the concurrent exposure to two organophosphorus compounds in rats (Society for Neuroscience Abstracts, 2002). The present work updates the neuropathologic findings. The toxicants used were the insecticide chlorpyrifos (as a single 60 mg/kg subcutaneous dose) and the classical delayed neurotoxicant tri-ortho-tolyl phosphate (TOTP, given seven times over a two-week period as 75, 150 or 300 mg/kg gavage doses). Stress was mimicked by administration of corticosterone in the drinking water (400 ug/ml) over a 63-day period. The toxicants were given in two courses between days 7-27 and days 42-62, with sacrifice on day 63 (experimental groups n=12). Neurochemical findings are reported in an abstract by M. Ehrich et al. at this meeting. Neuropathologic studies demonstrated an axonopathy in the distal (medullary and cervical) levels of the gracile fasciculus, which progressed to overt myelinated fiber degeneration. Lesions included swollen or collapsed axons and myelinated fiber breakdown. These were primarily related to the TOTP dosage, with the 300 mg/kg group having a higher incidence and more extensive changes (including enhanced proximal extent of the nerve fiber lesions) than those animals given 150 mg/kg. Stress did not appear to affect the expression of the neuropathic process. Preliminary studies suggest that chlorpyrifos enhanced the severity of the lesions. The nature and distribution of this nerve fiber degeneration are consistent with this being a rat model of organophosphorus ester-induced delayed neuropathy (OPIDN). (Supported by: US Army Medical Research and Materiel Command DAMD17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1482 THE TOXICOKINETICS OF PERIPHERAL CHOLINESTERASE INHIBITION FROM ORALLY ADMINISTERED CARBOFURAN IN RATS.

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This study was performed with the carbamate pesticide carbofuran to determine the time course of inhibition and recovery of cholinesterase activity (CA) after oral administration. A single dose of carbofuran in corn oil vehicle was administered *via* oral gavage to cannulated adult male and female CD rats at one of three dose levels: 0, 0.5, or 1.0 mg/kg. Blood samples were collected at -15 (predose baseline), 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360 and 480 minutes after dosing for determination of plasma and erythrocyte (RBC) CA. Carbofuran did not inhibit plasma CA at either dose level when compared to controls. Following a single oral administration of 0, 0.5, or 1.0 mg/kg, RBC CA was inhibited at the low and high dose levels in both the males and females. To account for inherent variability as seen in the control values, the percent of baseline RBC CA was calculated at each time point (for each animal in each dose group). For the 0.5 mg/kg dose group, the percent of baseline activity was not statistically different from controls from 150 through 480 minutes for males and from 75 through 480 minutes for females (with the exception of the 240 min time point in males and the 360 min time point in females). For the 1.0 mg/kg dose group, percent of baseline RBC CA was not significantly different from controls at the 180 and 360 minute time points for males and the 240 and 480 minute time points for females. When normal variability is taken into account by evaluating the data as percent of baseline value, a return to normal RBC CA occurred by 150 minutes (males) and 75 minutes (females) in the low dose group.

1483 DEVELOPMENT OF A NEONATAL RAT PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MODEL FOR CHLORPYRIFOS.

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Juvenile rats are more susceptible than adults to the high dose effects of organophosphate (OP) insecticides, like chlorpyrifos (CPF); therefore, it is assumed that infants and children are likewise more susceptible. Age-dependent differences are primarily a function of metabolic capacity for several important enzyme systems. This includes CYP450 activation to CPF-oxon, and detoxification to trichloropyridinol (TCP), as well as B-esterase (B-EST) and A-esterase (A-EST) detoxification of CPF-oxon to TCP. A PBPK/PD model describing the time-course of CPF, CPF-oxon, and the inhibition of acetylcholinesterase (AChE) in adult rats and humans was modified to allometrically scale (based on body weight) the age-dependent development of CYP450 and A-EST enzyme activity. The model provided a good simulation of brain and RBC AChE inhibition in post-natal day (PND) 17 rats orally administered 15 mg/kg CPF. The model was used to simulate concentrations of CPF-oxon in the brain of neonatal rats (PND4) following single acute oral exposure to a range of CPF doses (0.5-50 mg/kg). Doses greater than 5 mg/kg resulted in theoretical concentrations of CPF-oxon in the brain of PND4 rats that was greater than in adults, this difference increased with dose. Doses less than 1 mg/kg resulted in brain CPF-oxon concentrations in PND4 rats that were similar to theoretical adult AUCs. The model simulation suggests that neonatal rats are more sensitive than adults to the effects of acute high dose exposures. However, the model also indicates that even though neonatal rats have lower metabolic capacity, it is adequate to detoxify CPF at relevant environmental exposure levels. These simulations are consistent with differences in the acute toxicity response noted between neonatal and adult rats following exposure to CPF. This model represents an important starting point in the development of a PBPK/PD model to better define the potential of CPF to cause neurotoxicity in sensitive populations, such as infants and children. (Supported by EPA grant R828608)

1484 POTENTIAL UTILITY OF SALIVA BIOMONITORING FOR ORGANOPHOSPHATE INSECTICIDE DOSIMETRY AND ESTERASE INHIBITION.

A. Kousba, T. S. Poet and C. Timchalk. *Molecular Biosciences, Pacific Northwest National Laboratory, Richland, WA.*

Chlorpyrifos is a phosphothioate, which is a commonly used organophosphate (OP) insecticide. Its active metabolite CPF-oxon is a potential inhibitor of cholinesterase enzymes (ChE), such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Whereas the inhibition of AChE is associated with neurotoxicity, BuChE inhibition represents a detoxification mechanism and a potential biomarker of exposure/response. In the current study CPF, dissolved in corn oil, was administered orally as a single dose (0, 1, 10, and 50 mg/kg) to male Sprague-Dawley rats followed by saliva and blood collection at 0, 3, 6, and 12 hour post-dosing. CPF and its major metabolite trichloropyridinol (TCP) were quantified in blood and saliva using gas chromatography (GC) and a modified Ellman method was used to monitor the resultant ChE inhibition. A preliminary *in vitro* study, using both AChE and BuChE specific inhibitors, indicated that the vast majority (>90%) of rat salivary ChE activity was due to BuChE. Utilizing saliva as a source of BuChE, the bimolecular inhibition rate constant (Ki) and the first-order reactivation rate (Kr) were ~8000 $\mu M^{-1}\ h^{-1}$ and 0.08 h^{-1}, respectively. Oral administration produced a dose-dependent inhibition of ChE in plasma and saliva. At all dose levels the maximum salivary ChE inhibition (3-6 hr post-exposure) was slightly less than that of the plasma, and saliva exhibited a faster ChE recovery. CPF and TCP

were quantified in blood, whereas TCP was detected in saliva and only at higher doses. Although the saliva TCP concentrations were significantly less than the plasma concentrations, the TCP pharmacokinetics were comparable (i.e. similar half-life). These results suggest that saliva may be a useful biological matrix for monitoring CPF exposure and response either through measuring the metabolite levels or the degree of ChE inhibition. These data will be used for further validation of an already constructed pharmacokinetic/pharmacodynamic model for CPF. (Sponsors EPA grant R828608 and CDC/NIOSH R01OH03629-01A2).

1485 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC AND PHARMACODYNAMIC (PBPK/PD) MODEL FOR THE ORGANOPHOSPHATE PESTICIDE, DIAZINON.

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Organophosphate (OP) pesticides, like diazinon (DZN), constitute a large class of insecticides that are widely utilized, and the potential exists for significant exposures from multiple routes. The objective was to develop a PBPK/PD model capable of predicting the relationships between exposure, bioactivation, detoxification, and the inhibition of target esterases (EST). In this model, CYP450 metabolism of DZN to the oxon and detoxification to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) are both mediated by CYP450s in the liver. Hydrolysis of the oxon *via* A-EST occurs in the liver and blood and interactions with target B-EST (acetyl-, butyryl- and carboxyl-) were modeled as second order processes occurring in the liver, blood, diaphragm and brain. Metabolic rate constants for the CYP450- and A-esterase-mediated metabolism were measured *in vitro*. B-EST inhibition and regeneration rates have been determined *in vitro* and model optimization against cholinesterase (ChE) inhibition data. To facilitate model development, single oral-dose pharmacokinetic studies were conducted in rats (1 - 100 mg/kg) and the kinetics of DZN and IMHP as well as the extent of plasma ChE and RBC and brain acetylcholinesterase (AChE) inhibition were determined. In blood, the concentration of IMHP was greater than DZN and the kinetic time-course was linear over the dose-range and reasonably simulated by the model. Peak ChE inhibition occurred at ~6 hr post-dosing and the model accurately simulated the dose-dependent inhibition of plasma ChE, RBC AChE and brain AChE. This DZN PBPK/PD model quantitatively estimates target tissue dosimetry and ChE inhibition and will be integral to risk assessments for DZN and OP mixture exposures under a variety of scenarios. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2).

1486 MASS SPECTRAL EVIDENCE THAT MIPAFOX-INHIBITED NEUROPATHY TARGET ESTERASE (NTE) DOES NOT UNDERGO DEALKYLATION.

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Organophosphorus compound-induced delayed neurotoxicity (OPIDN) is thought to be initiated by inhibition and aging of neural NTE. Aging of phosphylated NTE and other serine esterases involves progressive resistance to reactivation, attributed to anion formation *via* time dependent dealkylation of the active site OP adduct. Because N, N? diisopropylphosphorodiamidofluoridate (mipafox, MIP) inhibits NTE and produces OPIDN, it has been assumed that MIP inhibited NTE undergoes aging *via* dealkylation. Recent work, however, showed that MIP-inhibited NTE could be reactivated at low pH after allowing time for aging. In contrast, diisopropylphosphorofluoridate (DFP) inhibited NTE and DFP- or MIP inhibited butyrylcholinesterase (BChE) could not be reactivated after allowing time for aging. These observations suggest the hypothesis that DFP- or MIP-inhibited BChE and DFP-inhibited NTE undergo aging *via* dealkylation, whereas MIP-inhibited NTE does not. This hypothesis was tested by inhibiting horse serum BChE or human recombinant NTE esterase domain (NEST) with MIP or DFP. Using peptide mass mapping with surface enhanced laser desorption/ionization mass spectrometry, *m/z* peaks corresponding to active site peptides and their intact or dealkylated adducts were examined in control and treated samples at 0, 1, 2, 12, 24, and 36 h after inhibition. Time-dependent mass shifts representing a change from intact to dealkylated active site adducts were found for MIP- and DFP-inhibited BChE. Moreover, a peak corresponding to dealkylated active site adduct was found at all times for DPF inhibited NEST. In contrast, a peak representing intact active site adduct was found at all times for MIP-inhibited NEST, showing that dealkylation did not occur. The results suggest that MIP produces OPIDN through a mechanism other than dealkylation of MIP-inhibited NTE. If an anionic active site adduct is required, it is possible that this arises from removal of the acidic phosphoramido proton. (Supported by DAAD19-02-1-0388).

1487 TENTATIVE MODELS FOR THE THREE-DIMENSIONAL STRUCTURE OF THE NTE ESTERASE DOMAIN (NEST): PREDICTIONS FROM THREADING AND DOCKING.

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Neuropathy target esterase (NTE), the primary target for initiation of organophosphorus compound-induced delayed neuropathy, is a 1327-amino acid integral membrane protein, whose three-dimensional structure is intractable to experimental determination. Moreover, sequence analysis shows that NTE is a member of a novel protein family, so that its theoretical model cannot currently be obtained by homology modeling. The NTE esterase domain (NEST) corresponds to NTE residues 727-1216 and is the minimum NTE construct with full esterase activity. The threading program PROSPECT was employed to conduct fold recognition and sequence structure alignments for NEST. Based on the alignments obtained from threading, atomic structures of NEST were generated using the program MODELLER. Resultant models were refined in the CHARMm module of InsightII 2000. Finally, candidate structures were evaluated by docking the neuropathic compounds diisopropylphosphorofluoridate and ethyl 4-nitrophenyl phenylphosphonate as ligands into the NEST model using the Affinity module of InsightII 2000. This strategy yielded three putative structures of NEST for further study. The models were consistent with experimental data from ligand binding and site-directed mutagenesis. Namely, they predicted Ser^{966} as the active-site serine, Asp^{1086} and Asp^{960} as possible critical residues for catalysis, and Asp^{1044} or Asp^{1004} as possible acceptor residues for the intramolecular transfer of an alkyl group during aging of phosphylated enzyme. These models for NEST provide a starting point for gaining atomic-level insight into interactions of NTE with small molecules and could be further refined and validated through interative modeling and experimental validation. (Supported in part by DAAD19-02-1-0388).

1488 DECREASE OF 5-HT LEVELS AFTER PYRETHROID TREATMENT.

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Deltamethrin, cyfluthrin and cyhalothrin, Type II pyretroid insecticides, are used topically for the control of ectoparasites. Type II pyrethroids when injected peripherally to rat produced a severe syndrome characterized by salivation and choreoathetosis. Because of a variety of putative biochemical and physiological target sites may contribute to pyrethroid toxicity, the objective of the present study was to investigate neurochemical effects following the administration of deltamethrin (40 mg/kg, i.p. for 6 days), cyfluthrin (14 mg/kg i.p. for 6 days) and cyhalothrin (8 mg/kg, per os for 6 days) in male Wistar rats(n =6/group). Animals were sacrificed 24 hours following pyrethroid administration and their brains were rapidly removed. The frontal cortex, hippocampus, midbrain and striatum were dissected and analyzed for content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. A serotonin depleting effect was produced by these pyrethroids. Deltamethrin decreased 5-HT and 5-HIAA levels in midbrain (38%; $P<0.05$; 17%, $P<0.05$) and striatum (46%, $P<0.001$; 21%, $P<0.05$) and decreased 5-HIAA levels in frontal cortex (62%, $P<0.001$) and hippocampus (48%, $P<0.001$) respect to corn oil controls. Cyfluthrin decreased 5-HT and 5-HIAA levels in frontal cortex (25%, $P<0.05$; 30%, $P<0.01$), hippocampus (20%, $P<0.05$; 19%, $P<0.05$) and striatum (31%, $P<0.01$; 36% $P<0.01$) respect to corn oil controls. Cyhalothrin decreased 5-HT levels in frontal cortex (35%, $P<0.001$), hipoccampus (26%, $P<0.05$), midbrain (28%, $P<0.05$) and striatum (24%, $P>0.001$) and decreased HIAA levels in frontal cortex (36%, $P<0.01$) and midbrain (27%, $P<0.05$). The data presented herein suggests that a lower activity of serotonergic system exists in the action of Type II pyrethroids. This work has been supported by projects No. PB9701236, (DIGICYT), No. 08.8/0002/98 (CAM) & No. 99/0936 (FIS), Spain.

1489 CONVERSION OF DELTA PH AND ELLMAN VALUES FOR CHOLINESTERASES.

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Intensive use of anticholinergic pesticides such as organophosphate esters and threat of chemical warfare establish the need for rapid, high throughput, reliable and standardizable determinations of blood cholinesterase levels to provide early warning of exposures to neuroactive chemicals. Many clinical and research labora-

tories use the colorimetric Ellman assay based on hydrolysis of acetylthiocholine. CHPPM (US Army Center for Health Promotion and Preventive Medicine) uses a slower method, the delta pH based on that of Michel to monitor approximately 25, 000 DOD personnel annually. One of the goals of this project is to establish a conversion factor between the pH and colorimetric assays applicable to monitoring studies and field tests. Blood drawn under the appropriate regulations by CHPPM was centrifuged and detergent-lysed before being subjected to the delta pH assay with acetylcholine as substrate. Duplicate RBC samples were sent to UC Davis to be assayed with acetylthiocholine by the Ellman method. Samples were lysed, diluted with buffer and run with and without quinidine to separate activities due to acetylcholinesterase and non-specific cholinesterases. For example, slopes of delta pH vs Ellman for three of five sets of samples yielded r^2 correlations of 0.74 to 0.8. Comparisons continue to establish critical assay conditions and Ellman equivalents of the delta pH assay. Supported by DOD (DAMD17-01-1-0772), NIOSH (#CDC U07/CCU906162-06) and NIEHS (#ES05707).

1490 DIFFERENTIAL PROFILES OF CHOLINESTERASE INHIBITION AND NEUROBEHAVIORAL EFFECTS IN RATS EXPOSED TO FENAMIPHOS AND PROFENOPHOS.

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The relationship between cholinesterase (ChE) inhibition and neurobehavioral changes was examined using two ChE-inhibiting organophosphorus pesticides, fenamiphos and profenophos. Both pesticides inhibit blood ChE, yet brain ChE is relatively spared (little to no inhibition up to lethal doses). Interestingly, pronounced neurobehavioral signs were observed following fenamiphos but not profenophos. A direct comparison was then undertaken to evaluate the influence of brain ChE on the behavioral signs observed. After a single oral dose, both pesticides greatly inhibited blood ChE (87-98% inhibition), yet whole brain ChE was only inhibited by 9-14% at the highest doses. Fenamiphos produced dose-dependent lacrimation, salivation, tremors, gait abnormalities, and decreased motor activity and tail pinch response. Despite the similar ChE inhibition profile, profenophos produced no changes in any of these measures. Thus, the neurobehavioral effects of fenamiphos could not be explained based on brain ChE inhibition alone. Pretreatment with anticholinergic drugs was used to evaluate the contribution of peripheral vs central ChE inhibition. Scopolamine (SCO) and methylscopolamine (MSC) were used as central/peripheral and peripheral-only cholinergic receptor blockers, respectively, in combination with fenamiphos. Neither drug altered fenamiphos effects on ChE. Some effects of fenamiphos were blocked or attenuated only by SCO, whereas other effects were blocked by both drugs. These data indicate that some of the pronounced neurobehavioral changes observed following fenamiphos may be centrally mediated (blocked by SCO only), despite the relative sparing of brain ChE. Regionally specific ChE inhibition or direct cholinergic receptor activation may be responsible for these effects. Other behavioral changes may be mediated more peripherally (blocked by both MSC and SCO), yet the contrast between profenophos and fenamiphos indicates that these neurobehavioral effects cannot be predicted on the basis of blood ChE. This abstract does not necessarily reflect EPA policy

1491 CHRONIC DIETARY EXPOSURE WITH INTERMITTENT SPIKE DOSES OF CHLORPYRIFOS FAILS TO ALTER BRAINSTEM AUDITORY EVOKED RESPONSES (BAERS) IN RATS.

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Human exposure to pesticides is often characterized by chronic low level exposure with intermittent spiked higher exposures. Cholinergic transmission is involved in auditory structures in the periphery and the brainstem and is altered following chlorpyrifos exposure. This study examined the effects of chronic (1 year) dietary exposure (0, 1, or 5 mg/kg/day) to chlorpyrifos in male Long Evans rats (100-110 days old at study initiation) on BAERs. The chlorpyrifos doses were chosen to produce minimal and approximately 50% inhibition of brain cholinesterase activity, respectively. In addition to dietary exposure, half of the animals received an oral bolus of 45 mg/kg chlorpyrifos (in corn oil) every other month (n = 16-18 rats/treatment). Subjects were weight maintained at 350g throughout the study. After the final spiked exposure, the animals were allowed to recover for about 2.5 months, so only irreversible effects would be examined. Subjects were surgically implanted with screw electrodes over the cerebellum and allowed to recover for one week. Unanesthetized animals were placed in a restrainer and presented with the following auditory stimuli (presented at 5.6 hz): rarefaction click, 4 and 16 kHz pure tone pips presented at 50, 65, and 80 dB SPL, and 64 kHz pure tone pip presented at 65, 70 and 80 dB SPL. Dietary exposure to chlorpyrifos (alone or in combination with oral spike doses) did not produce changes in brainstem auditory evoked responses. The evoked responses showed the expected intensity and frequency-dependent changes, indicating that the animals responses were under stimulus control. Thus, chronic exposure to chlorpyrifos did not appear to alter auditory responses at the level of the brainstem in adult animals. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

1492 COMPARATIVE EFFECTS OF METHYL PARATHION (MPS) AND ITS METABOLITE METHYL PARAOXON (MPO) ON ACETYLCHOLINE (ACH) RELEASE AND MUSCARINIC AUTORECEPTORS IN JUVENILE AND ADULT RATS.

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Previous studies have demonstrated that young rats are more sensitive than adults to acute toxicity of high dosage of MPS. The present study investigated the relationship between the toxicity of MPS and ACh release and its muscarinic receptor mediated regulation in juvenile and adult rat brain. AChE activity and QNB binding from the same rats and effects of MPS and MPO *in vitro* on muscarinic receptors were also evaluated. The results showed that in absence of physostigmine (PHY) and atropine (ATR) in buffers, MPO *in vitro* reduced ACh release in a concentration dependent manner (20-40% in 21 day rats, 10-40% in adult rats); in presence of PHY and in absence of ATR in buffers, MPO had no effect on ACh release in both age groups; and PHY was always included in the perfusion buffers and ATR was added 10 min before the second stimulus, MPO also decreased release as before; but MPS *in vitro* did not show any effect on the ACh release. The ChE activity in juvenile rats showed a quicker recovery by 24h and 96h than that in adult rats. Different quantitative reductions in QNB binding were noted in both age groups 24h and 96h after exposures to both dosages of MPS, by 4 hours, however, a significant reduction of QNB binding was noted in juvenile rats only. DSAR or S1 was significantly reduced 24h after LD10 exposure in juvenile striatum, but was no remarkably affected at any other timepoint or with lower dosage. Different quantitative reductions in S2/S1 release ratios were noted in both age groups 96h after exposures to both dosages of MPS, but no significant reduction was noted at other timepoints. The results suggested that MPO may have potential direct effects on muscarinic receptor function and effects of MPS on ACh release and its muscarinic receptor-mediated regulation during maturation as a possible contributing factor to age-related differences in sensitivity.

1493 PYRIDOSTIGMINE BLOCKS PARAOXON-INDUCED BLOOD-BRAIN BARRIER LEAKAGE.

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Increased entry of pyridostigmine (PYR) into the brain has been hypothesized as a contributing factor to unexplained Gulf War Illnesses. Some organophosphorus (OP) cholinesterase (ChE) inhibitors can compromise blood-brain barrier (BBB) integrity, generally through elicitation of convulsions. Preliminary results suggested, however, that low-level paraoxon exposure (0.1 mg/kg, im, causing roughly 50% ChE inhibition) could increase BBB leakage as evidenced by the enzymatic marker horseradish peroxidase (HRP) in 25-30 day old Long Evans rats. We evaluated the interaction between acute paraoxon and acute PYR on HRP accumulation and brain ChE activity. PYR (30 mg/kg, po, or saline) was administered 50 minutes prior to paraoxon (or vehicle, 0.4% DMSO in saline). Rats (n=3/trt) were anesthetized (diethyl ether) 7.5 minutes later and heart was exposed for intracardiac HRP injection (40 mg/ml in 2% Evans Blue, 200 ml/rat) through the left ventricle, beginning exactly 10 min after paraoxon. One minute after HRP injection, rats were sacrificed, whole brain was dissected and placed in 2.5 % glutaraldehyde for 48 hours. Cortical (temporal and frontal) regions were sectioned at 70 mm on a vibrotome and then histochemically processed and counted for number of BBB leaks per section as evaluated by HRP staining. Brain ChE activity was measured under the same treatments except rats were not injected with HRP. Paraoxon caused a significant >4.5 fold increase in number of BBB leaks in the temporal cortex. Rats treated with PYR alone or PYR prior to paraoxon exhibited minimal leaks, similar to controls. Little brain ChE inhibition was noted after PYR exposure while paraoxon alone caused 55-58% inhibition. Interestingly, brain ChE inhibition was somewhat lower (40-42% inhibition) in rats exposed to both PYR and paraoxon. The results suggest that paraoxon-induced BBB leakage does not facilitate PYR entry, but that PYR may inhibit BBB leakage by paraoxon. (Supported by grant DAMD17-00-1-0070 from US Army)

1494 INTERACTIVE TOXICITY OF CHLORPYRIFOS AND PARATHION IN NEONATAL RATS.

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Organophosphorus insecticides (OPs) elicit their effects by inhibiting the enzyme, acetylcholinesterase. Young mammals are generally more sensitive to acute OP poisoning compared to adults. There is very little information on the combined action of multiple OP exposures, however. Recently, we reported interactive effects of OPs, chlorpyrifos (CPF) and parathion (PS) in adult rats (Karanth et al., 2001). In the present study, we evaluated the *in vivo* effects of sequential and concurrent exposures to CPF and PS in neonatal (7 days old) rats. LD1 values for CPF (8 mg/kg, po) and PS (0.5 mg/kg, po) were estimated by dose-response studies. Rats were exposed to OPs either simultaneously or sequentially (separated by 4 h) and functional signs of toxicity and lethality were recorded. Animals were sacrificed at 4, 8 and 24 hr (after first exposure) for biochemical measurements. The concurrent group showed more lethality (9/12) than either of the sequential dosing groups. Among the sequential groups, CPF-first showed higher lethality (3/8) than PS-first (1/8). After 4 hr of first dose, brain cholinesterase (ChE) inhibition was significantly higher in concurrent group (80%) compared to CPF-first (60%) and PS-first (30%) while at 8 hr, ChE inhibition was higher in CPF-first group (90%) than in PS-first group (79%) or concurrent group (78%). At 24 hr after exposure, recovery from inhibition was faster in concurrent group compared to sequential groups. ChE inhibition in plasma and diaphragm were also higher in concurrent group compared to sequential groups after 4 hr of exposure. Carboxylesterase inhibition was relatively similar among the groups in plasma (52-78%) and liver (77-84%) at different time-points. These results suggests that neonatal rats, in contrast to adult rats, are more sensitive to concurrent than sequential exposures. The mechanisms contributing to age-related sequence-dependent differences in interactive toxicity are unclear.

1495 AGE AND STRAIN COMPARISONS OF CARBACHOL-STIMULATED INOSITOLPHOSPHATE (IP) RELEASE IN RAT RETINA AND FRONTAL CORTEX.

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PURPOSE: This study investigated age- and strain-related differences in retinal and brain IP second messenger pathways. This work is a prelude to further experiments on age-related effects of organophosphorous pesticides (OP) on retinal toxicity. Acute OP exposure in young adult rats produced a long-lasting down-regulation of the IP pathway in retina but not in brain; we hypothesize that this effect in aged rats may result in permanent retinal damage. Strain comparisons were necessary because of the limited availability of aged rats. This study compared IP release in retina and frontal cortex. METHODS: Tissue was taken from young and old rats of different strains: Fisher-344 (F-344, albino; 70-day, 1- or 2-year old), Long-Evans (LE, pigmented; 70-day old only), and Brown Norway (BN, pigmented; 70-day and 2-year old only). 3H-myo-inositol (3H-MI) was incorporated into whole retina and cortical slices. IP released during incubation with carbachol was separated by anion exchange column chromatography and is reported here as the fraction released of the total amount of 3H-MI incorporated. RESULTS: In the retina, F-344 and LE had higher levels of stimulated IP release compared to BN. No age-dependent differences in IP release were noted. In the frontal cortex, as in the retina, stimulated IP release was higher in F-344 than in BN. In the young LE tested, LE had even higher levels of IP release than F-344. No age-related differences were noted in within-strain comparisons. DISCUSSION: In summary, carbachol-stimulated IP release in retina and frontal cortex was higher in rats from the F-344 and LE strains compared to the BN strain. In addition, within-strain comparisons indicate that IP release in both tissues is similar in young and aged F-344 and BN rats. These results suggest that carbachol-stimulated IP release depends on strain, but not on age or pigmentation. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1496 DIFFERENTIAL SENSITIVITY TO ANTICHOLINESTERASE PESTICIDES IN THE JUVENILE RAT: EFFECTS ON THERMOREGULATION.

C. M. Mack and C. J. Gordon. *USEPA, Research Triangle Park, NC.* Sponsor: D. Herr.

Organophosphate (OP) and carbamate (CB) pesticides inhibit cholinesterase (ChE) activity and induce acute hypothermia in adult rats. Studies have shown that juveniles are generally more susceptible to neurotoxic insult than adults, however, the effects of OP and CB pesticides on thermoregulation in developing animals are relatively unknown. Thus, we investigated alterations in core body temperature (Tc) in juvenile animals exposed to an OP or CB. Male rats were implanted on postnatal day (PND) 15 with a radio transmitter to monitor Tc and motor activity. PND 17 pups received a single dose of the OP chlorpyrifos (CHP) (1, 5, 10 or 15 mg/kg) or the CB carbaryl (CAR) (10, 20, 80, 120, or 160 mg/kg) or corn oil, po. Pups were returned to their dams and littermates immediately after dosing. CHP doses of 10 and 15 mg/kg resulted in 1.0 °C and 2.5 °C reductions in Tc, respectively. Tc recovered 16 hr after dosing. MA was significantly reduced following 15 mg/kg CHP. Conversely, the highest dose of CAR (160 mg/kg) resulted in a 1.3 °C reduction in Tc that recovered in 9 hr. In contrast, adult rats dosed with 50 mg/kg CHP underwent a 2.2 °C hypothermia while 75 mg/kg CAR led to a 1.8 °C hypothermia. Therefore, compared to adults, 17 day old rats are 5-times more sensitive to CHP, an irreversible ChE inhibitor but approximately one-half as sensitive to CAR, a reversible inhibitor. Overall, it appears that during development in the rat from pre-weanling to adult, there is a progressive attenuation in sensitivity to CHP, but an exacerbation in sensitivity to CAR. This abstract does not necessarily reflect USEPA policy.

1497 INDUCTION AND PROMOTION OF DELAYED POLYNEUROPATHY BY PHOSPHOROAMIDATES. *IN VITRO* AND *IN VIVO* STUDIES.

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Certain esterase inhibitors, such as sulfonyl fluorides, carbamates and organophosphorus compounds (OP), exacerbate the clinical/morphological expression of axonopathies (promotion). The model for studying promotion is OP induced delayed polyneuropathy (OPIDP) in the hen. Promotion was associated with inhibition of a phenyl valerate esterase (PV) activity in whole nerve homogenate (M200) and of a PV activity associated with soluble nerve proteins (approx. MW 70 kDa) (S-PV). Inhibition and 'aging' (a molecular rearrangement after inhibition) of a PV called neuropathy target esterase (NTE) trigger OPIDP. Promoters inhibit NTE but do not cause OPIDP because they do not 'age' NTE. D(+) and L(-) methamidophos (O, S-dimethyl phosphorothioamidate) which caused promotion and, at higher doses, OPIDP were exceptions. In fact, NTE sensitivity to inhibition by methamidohos isomers was lower than that of S-PV. We assessed the ability of some methamidophos analogs to cause OPIDP, promotion or both. The O-ethyl and O-isopropyl analogs could not be tested *in vivo* because of high acute toxicity. In fact, they were more potent inhibitors of acetylcholinesterase (the target of the acute toxicity of OPs) than of NTE and S-PV. NTE, M200 and S-PV were inhibited by the O-n-hexyl analog (10 mg/kg sc) by 73, 62 and 87%, by the O-n-butyl analog (8 mg/kg sc) by 74, 63 and 89%, and by the O-n-propyl analog (15 mg/kg sc) by 40, 28 and 64%, respectively. The O-n-hexyl analog did not cause OPIDP (suggesting that NTE did not age) and promoted OPIDP caused by dibutyl dichlorvos (0.4 mg/kg sc): the median clinical score (range) increased from 1(0-3) to 7(4-8) (0-8 point scale). The O-n-butyl analog caused mild OPIDP (score 1) and, when given after dibutyl dichlorvos, it increased the score to 5(4-7). However, it was not clear whether this was promotion or cumulative effect. The O-n-propyl analog neither caused OPIPD nor promotion. These data indicate that phosphoroamidates are a particular class of compounds and support the association between promotion and substantial inhibition of S-PV activity.

1498 EXPLORING THE EXPLANATION OF AGE-RELATED SENSITIVITY TO A PYRETHROID INSECTICIDE, DELTAMETHRIN, IN RATS.

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This study was designed to assess the age-related sensitivity of a pyrethroid insecticide, deltamethrin [(S)- -cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromomovinyl)2, 2-dimethylcyclopropanecarboxylate], in Long Evans, hooded, male rats. Previously, in the literature, deltamethrin has been demonstrated to be more toxic to young rats (Sheets et al., Toxicol Appl. Pharmacology, 1994). These studies are designed to explore the reasons for this increased sensitivity. Our hypothesis is that weanling rats are more sensitive than adults to the toxic effects of deltamethrin due to pharmacokinetic differences. To test this hypothesis, we first validated a high performance liquid chromatography (HPLC) method to quantitate deltamethrin in tissues from both weanling and adult rats (modified from Anad n et al., Toxicol Appl. Pharmacology 1996). Advantageous mobile phase, extraction solvent, limit of detection, and recovery of deltamethrin was determined in plasma. To estimate the time of peak deltamethrin concentration in plasma, adult (90 days) and weanling (22 days) male rats were gavaged (n = 3-5) with a single dose of either 0 (corn oil vehicle) or 4 mg/kg of deltamethrin, and plasma collected between 1 and 24 hours after dosing. HPLC analysis of the plasma revealed that the time of peak deltamethrin concentration in both age groups approximately 2 hours after dosing, and there does not appear any difference between weanling and adult deltamethrin concentrations at this time. Thus, it seems that the plasma concentration of deltamethrin does not explain the age-related differences in sensitivity. This abstract does not reflect EPA policy.

1499 DOES CHRONIC CHLORPYRIFOS TOXICITY COMPROMISE DOPAMINERGIC FUNCTION IN THE RAT STRIATUM?

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Although the specific etiology is not known, epidemiological studies implicate pesticide exposure as a major risk factor for Parkinson's disease. Acetylcholine's ability to reverse parkinsonian symptoms suggests that dopamine and acetylcholine exert opposing effects on extrapyramidal function. Recognition of this reciprocal balance has prompted speculation as to whether perturbations of cholinergic transmission might initiate dopaminergic dysfunction. Chlorpyrifos (CPF) is a commonly used anti-cholinesterase (anti-ChE) insecticide. We hypothesized that chronic, oral exposure to CPF would depress striatal dopaminergic function. Adult, male, Long Evans rats were given CPF for one year at three levels of dietary exposure (0, 1, or 5 mg/kg/day; n=10/group); half of the rats in each dietary exposure group received high dose bimonthly CPF challenges (spikes) by oral gavage and the other half received vehicle. Animals were sacrificed and tissues were taken for analysis at 6 or 12 months and also 3 months after dosing ceased. Cholinergic endpoints (i.e., ChE inhibition), as well as measures of dopaminergic function (i.e., [3H]mazindol binding; concentrations of dopamine and its metabolites) were assessed in the striatum. During dosing, there was substantial striatal ChE inhibition in all spiked groups and in the 5mg/kg CPF feed group, but three months after dosing ended, ChE had returned to control levels in all groups. There appear to be no changes in dopamine transporter binding or in concentrations of dopamine and its metabolites at any time or in any of the treatment groups. These data indicate that chronic exposure to CPF would not be expected to compromise dopaminergic function. This abstract does not reflect EPA policy. S. Oxendine is funded through a Minority Fellowship in Neuroscience.

1500 INHIBITION OF CHOLINESTERASE AND CARBOXYLESTERASE FOLLOWING *IN VIVO* EXPOSURE OF RATS TO MIXTURES OF PARATHION AND AZINPHOSMETHYL.

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The patterns of inhibition of brain and serum cholinesterase (ChE) and serum carboxylesterases (CbxE) were determined after oral administration of two OP insecticides singly or in simultaneous or sequential mixtures. The effective dose levels that would inhibit 10% of brain ChE at the time of peak inhibition were determined to be 1.0 mg/kg parathion (P) and 1.5 mg/kg azinphosmethyl (A) at 10 and 2 hr, respectively. Sequential exposures were performed with the first insecticide administered followed by the second insecticide at the peak inhibition time for the first. Tissue samples were collected at 2, 4, 8, 10, and 24 hr. Brain and serum ChE levels were determined by continuous spectrophotometric assay, and serum CbxE inhibition was determined by discontinuous spectrophotometric methods. Inhibition of brain ChE for A was 9% at 2 hr, 4% at 4 hr, and 0% at 8, 10, and 24 hr; for P was 7% at 8 hr, 12% at 10 hr, and 0% by 24 hr. Simultaneous exposure prolonged inhibition (from 2% at 2 hr to 17% at 10 hr, with a peak of 23% at 8hr). Sequential exposure (A followed by P) indicated no significant greater-than-additive effects; inhibition ranged from 7% at 2 hours to 4% at 24 hr with peak inhibition at 8 hours (20%). P followed by A produced a similar trend; 0% at 2 hr, peak at 8 hr (23%), and 0% at 24 hr. Inhibition of serum ChE levels decreased at successive time points, and simultaneous and sequential exposures showed no greater-than-additive effects. CbxE was greatly inhibited by P (ranged from 81% at 2 hr to 28% at 24 hr). Peak inhibition of CbxE by A was only 13% at 8 hr. Simultaneous exposure resulted in prolonged inhibition compared to the single compounds. Sequential exposures showed no greater-than-additive effects. There was no evidence of interactions in the exposure to binary mixtures of these two insecticides administered at relatively low dosages. (Supported by American Chemistry Council CRAM 2a-99).

1501 INVESTIGATION OF THE COMBINED TOXICITY OF A MIXTURE OF CHLORPYRIFOS AND METHYL PARATHION.

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The organophosphorus insecticides chlorpyrifos (CPS) and methyl parathion (MPS) exert their toxic effects through a common mechanism of toxicity, the inhibition of cholinesterase (ChE). This study compared the effects of low level exposure to CPS, MPS, and a mixture of the two compounds on brain and serum ChE and serum carboxylesterases (CbxE). The time to peak inhibition of brain ChE was determined for CPS and MPS and the effective dosages (ED) to inhibit 10-15% of brain ChE for each compound were determined. Adult male rats were orally exposed to either the ED for CPS (5 mg/kg), the ED for MPS (1.75 mg/kg), or a mixture of the two and brain and blood were collected at 30 min, and 2, 4, 8, 12, and 24 hours post exposure. With CPS alone, inhibition of brain ChE was not present at 30 min or 2 hours but increased to peak at 11% by 12 hours. Serum ChE inhibition was 33% at 30 min and increased to peak at 58% by 12 hours. Serum CbxE inhibition was over 90% at 30 min and remained so through 12 hours. With MPS alone, inhibition of brain ChE was 10% at 30 min and increased to 16% at 12 hours. Serum ChE and CbxE inhibition was 51-52% at 30 min but decreased thereafter. With simultaneous exposure to CPS and MPS, inhibition of brain ChE was 14-15% at 30 min to 2 hours with peak inhibition of 25% occurring at 12 hours. The pattern of serum ChE inhibition reflected the early inhibition similar to MPS and this inhibition increased with time similar to that of CPS. The pattern of serum CbxE inhibition was similar to that of CPS. The patterns of ChE and CbxE observed following simultaneous exposure were consistent with *in vitro* potencies as esterase inhibitors and did not give evidence of greater-than-additive effects. (Supported by American Chemistry Council CRAM 2a-99).

1502 THE EFFECT OF EARLY POSTNATAL EXPOSURE TO CHLORPYRIFOS AND CHLORPYRIFOS-OXON ON NEUROTROPHIN LEVELS IN THE RAT FOREBRAIN.

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Recent studies have demonstrated that young animals are more susceptible to the toxic effects of organophosphorus insecticides such as chlorpyrifos (CPS) than are adults. CPS exerts its toxicity through the inhibition of cholinesterase (ChE) but it has been suggested that CPS may exert its neurotoxicity in juveniles through non-cholinergic mechanisms. The development of the brain is a highly coordinated process in which neurotrophins play an essential role in neuronal proliferation and differentiation. It is possible that the neurotoxic effects of CPS in juveniles may be related to interference with the levels of the neurotrophins in the brain. This study investigated the effects of repeated exposure of neonatal rats to either CPS or its active metabolite chlorpyrifos-oxon (CPO) on the levels of two neurotrophins, nerve growth factor (NGF) and brain derived neurotrophic factor (BNDF). Two dosages of (1.5 and 3.0 mg/kg) and CPO (0.25 m/kg and 0.35 mg/kg) that produced similar degrees of brain ChE inhibition were administered daily from postnatal day (PND) 1-6. Rats were sacrificed on PND 4, 7, and 12 and forebrain ChE, NGF, and BDNF levels were determined. ChE activity was persistently inhibited with CPS but transiently inhibited with CPO. Forebrain NGF levels were decreased on PND 4 with the high dosage of CPS and on PND 7 with both dosages of CPS with recovery by PND 12. No effects on NGF were observed with CPO. No effects on BDNF levels were observed with either CPS or CPO. The basis for the decrease in NGF levels following neonatal exposure to CPS but not CPO appears to be related to the persistent inhibition of ChE induced by CPS. The compensatory down regulation of muscarinic receptors in response to the persistent inhibition may be involved.

1503 ROUTE OF EXPOSURE, NITROGEN SUBSTITUTION AND ACID STABILITY INFLUENCE THE HAZARDS AND COVALENT PROTEIN MODIFICATIONS PRODUCED BY DITHIOCARBAMATES.

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Dithiocarbamates and their disulfides have a broad spectrum of applications in agriculture, industry and medicine and the reported biological effects of dithiocarbamates have also been quite varied. The nitrogen substituents and oxidation state of a dithiocarbamate influence its rate of decomposition, decomposition products and metabolic pathways. Similarly, due to differences in acid stability and the potential for acid hydrolysis to occur following oral exposure, the route of exposure may also influence the disposition and biological effects of a dithiocarbamate *in vivo*. In this study rats were exposed by oral and parenteral routes of administration to representative dithiocarbamate compounds with differing nitrogen substituents and oxidation states for acute and subchronic durations. Morphological assessments, clinical chemistry, covalent protein modifications and urinary metabolites were then used to characterize the target cells and disposition of the dithiocarbamates. Oral administration of acid labile N, N-diethyldithiocarbamate (DEDC) produced a neurofilamentous axonopathy and covalent protein cross-linking consistent with *in vivo* release of carbon disulfide. In contrast, parenteral administration of DEDC, oral administration of the bis disulfide of DEDC, disulfiram, or the

acid stable pyrrolidine dithiocarbamate produced a Schwannopathy accompanied by segmental demyelination and the formation of S-(dialkylaminocabonyl) cysteine adducts. Oral administration of the monoalkyldithiocarbamate N-methyldithiocarbamate resulted in acute hepatotoxicity characterized by centrilobular necrosis and the formation of S-(methylaminothiocarbonyl) cysteine adducts consistent with the generation of a methylisothiocyanate intermediate. These data provide relationships useful for predicting the potential hazards associated with a particular dithiocarbamate and route of exposure. Supported by ES06387, ES00267 and ES07028

1504 FIPRONIL BLOCK OF GLUTAMATE-ACTIVATED CHLORIDE CURRENTS IN COCKROACH NEURONS.

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Fipronil is a highly effective broad-spectrum insecticide with many applications in agriculture, public health and animal health, and good efficacy against many resistant strains. While fipronil is known to be a highly effective and selective blocker of insect GABA-gated chloride channels, its activity against other ligand-gated chloride channels has been less well characterized. We studied the effects of fipronil on the inhibitory glutamate receptor-chloride channel complex, which is found only in invertebrate nerve and muscle. Glutamate-activated chloride currents were recorded from neurons isolated from cockroach thoracic ganglia, using the whole-cell patch clamp technique and symmetrical chloride concentration. The application of 1 mM glutamate to most neurons evoked rapidly-desensitizing inward currents with an EC50 of 36.8 ≥ 3.0 μM and a Hill coefficient of 1.56 ≥ 0.17. The time constant of desensitization was approximately 500 ms. The similarity between the reversal potential and the chloride equilibrium potential indicated that glutamate-induced currents were carried by chloride ions. Fipronil suppressed the glutamate-induced peak currents in a dose-dependent manner with an IC50 of 0.73 ≥ 0.27 μM and a Hill co-efficient of 0.68 ≥ 0.15, and greatly slowed the rate of desensitization. Picrotoxinin at 100 μM slightly suppressed glutamate-induced currents, to 87.8 ≥ 3.7 % of control, while dieldrin at 100 μM had no effect (96.7 ≥ 3.1%). AP5 and CNQX, mammalian excitatory glutamate receptor antagonists, were without effect on glutamate-activated Cl- currents. It was concluded that fipronil block of the glutamate receptor-chloride channel complex may play a role in the higher toxicity against insects compared to mammals. Supported by NIH Grant NS14143.

1505 STATE-DEPENDENT BLOCK OF MAMMALIAN AND INSECT SODIUM CHANNELS BY THE INSECTICIDES INDOXACARB AND DECARBOMETHOXY-INDOXACARB.

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Indoxacarb, the only commercial pyrazoline-type insecticide, is effective against insects resistant to various insecticides. We compared the effects of indoxacarb and its decarbomethoxyllated metabolite DCJW on Na+ channels in rat dorsal root ganglion (DRG) neurons and cockroach thoracic ganglion neurons, using the whole-cell patch clamp technique. In DRG neurons, indoxacarb and DCJW at 1-10 mM slowly and irreversibly blocked both tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na+ channels in a voltage-dependent manner. TTX-S Na+ channels were more sensitive than TTX-R Na+ channels to both compounds at the resting potential near −80 mV, but this difference was due largely to their different inactivation kinetics. Cockroach neurons had two types of TTX-S Na+ currents, also differing in inactivation kinetics and insecticide sensitivity. At −100 mV, type-I Na+ currents were inhibited reversibly by 100 nM indoxacarb and irreversibly by 100 nM DCJW. In contrast, type-II Na+ currents, which inactivate at more positive potentials compared to type I, were not affected by indoxacarb or DCJW at −100 mV, but were inhibited by both at more depolarized potentials, from −60 mV to −40 mV. In both mammalian and insect neurons, the slow inactivation curves of Na+ channels were shifted in the hyperpolarizing direction by indoxacarb and DCJW. For example, the shift amounted to 4.27 mV by 100 nM DCJW in cockroach type I Na+ channels and to 8.1 mV by 1 μM DCJW in rat TTX-S Na+ channels. It was concluded that indoxacarb insecticides bind specifically to inactivated states of sodium channels, and that their differential blocking actions on different Na+ currents result in large part from differences in the voltage dependence of inactivation. Cockroach Na+ channels appear to be more sensitive to the indoxacarb insecticides than rat Na+ channels. Supported by NIH Grant NS14143.

1506 THE INTERACTION OF PARAOXON WITH HUMAN RECOMBINANT ACETYLCHOLINESTERASE.

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Previous studies from this laboratory have reported that the inhibitory constant, k_i, for the inhibition of mouse or rat acetylcholinesterase by the organophosphate paraoxon (*O*, *O*-diethyl *p*-nitrophenyl phosphate) changes as a function of paraoxon concentration. The current study extends these observations by characterizing the kinetic interaction of paraoxon with human recombinant acetylcholinesterase over a wide range of oxon concentrations, including those that yielded pseudo-first order conditions with respect to enzyme, as well as second order conditions. The k_i was calculated by application of continuous systems modeling to experimentally determined residual acetylcholinesterase activity following addition of various paraoxon concentrations (Kardos and Sultatos, 2000, *Tox. Sciences.*, 58: 118-126). Incubation of 100 nM paraoxon with 0.4 nM human recombinant acetylcholinesterase at room temperature revealed a k_i of 0.066 $nM^{-1}h^{-1}$. In contrast, 0.156 nM paraoxon under the same incubation conditions gave a k_i of 0.550 $nM^{-1}h^{-1}$. These data suggest that individual paraoxon molecules at the lower concentration have a greater capacity to inhibit acetylcholinesterase than individual paraoxon molecules at the higher concentration. Furthermore, this observation supports the hypothesis that paraoxon binds to a site separate from the active site, and modifies the reactivity of the active site towards additional paraoxon molecules.

1507 TOXICOKINETIC-TOXICODYNAMIC RELATIONSHIPS IN CASES OF CYHALOTHRIN EXPOSURE.

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The primary site of action of the pyrethroid insecticides is the nervous system. Type II pyrethroids, such as cyhalothrin, are characterized by blocking nerve conduction and by causing convulsions and paralysis. The nerve-blocking action of Type II pyrethroids has been associated with the release of neurotransmitters. Because the value of Toxicokinetic-Toxicodynamic (TK-TD) relationships in medical toxicology is now recognized, the objective of the present study was to correlate the dopamine (DA) and 5-hydroxytryptamine (5-HT) levels and the corresponding cyhalotrin concentrations in nervous tissues of rats exposed to cyhalothrin. Two experiments were carried out: (1) male Wistar rats treated with cyhalothrin (single dose of 20 mg/kg, per os) were killed at different time period after treatment and nervous tissue samples isolated, homogenized and extracted in acetonitrile to determine cyhalothrin levels by HPLC-UV; (2) male Wistar rats treated with cyhalothrin (8 mg/kg, per os, for 6 days) and male Wistar rats treated with corn oil (control animals) were killed 24 h after dosing and nervous tissue samples isolated and contents of DA and 5-HT quantified by HPLC-ED. Our results suggest that TK-TD relationships should be considered for the risk assessment of pyrethroids. This work has been supported by projects No. PB9701236, (DIGICYT), No. 08.8/0002/98 (CAM) and No. 99/0936 (FIS), Spain.

Toxicokinetic-Toxicodynamic (TK-TD) relationships for cyhalothrin

	Kinetic parameters			DA and 5-HT decreasing effect	
Brain regions	C_{max} (μg/mL)	AUC (mg/mL·h)	$t_{1/2}$ (h)	DA (% of control)	5-HT (% of control)
Hippocampus Striatum Frontal cortex Hypothalamus	15.1 ± 18.1 17.4 ± 25.6	215.2 ± 258.2 276.6 ± 0.21	23.0 ± 2.3 18.7 ± 1.6	-32% ± 3% -36% ± 4% -53% ± 3% -54%	-20% ± 3% -45% ± 3% -35% ± 3%

Results are significantly different from the control value at $P<0.05$, $P<0.01$ or $P<0.001$.

1508 COMBINED EXPOSURE TO DIETHYLDITHIOCARBAMATE (DDC) AND IRON (FE): EFFECTS ON THE NIGROSTRIATAL DOPAMINERGIC SYSTEM.

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Environmental neurotoxicants, in particular pesticides and heavy metals have been implicated in the etiology of Parkinson's disease (PD). The purpose of the present study was to investigate a possible mechanism for the accumulation of Fe in the substantia nigra pars compacta (SNpc) in PD, and to propose an animal model for the disease. It hypothesized that DDC, a common dithiocarbamate fungicide,

when administered in conjunction with Fe, would chelate this metal and selectively increase its deposition in SNpc. Fe accumulation can increase oxidative stress, and result in dopaminergic (DA) neuronal degeneration and associated decreases in DA and metabolite levels in the striatum. Male C57BL/6 mice of 8 weeks of age were treated i.p. twice weekly for 4 weeks with saline, 100 mg/kg DDC, 10mg/kg (low Fe), 25 mg/kg (high Fe) ferric citrate or with the combination of 100 mg/kg DDC and 10 mg/kg or 25 mg/kg ferric citrate. Combined DDC + Fe produced progressive decreases in locomotor activity over the course of exposure when measured either immediately or 24 hours after treatment. Decreases in locomotor activity were still evident 7 days after the final treatment only in the combined exposure groups, with the DDC + high Fe group showing the greatest decrease. DDC + Fe also significantly decreased levels of striatal DA, DOPAC and HVA. In frontal cortex, the levels of DA and its metabolites were generally decreased by Fe alone, but showed a trend towards higher levels in the combined DDC + high Fe group. These results could indicate a protective role for DDC in the frontal cortex, acting to remove Fe from this region, while having the opposite effect in the nigrostriatal DA system, possibly resulting in an accumulation of Fe in the SNpc. Alternatively, these results could suggest earlier stages of DA dysfunction occurring in frontal cortex. Combined exposure to DDC and Fe may serve as a useful model to study the mechanisms of pathogenesis associated with PD. Supported by ES01247 & ES10791.

1509 NEUROBEHAVIORAL EVALUATION OF HOUSEHOLD EXPOSURE TO DURSBAN.

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Exposure to Dursban has been associated with chronic neurobehavioral effects (headaches, fatigue, problems with memory and concentration, irritability etc.) EPA states that the application of termiticide post construction results in exposures to applicators and to residents that exceed acceptable toxicity limits. Subject: Male, 41 years, high school graduate, married, 3 children Exposure: Approximately 70 gallons of Dursban applied in a structure with foundation cracks. 14 pet fish immediately died. All family members had acute symptoms, including nausea, diarrhea, chest tightness, anxiety. Subject was involved with clean-up intermittently over a period of months. Dust samples one month after the application showed 810, 000 mcg/kg of chlorpyrifos. Air sampling another month later showed elevated VOC's consistent with Dursban exposure. ACHE testing was inconclusive. Later and persistent symptoms included fatigue, memory loss, personality changes, and impaired executive function. Assessment approach: Evaluation 4 years post-incident included an extensive interview, administration of tests which have established normative bases and which are known to be sensitive to neurotoxicity, tests for malingering and distortion, and record review. Findings: Prior IQ was at the 50th%tile, or in the average range. The Neurotoxicity Screening Survey showed results consistent with those of patients diagnosed with neurotoxicity. Deficits included Full-scale IQ 6th%tile, Working Memory at the 2nd%tile, verbal fluency (COWAT) (10th%tile); manual dexterity (Grooved Pegboard) (2nd%tile); auditory information processing and tracking (PASAT) (1st%tile); Stroop Color and Word Test (evaluates mental flexibility) 7th%tile; Visual Search and Attention Test (visual detection skills) 2ndtile. Malingering was ruled out by 5 separate tests. Personality testing using the NEO Personality Inventory found significant personality disorder, but there was no evidence of such disorder pre-incident. Record review found no competing explanations of his illness. Results were consistent with Dursban neurotoxicity.

1510 CHARACTERIZATION OF A MONKEY MODEL OF LASER-INDUCED CHOROIDAL NEOVASCULARIZATION (CNV).

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Capillary growth into the subretinal space through breaks in Bruch's membrane occurs in the exudative form of age-related macular degeneration (AMD). The primate CNV model has been used to evaluate potential therapies for AMD. The objective of this study was to characterize the progression of the CNV sites with respect to vascular leakage and to compare these data with histopathologic observations. Seven cynomolgus monkeys underwent laser treatment. Nine laser sites were placed in the macula of each eye with a diode laser (532 nm). Fundus photography and fluorescein angiography were performed at weekly intervals for 6 weeks following laser treatment. Animals were then euthanized. The eyes, perfusion fixed in situ, were collected, embedded in epoxy resin, sectioned at 1 micron, stained with toluidine blue, and examined microscopically. Laser sites were scored according to the intensity of vascular leakage, with Grade IV representing the most severe and clinically relevant form. Grade IV vascular leakage occurred in 22% of individual laser sites involving 63% of eyes. Microscopically, focal neural retina injury and obliteration of Bruch's membrane and the choriocapillaris were observed for most laser sites. In many sites, choroidal capillaries extended into the subretinal space or in a space created by duplication of the retinal pigment epithelial basal lamina. The presence of capillaries in either circumstance generally correlated with Grade IV vascular leakage. Additionally, some sites with less severe vascular leakage also had capillary growth. Intravitreal treatment with a recombinant antigen binding fragment of a humanized monoclonal antibody to vascular endothelial growth factor prevented Grade IV vascular leakage and most capillary proliferation. This study corroborates the usefulness of the monkey CNV model in assessing the efficacy and safety of potential therapies for diseases with subretinal neovascularization such as AMD.

1511 LASER-INDUCED CHOROIDAL NEOVASCULARIZATION IN DRUG DEVELOPMENT — EXPERIENCES WITH THE PRIMATE MODEL.

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Introduction Age-related macular degeneration (AMD) is the most common cause of vision loss in individuals over the age of sixty. Dry AMD accounts for about 90 percent of all cases, but results only in minimal visual symptoms. The wet form of AMD results in severe vision impairment and is characterized by choroidal neovascularization (CNV): vessels grow from the vascular choroid through Bruch's membrane and invade the outer retina, which subsequently leads to degeneration of the RPE- and photoreceptor cells. Among the models used for AMD, non-human primates are the only laboratory mammals having a macula. The experiences with laser induced CNV in primates are described and discussed. Material and Methods Twelve cynomolgus monkeys were used in three studies. Eight to ten lesions were placed around the macula. Lesions were placed in one or both eyes. A green laser operating at 532 nm and a red laser at 810 nm were utilized. The quality of the lesions is influenced by the lesion size (75-100 μm), the laser power (450-900 mW) and the exposure time (0.1-0.2 s). Eyes received either no treatment or an intravitreal injection of control article or Pigment Epithelium-Derived Factor (PEDF). Primary evaluation was achieved with fluorescein angiography. Lesions that developed a clear luminescent circle were counted as CNV. Results The laser-induced AMD model in cynomolgus monkeys has provided important information about CNV. Verteporfin administration prevents angiographic leakage for at least 4 weeks in this monkey model. Our studies confirmed inhibiton of CNV by Pigment Epithelium-Derived Factor (PEDF). The laser lesions are not permanent and start to regress between one and two months. CNV can be re-induced following regression of initial lesions. Discussion While the model differs from clinical AMD in that the CNV regresses, it provides a valid model of neovascularization originating from the choroid. Efficacy and safety of anti-angiogenic compounds can be tested with this model.

1512 ENHANCED EXPRESSION OF VASCULAR COAGULATION ADHESION MOLECULE (VCAM-1) IN 2-BUTOXYETHANOL-INDUCED HEMOLYSIS AND THROMBOSIS IN FEMALE RATS.

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In previous studies we found that exposure of rats to 2-butoxyethanol (BE) was associated with morphological changes in red blood cells; hemolytic anemia; and disseminated thrombosis and infarction in bone, heart, lung, brain, eye, liver, tooth, and nasal mucosa. Females are more susceptible to the toxic effects than males. Histopathological changes noted in the eyes included hemorrhages in the retina, photoreceptor degeneration, and thrombi in ciliary processes and limbal blood vessels. Several mechanisms for the thrombosis formation were proposed, such as hemolysis with potential release of protocoagulant factors from destroyed erythrocytes, direct endothelial damage induced by BE, and disturbed blood flow secondary to changes in erythrocytic rheological factors. Vascular cell adhesion molecule-1 (VCAM-1), a membrane glycoprotein belonging to the immunoglobulin supergene family, is functionally important for the adhesion of leukocytes to activated endothelium. P-selectin, a membrane glycoprotein contained within platelets and endothelial cells, is rapidly mobilized to the plasma membrane following cell activation and acts to regulate transient interactions of endothelial cells and leukocytes. In the present work we investigated VCAM-1 and P-selectin expression and distribution over time in our rat BE hemolysis and thrombosis model. Female Fischer F344 rats were exposed to two, three, or four daily doses of BE at 250 mg BE/kg body weight and studied histologically and immunohistochemically using specific monoclonal antibodies. Strong VCAM-1 expression was seen in the cytoplasm of epithelial and stromal ciliary processes and retinal pigment epithelium, only in eyes of rats exposed to 3 and 4 doses of BE, correlating with the appearance of thrombosis in the iris and retina. No change in P-selectin expression was seen. This immunolabeling distribution suggests that VCAM-1 plays a role in the pathogenesis of BE-related thrombosis by promoting adhesion of the circulating cell to endothelium.

1513 D2 AGONIST INDUCED RETINAL DEGENERATION CONFINED TO ALBINO RATS.

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The non-ergoline dopamine agonists pramipexole and ropinirole with *in vitro* selectivity and full intrinsic activity at the D2 and D3 dopamine receptor subtypes both induce retinal degeneration that is confined to albino rats in 2-year carcinogenicity studies. For pramipexole, we observed retinal degeneration with a dose-dependent increase of severity in albino Wistar rats under daily administration (2 and 8 mg/kg/day) over two years. No such effects were seen at 0.3 mg/kg/day. The retinal degeneration was morphologically characterized by a reduced thickness of the outer nuclear layer, in particular of the inferior hemisphere. While the observations can be attributed to an age related light-induced mechanism, a possible ocular toxicity could not conclusively be excluded. In a mechanistic study, the effects of continuous light exposure (24 h/day) and concomitant pramipexole administration were further investigated in albino and pigmented rats. Slightly increased retinal degeneration after 13 weeks of treatment was found in albino Wistar rats, but not in pigmented Brown Norway rats. Thus light does not exacerbate retinal degeneration in pigmented rats. In addition, pigmented Brown Norway rats were given pramipexole for 2 years. No retinal degeneration was detected by routine light microscopy after the end of treatment. By means of a highly sensitive image analysis, morphometry revealed only a slight reduction in thickness of the outer nuclear layer. Species-specificity was confirmed for both pramipexole and ropinirole, as no drug-induced retinal degeneration was found in 2-year albino mice carcinogenicity, in 52-week toxicity study in albino (Wistar) rats and 52-week study in non-human primates. The retinal degeneration found in long term studies in albino rats administered pramipexole and ropinirole was not observed in human patients. Based on available data, both compounds therefore produce no greater clinical risk of ocular toxicity than L-dopa or other dopamine agonists.

1514 THE CYNOMOLGUS MONKEY AS A MODEL FOR OCULAR TOXICITY TESTING: INCIDENCE AND CHARACTERIZATION OF SPONTANEOUS LESIONS IN THE OCULAR FUNDUS.

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Man and nonhuman primates share many ocular similarities such as retinal cell anatomy, blood vessel system and presence of a fovea. Because of these similarities ocular function can be investigated satisfactorily in nonhuman primate models in toxicity studies. The cynomolgus monkey (Macaca fascicularis) has proven to be a highly suitable and well established animal model for examining the ocular toxicity of drugs in preclinical research. The spectrum of ophthalmoscopic techniques for evaluation of the posterior part of the eye is comparable to that of man and comprises indirect ophthalmoscopy, fluorescein angiography, fundus photography and electro-retinography. Since it is necessary to be aware of spontaneously occurring ocular diseases and abnormalities to assess the influence of test articles on the eye, the fundus of 1829 animals was examined in otherwise untreated animals. Altogether 134 (7.3%) spontaneous findings were seen, such as optic nerve variations or differences in the retinal pigmentation. Specifically, drusen, variable macula pigmentation, altered translucence, vascular variability, microaneurisms, punctiform hemorrhages and optic nerve disk variability were encountered. Systematic photographic documentation has been initiated using a digital fundus camera in order to establish a photo database to enable standardized diagnosis and description of findings. These spontaneous lesions must be clearly distinguished from traumatic injuries such as chorioretinal or vitroretinal scarring. In summary, extensive background data for the cynomolgus monkey in regard to spontaneous ocular lesions are available and allow the proper assessment of possible drug-related findings in toxicity studies In addition, photo documentation is important to objectively demonstrate the drug-related findings and also document changes over a certain time period.

1515 EVALUATING THE EYE IRRITANCY OF SOLVENTS IN A SIMPLE FRAGRANCE MIXTURE WITH THE BOVINE CORNEAL OPACITY AND PERMEABLITY (BCOP) ASSAY.

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Fragrances are complex mixtures used in many consumer products. Organic solvents are major components of fragrance formulations functioning mainly as solubilizers and fragrance delivery mechanisms. Solvents appear to have an additive effect on the level of irritation of these formulations. In order to understand this solvent effect, we evaluated the use of the BCOP for predicting the irritancy potential of simple fragrance mixtures containing six commonly used solvents. Additionally, we wanted to correlate the *in vitro* corneal depth of injury with *in vivo* results using histological evaluation. Abattoir-derived corneas were exposed to test materials for 1 and 3 minutes and opacity and permeability end points measured to calculate an *in vitro* score at 20 hours. Negative (medium) and positive (100% ethanol) controls were tested concurrently with all formulations. For comparison to BCOP, potential irritant effects of the test articles were also evaluated in a primary eye irritation study (EPA-OPPTS 870.2400). Animals were scored at 1, 4, and 24 hours followed by a fluorescein exam. Both *in vivo* and *in vitro* tissues were taken for histology. Fragrance/solvent mixtures, except ethanol, showed an increase in irritation *in vivo* and in the BCOP in comparison to solvent alone and fragrance alone. Histological changes included epithelial damage/loss, stromal swelling, and inflammatory infiltration (in vivo) or keratocyte changes (in vitro). These data show the need for using formula specific benchmarks to accurately assess ocular irritation in the *in vitro* BCOP assay, thus eliminating the need for *in vivo* testing of product evaluations.

1516 CORNEAL PERMEABILITY IN AN IMPROVED HOLDER FOR THE BOVINE CORNEA OPACITY AND PERMEABILITY (BCOP)ASSAY.

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In the BCOP assay corneas are mounted in a holder between two saline-filled chambers. After exposure to a test material, light absorbance and fluorescein permeability to are measured to assess damage to the cornea. Problems with this assay are, 1) the flat surfaces of the holder clamp directly onto the cornea, causing edge damage to the epithelium and the endothelium, and 2) since the bovine cornea is convex and non-spherical, clamping the cornea in the circular opening between the chambers of the holder wrinkles the cornea. We developed a new holder for the BCOP assay that does not damage the cornea because it contacts the sclera and maintains normal curvature. Opacity tests showed that the new holder is a significant improvement over the standard holder. The purpose of this study was to compare corneal permeability to fluorescein between the new holder and standard holders. The permeability (pg/cm^2/minute) of intact corneas, corneas without epithelium and corneas treated with isopropyl alcohol, 1% NaOH, acetone, 30% trichloroacetic acid or benzalkonium chloride (0.01% or 0.1%) for 10 min was determined by measuring fluorescence of samples from the endothelial chamber during a 90 min exposure of the epithelium to 0.0008% Na-fluorescein solution. Permeability in the new holders is lower than in the standard holders for all conditions tested except corneas without epithelium or after acetone treatment. Variability of permeability data from the new holders was markedly less than that from standard holders for all conditions tested. Lower corneal permeability and decreased data variability in the new holders may be due to improved structural integrity of the cornea. Corneal edge damage and wrinkling in the standard holder may increase permeability to a variable extent depending on the degree of trauma to the tissue during the mounting process. Data collected using the new holder appear to be more reliable because the assay begins with an intact cornea. A new BCOP assay protocol should be developed using the improved corneal holder.

1517 ISOLATION AND CHARACTERIZATION OF UDP-GLUCURONOSYLTRANSFERASES FROM FEMALE RHESUS MONKEY.

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Three isoforms of the UDP-glucuronosyltransferase (UGT) family have been cloned and sequenced from a female rhesus monkey liver cDNA library using primers designed for human and cynomolgus monkey UGTs. The clones were expressed in the HK-293 cell line and microsomes were prepared for activity assays. The first isoform, a UGT1A family member, has sequence homology and substrate specificity very similar to human UGT1A1 and has been named rhesus UGT1A01. The activity of UGT1A01 was assayed by a general UGT substrate [7-hydroxy-4-(trifluoromethyl)-coumarin], in addition to more specific substrates (β-estradiol, bilirubin, 1-naphthol and 17α-ethinylestradiol). UGT1A01 was also detected in a western blot utilizing a polyclonal antibody developed against the human UGT1A family. The two other isoforms are from the 2B family of UGTs, each a product from a single gene, and have been given the names UGT2B9*2 and UGT2B33. Microsomal preparations of the UGT cell lines catalyzed the glucuronidation of 7-hydroxy-4(trifluoromethyl)-coumarin in addition to selected estrogens (17α-ethinylestradiol, β-estradiol and estriol) and morphinan (morphine, naloxone, and naltrexone) substrates. Substrate specificity and kinetic parameters (Km and Vmax)

are similar in comparison to human UGT2B7 and cynomolgus monkey UGT2B9. Kinetic parameters for the estrogen substrates were also determined in microsomes isolated from rhesus liver and kidney. Comparison of Km values for the individual isoforms to that of female liver microsomes suggest the use of β-estradiol-3-glucuronidation, β-estradiol-17-glucuronidation, and estriol-17-glucuronidation to assay UGT1A01, UGT2B9*2 and UGT2B33 activity in rhesus liver microsomes, respectively.

1518 EFFECTS OF MICROSOMAL ENZYME INDUCERS ON THYROID HORMONE GLUCURONIDATION: CHARACTERIZATION OF THE ROLE OF UDP-GLUCURONOSYLTRANSFERASE 1A FAMILY OF ENZYMES.

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Microsomal enzyme inducers (MEI) that upregulate UDP-glucuronosyltransferases (UGTs) may also increase the hepatic glucuronidation of thyroxine (T_4), resulting in a reduction of serum T_4 concentration, and a subsequent increase in thyroid stimulating hormone (TSH). Increased TSH can result in increased thyroid cell proliferation, and thus promote thyroid tumors in rodents. Enhanced T_4 glucuronidation, presumably catalyzed by UGT1A1 and 1A6, is suggested to increase TSH levels in rats. The aim of this study was to determine whether increased T_4 glucuronidation, decreased serum T_4, and increased TSH following MEI treatment are dependent on the UGT1A family of enzymes. Male Wistar and UGT1A-deficient Gunn rats were fed control diet or a diet containing pregnenolone-16α-carbonitrile (PCN; 800 ppm), 3-methylcholanthrene (3-MC; 200 ppm), or Aroclor 1254 (PCB; 100 ppm), chemicals known to increase UGTs and decrease serum T_4 levels, for 7 days. Serum free and total T_4 and triiodothyronine (T_3), serum TSH, and hepatic glucuronidation activities toward T_4 and T_3 were determined. T_4 glucuronidation was increased in Wistar rats after MEI treatment: PCN (298%), 3-MC (85%), and PCB (450%). In Gunn rats, T_4 glucuronidation was minimal, and remained unchanged after MEI treatments. T_3 glucuronidation was significantly increased after PCN (121%) or PCB (58%) treatment in Wistar rats, but only PCN increased T_3 glucuronidation in Gunn rats (43%). In both rat strains, PCN, 3-MC, and PCB treatments decreased both total and free serum T_4, whereas serum T_3 was relatively maintained. TSH was increased in Wistar and Gunn rats after PCN (130 and 277%) or PCB treatment (72 and 60%). From these data, it can be concluded that T_4 glucuronidation is catalyzed primarily by UGT1A enzymes. However, the decrease in serum T_4 concentration and increase in TSH are not dependent on increased T_4 glucuronidation, and therefore cannot be attributed to the UGT1A family of enzymes. (Supported by NIH grants ES-08156 and ES-07079)

1519 NUCLEAR RECEPTOR PXR IS REQUIRED FOR INDUCTION OF UDP-GLUCURONOSYLTRANSFERASES IN MOUSE LIVER BY PREGNENOLONE-16α-CARBONITRILE.

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Treatment of mice with the prototypical microsomal enzyme inducer pregnenolone-16α-carbonitrile (PCN) alters the hepatic expression of many genes involved in biotransformation and transport of xenobiotics and endogenous compounds, such as cytochrome P450s 3a and 7a1, and organic anion transporting polypeptide 2 (Oatp2), *via* the nuclear receptor pregnane X receptor (PXR). PCN also induces the glucuronidation of a number of substrates in rat liver. However, the role of PXR in the induction of UDP-glucuronosyltransferases (UGTs) by PCN is not known. Therefore, the aim of this study was to determine the role of PXR in the induction of UGTs by PCN. Four to 6 month-old male PXR-null and wild-type mice received control or PCN-treated (1500 ppm) diet for 21 days. On day 22, livers were taken to prepare microsomes and total RNA to determine UGT activity and mRNA levels, respectively. In wild-type mice, PCN treatment significantly increased UGT activity toward bilirubin, 1-naphthol, chloramphenicol, thyroxine, and triiodothyronine. On control diet, the UGT activity towards the above substrates (except for 1-naphthol) in the PXR-null mice was significantly higher than that of wild-type mice. However, UGT activity in PXR-null mice was not increased by PCN. In agreement with the above findings, mRNA levels of mouse UGT 1A1 and 1A9, which are involved in the glucuronidation of bilirubin and phenolic compounds, were increased about 100% in wild-type mice following PCN treatment, whereas UGT 1A2, 1A6 and 2B5 were not affected. In contrast, PCN treatment had no effect on the mRNA levels of these UGTs in PXR-null mice. Taken together, these results indicate that PCN treatment induces glucuronidation in mouse liver, and that PXR is involved in the regulation of constitutive expression and induction of some UGTs. (Supported by NIH grant ES-08156)

1520 STABLE AND UNSTABLE GLUCURONIDES IN CURCUMIN METABOLISM.

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Curcuminoids are constituents of the rhizome of Curcuma longa, commonly called turmeric, and have recently gained much interest due to their putative anti-inflammatory and anti-carcinogenic activities. Since very little is known about the metabolism of curcuminoids in the mammalian organism, we have studied the biotransformation of the major curcuminoids, i.e. curcumin, monodemethoxy-curcumin and bisdemethoxy-curcumin in liver microsomes, liver cytosol and precision-cut liver slices from non-induced male Sprague-Dawley rats, as well as in microsomes and cytosol from human liver. Hydroxylated or demethylated products were not detected in any of these systems. The major phase I metabolites were identified as hexahydrocurcuminoids by capillary gas chromatography-mass spectrometry. These reductive metabolites were formed in slices and cytosol but not microsomes of rat and human liver. In slices, most of the hexahydrocurcuminoids were present as polar and stable glucuronides, which were also formed when hexahydrocurcuminoids were incubated with microsomes from rat and human liver in the presence of uridine diphosphoglucuronic acid. Rat hepatic microsomes and curcuminoids gave rise to the formation of lipophilic (i.e. ethylacetate-extractable) glucuronides, which were chemically unstable and decomposed rapidly to the curcuminoids in aqueous solution. In contrast, microsomes from human liver converted the curcuminoids to two glucuronides, one of which was lipophilic and the other hydrophilic. Based on the comparison with structurally related glucuronides and on chemical considerations, we postulate that the unstable and lipophilic glucuronides of the curcuminoids carry the glucuronic acid group at the enolic hydroxyl group of the aliphatic moiety, whereas the stable and hydrophilic glucuronides of the hexahydrocurcuminoids represent phenolic glucuronides. Supported in part by NIH Grant P50 AT00474 to the Arizona Center for Phytomedicine Research.

1521 EFFECT OF ESTRAGOLE, SAFROLE AND MYRISTICIN ON *IN VITRO* HUMAN CYP3A4, CYP2D6 AND CYP1A ACTIVITIES.

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Some allylbenzene compounds found in essential oils and food flavorings (e.g. basil, anise, fennel, nutmeg) are known to be liver carcinogens in rodents after metabolic activation. The current study was performed to investigate the effect of three of these compounds, estragole, safrole, and myristicin on *in vitro* CYP3A4, CYP2D6 and CYP1A activities in human liver microsomes. Pooled microsomes were incubated with estragole, safrole or myristicin in concentration ranges of 10 nM to 150 μM. CYP3A4, CYP2D6 and CYP1A activities were measured using standard assays - testosterone 6-β-hydroxylation, bufuralol 1'-hydroxylation and 7-ethoxyresorufin *O*-deethylase (EROD), respectively. Appropriate controls were included, including ketaconazole (5 μM) and quinidine (2 μM) as inhibitors of CYP3A4 and CYP2D6 activities. None of the three compounds influenced CYP2D6 activity in the concentration ranges studied. Safrole was a strong inhibitor of CYP3A4 (IC_{50}=0.43 μM) and CYP1A (IC_{50}=2 μM). Estragole and myristicin were also capable of inhibiting CYP3A4 and CYP1A at relatively higher concentrations [estragole - IC_{50} of 5.9 μM (CYP3A4) and 20 μM (CYP1A); myristicin - IC_{50} of 11 μM (CYP3A4) and 5 μM (CYP1A)]. These results indicate that estragole, safrole and myristicin may interfere with the metabolism of concomitantly administered therapeutic drugs, nutritional supplements or other food components that are substrates for CYP3A4 and CYP1A. Supported by funds from NIEHS contract (N01-ES-95437).

1522 *IN VITRO* DETERMINATION OF KINETIC CONSTANTS FOR 1, 3-DICHLOROPROPANE, 2, 2-DICHLOROPROPANE, AND 1, 1-DICHLOROPROPENE IN RAT LIVER MICROSOMES AND CYTOSOL.

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In 1998, the USEPA placed 1, 3-Dichloropropane (1, 3-DCP), 2, 2-dichloropropane (2, 2-DCP), and 1, 1-dichloropropene (1, 1-DCP) on the Contaminant Candidate List (CCL) and prioritized these drinking water contaminants for health effects research. We determined the kinetic constants of these compounds for the mixed-function oxidase (MFO) and glutathione (GSH) *S*-transerase (GST) pathways because they play an important role in the toxicity of halogenated hydrocarbons. Hepatic microsomal (M) and cytosolic (C) fractions were prepared from male

Fischer 344 rats and an automated equilibrium headspace technique was employed to assess substrate loss. Headspace concentrations were determined by gas-chromatography and concentration time-courses were modeled with a two-compartmental gas-liquid model and analyzed with Matlab®. We observed no metabolism for 2, 2-DCP *via* the GST pathway and only a minor level of clearance *via* the MFO pathway ($7x10^{-4}$ L/h/mg M-protein). The clearance of 1, 3-DCP *via* the MFO pathway was substantially greater than that of 2, 2-DCP ($4.1x10^{-2}$ L/h/mg M-protein; V_{max}=641 pmol/min/mg M-protein, K_m=0.95μM). The clearance of 1, 3-DCP *via* the GST pathway was minor relative to the MFO pathway ($2.4x10^{-4}$ L/h/mg C-protein; 0.6% of microsomal rate), and was consistent with the results of other α, ω-dihaloalkanes that show increased rates of GSH conjugation with increasing chain length. The clearance of 1, 1-DCP *via* the MFO pathway ($2.26x10^{-2}$ L/h/mg M-protein; V_{max}=3430 pmol/min/mg M-protein, K_m=2.5 μM) was substantially greater than for the GST pathway ($3.9x10^{-4}$ L/h/mg C-protein; 1.72% of microsomal rate). In summary, this *in vitro* approach has proven useful in evaluating the metabolism of volatile halogenated hydrocarbons. These rate constants may be incorporated in pharmacokinetic models to predict *in vivo* kinetics. *(This is an abstract of a proposed presentation and does not necessarily reflect United States Environmental Protection Agency (EPA) policy.)*

1523 METABOLISM OF RUTAECARPINE BY RAT LIVER MICROSOMES: A PARTIAL CHARACTERIZATION OF METABOLITES.

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Rutaecarpine is an alkaloid originally isolated from the unripe fruit of *Evodia rutaecarpa*. In addition to its traditional use in treatment of gastrointestinal disorders, rutaecarpine has recently been characterized to have anti-inflammatory activity through cyclooxygenase-2 inhibition. More recently, to develop rutaecarpine as an anti-inflammatory agent, total synthesis of rutaecarpine has successfully been established in our group. In the present studies, metabolic fate and cytochrome P450s involved in the metabolism of rutaecarpine was partially investigated in rat liver microsomes. When rutaecarpine was incubated with rat liver microsomes, 5 major peaks were detected on an HPLC. Four peaks (M1, M2, M4, M5) were believed to be a metabolite hydroxylated on the C-ring and one (M3) to be a metabolite hydroxylated on the phenyl moiety of E-ring. Using enriched rat liver microsomes, the anticipated isoforms of cytochrome P450s in the metabolism of rutaecarpine were partially characterized. The phenobarbital-induced microsomes greatly increased in the formation of the metabolites M1 and M3. The 3-methylcholanthrene-induced microsomes increased in the formation of metabolites M2, M3 and M4. (Supported by a grant of the Korea Research Foundation for the Institute for Drug Research, Yeungnam University).

1524 BIOTRANSFORMATION OF *N*-ETHYL-*N*-(2-HYDROXYETHYL)PERFLUOROOCTANESULFONAMIDE (N-ETFOSE) BY RAT LIVER MICROSOMES, CYTOSOL, AND SLICES.

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Perfluorosulfonic acid derivatives have been used in a range of commercial applications. Perfluorooctanesulfonic acid (PFOS) is environmentally and biologically stable, which has prompted studies about its formation from a range of precursors. The objective of this study was to elucidate the pathways for the biotransformation of the PFOS precursor N-EtFOSE. N-EtFOSE and several putative metabolites were incubated with liver microsomes and cytosol and with liver slices from male, Sprague-Dawley rats. Metabolites were analyzed and quantified by LC/MS/MS. Microsomal fractions fortified with NADPH catalyzed the *N*-deethylation (5.70 ± 0.30 pmol/min/mg protein) of N-EtFOSE to give *N*-(2-hydroxyethyl)perfluorooctanesulfonamide (FOSE alcohol). The broad-spectrum cytochrome P450 inhibitor *N*-benzylimidazole and the NADPH-cytochrome P450 reductase inhibitor diphenyliodonium chloride blocked the biotransformation of N-EtFOSE to FOSE alcohol, indicating a role for cytochromes P450. Microsomal fractions also catalyzed the *N*-dealkylation (117 ± 6 pmol/min/mg protein) of FOSE alcohol to give perfluorooctanesulfonamide (FOSA). Cytosolic fractions fortified with NAD^+ catalyzed the oxidation of FOSE alcohol to perfluorooctanesulfonamidoacetate (FOSAA) (3.12 ± 0.20 pmol/min/mg protein). The oxidation of N-EtFOSE to *N*-ethylperfluorooctanesulfonamidoacetate (N-EtFOSAA) was observed in liver slices, but not in cytosolic fractions. FOSA was biotransformed in liver slices to perfluorooctanesulfonic acid (PFOS), albeit at a low rate. The *O*-glucuronides of N-EtFOSE and FOSE alcohol and the *N*-glucuronide of FOSA were identified by LC/MS/MS. These results show that the major pathway for the biotransformation of N-EtFOSE is *N*-dealkylation to give FOSA. The biotransformation of FOSA to PFOS explains the observation that PFOS is formed in animals given N-EtFOSE. (Supported by the 3M Co.)

1525 *IN VITRO* DETOXICATION OF ORGANOPHOSPHATES AND THEIR MIXTURES BY RAT LIVER AND SERUM ESTERASES.

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Organophosphates (OPs), such as the oxon (bioactivated) metabolites of many common phosphorothionate insecticides, can be detoxified through the stoichiometric phosphorylation of B-esterases [carboxylesterases (CbxE) and cholinesterases (ChE)] or through the catalytic detoxication by calcium-dependent A-esterases. The detoxication of five OPs by liver and serum esterases was quantified using an exogenous source of cholinesterase (from bovine brain) to determine residual OP concentrations. The compounds studied were the oxons of three diethyl phosphorothionates, chlorpyrifos-oxon, diazoxon and paraoxon, and of two dimethyl phosphorothionates, methyl paraoxon and azinphosmethyl-oxon. Inhibition of CbxE (as monitored by the hydrolysis of the model substrate 4-nitrophenyl valerate) by the OPs was measured in parallel samples. With EDTA-treated samples to prevent A-esterase hydrolysis, in serum, OP detoxication correlated reasonably well with CbxE inhibition (194-310 pmol OP detoxified/unit CbxE inhibited), suggesting that stoichiometric detoxication by CbxE was responsible for the majority of the detoxication observed. In liver, however, detoxication of diethyl OPs (134-146 pmol/unit) was lower than that for dimethyl OPs (717-728 pmol/unit). It seems likely that, in liver, a second detoxication mechanism is operating on the dimethyl OPs. Detoxication of OP binary mixtures, whether added simultaneously or sequentially, was within the expected range, suggesting that no greater-than-additive interactions occurred *in vitro*. Only two compounds tested (chlorpyrifos-oxon and diazoxon) were substrates for calcium-activated A-esterase at the sub-micromolar concentrations assayed. Prior treatment with non-substrate OPs (i.e., paraoxon, methyl paraoxon and azinphosmethyl-oxon) had no measurable effect on hydrolysis of either of the substrate OPs. (Supported by American Chemistry Council CRAM 2a-99).

1526 COMPARISON OF HEPATIC *IN VITRO* METABOLISM OF THE PYRROLIZIDINE ALKALOID SENECIONINE IN SHEEP AND CATTLE.

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Pyrrolizidine alkaloids (PAs) are found in approximately 3% of the world's flowering plants and represent a significant group of plant toxins that affect livestock, wildlife and human health. In particular, tansy ragwort (Senecio jacobaea) is a plant found throughout the rangelands of the Pacific Northwest that produces PAs, including senecionine, which are toxic to the livestock that consume them. Sheep and cattle have been found to differ significantly in their response to PAs with sheep being relatively resistant to toxicity, while cattle are more susceptible. Resistant species abate PA toxicity by: producing a larger proportion of the non-toxic N-oxide metabolite versus the toxic DHP (6, 7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine) metabolite; conjugating the DHP product to glutathione; and eliminating the parent compound at a faster rate than susceptible species. In this study, we compared the *in vitro* hepatic metabolism of senecionine in sheep and cattle, two species commonly affected by PA-containing plants, using incubations of liver microsomes and cytosol and studied the production of the N-oxide and DHP metabolites as well as metabolism of the parent compound by HPLC. It was found that the two species differed in production of N-oxide ($p<0.0001$) and in the percent of parent compound metabolized ($p<0.0001$). Gender differences were found to be non-significant. The Km and Vmax for both N-oxide and DHP were studied and compared among the two species and between both genders. They differed on most comparisons. This data characterizes the metabolism of senecionine in two livestock species that are exposed to PA-containing plants and reaffirms the interspecies variability in metabolism of this group of toxins. Further, these results point to the importance of N-oxide production and the ability of the parent compound to be metabolized as important factors in sheep (a PA-resistant species) for abating PA toxicity.

1527 MECHANISM(S) OF DIFFERENTIAL INHIBITION OF HEPATIC AND PANCREATIC FATTY ACID ETHYL ESTER SYNTHASE ACTIVITY BY TRI-*o*-TOLYL PHOSPHATE METABOLITES.

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Fatty acid ethyl ester synthase (FAEES) is a family of enzymes involved in conjugation of fatty acids with endogenous and exogenous substrates having hydroxyl and/or amino group(s). In previous studies, we have reported that FAEES activity is expressed by beta-esterases, carboxyl esterase (CE) in the liver and cholesterol es-

terase (ChE) in the pancreas. The hepatic FAEES is structurally and functionally different than that in the pancreas. We also found that hepatic FAEES activity is inhibited by tri-o-tolyl phosphate (TOTP, beta-esterase inhibitor), and that metabolism of TOTP is prerequisite for such inhibition. However, no inhibition of FAEES activity was found in the pancreas. Therefore, we synthesized two putative metabolites of TOTP [2-o-(cresyl)-4H-1:3:2-benzodioxaphosphoran-2-one (CBDP; cyclic saligenin phosphate) and di-o-tolyl-o-(alpha-hydroxy) tolyl phosphate (hydroxylated-TOTP)] and studied their effect on FAEES activity in HepG2 (hepatocellular carcinoma) and AR42J (rat pancreatic tumor) cells. Both metabolites inhibited FAEES activity significantly in HepG2 cells within 15 min of incubation. However, no inhibition was observed in AR42J cells, similar to our previous findings in cell culture/in vivo studies using TOTP as inhibitor. To further unfold the chemistry of FAEES inhibition, we incubated commercial grade CE and ChE with CBDP or hydroxylated-TOTP, and found that both metabolites irreversibly inhibit FAEES as well as esterase activity of both CE and ChE. In order to identify the binding site of CE and ChE for CBDP, commercial grade CE or ChE was incubated with CBDP and subjected to 2-D gel electrophoresis for proteomic analysis. These studies will be helpful in elucidating the mechanism of FAEES inhibition and understanding its biochemical significance.

1528 MODULATION OF FATTY ACID METHYL ESTER FORMATION IN RATS USING TRI-*o*-TOLYLPHOSPHATE.

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Fatty acid methyl esters (FAMEs) are formed *via* conjugation of fatty acids with methanol, catalyzed by Fatty Acid Ethyl Ester Synthase (FAEES). Toxicity of these esters has been reported *in vitro* and *in vivo*. Since the same protein expresses FAEES and esterase activity, we hypothesize that inhibitors of esterases (FAEES) such as tri-o-tolylphosphate (TOTP) can modulate the formation of FAMEs. To test this, four groups of rats were used, group 1 served as control (vehicle only). Group 2 was treated with methanol only (3 g/kg *via* gavage), group 3 was given TOTP only (100 mg/kg i.p. in corn oil) and group 4 was administered TOTP as in group 3, followed by methanol after 18 hours. Three hours after exposure, animals were sacrificed and FAEES activity and FAME levels were measured in blood, liver, pancreas and brown fat. About 95% of FAEES activity was inhibited in the liver and whole blood of TOTP-treated rats (group 3) but no inhibition was observed in the pancreas. Total hepatic FAMEs were found to be lowest for the TOTP-treated group and highest in the methanol-treated animals (group 2). Total pancreatic FAMEs in different groups were unchanged (not statistically different), while significant increases in brown fat were observed in both methanol- treated groups. Low levels of FAMEs detected in the whole blood were found to be inconclusive. To verify that the oxidative metabolism of methanol was unaffected by TOTP, alcohol dehydrogenase activity was also measured and found to be unchanged in any group as compared to control. These results demonstrate that TOTP inhibits the formation of FAMEs in the liver *via* inhibition of FAEES, but not in the pancreas. These results confirm that hepatic metabolism of methanol to FAMEs can be modulated by esterase (FAEES) inhibitors.

1529 BIOTRANSFORMATION OF 1, 1, 1, 3-TETRACHLOROPROPANE IN RATS AFTER INHALATION EXPOSURE.

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1, 1, 1, 3-Tetrachloropropane is used in the production of chlorinated silicone fluids. Inhalation exposure of rats to 1, 1, 1, 3-tetrachloropropane results in myocarditis. The heart is a target organ not yet observed with chlorinated hydrocarbons. Likely, the organ-specific toxicity is based on biotransformation. To elucidate a mechanism explaining the organ-specific toxicity of 1, 1, 1, 3-tetrachloropropane, the biotransformation was studied in male and female rats (n = 5) after inhalation exposure to 100 ppm and 200 ppm for 6h. Urine samples were collected for 72 h after the end of the exposure and chlorine-containing metabolites in the urine were identified by GC/MS. In urine, 1, 1, 1-trichloropropane-3-ol was present as a major metabolite (likely as glucuronide) and 3, 3, 3-trichloropropionic acid and 3, 3-dichloroacrylic acid were excreted as minor metabolites in addition to very small amounts of the parent compound. Only minor parts of the calculated received doses of 1, 1, 1, 3-tetrachloropropane were recovered in urine as chlorine-containing metabolites. Elimination of these metabolites with urine was rapid. Inhalation of 1, 1, 1, 3-tetrachloropropane also resulted in an increase in the urinary excretion of acetone and of free fatty acids, and in the urinary excretion of 2-butanone, 2-pentanone and 2-hexanone. These results suggest a cytochrome P450 mediated oxidation of 1, 1, 1, 3-tetrachloropropane to give 3, 3, 3-trichloropropanal, which is reduced to 1, 1, 1-trichloropropane-3-ol or oxidized to 3, 3, 3-trichloropropionic acid. 3, 3, 3-Trichloropropionic acid and 3, 3-dichloroacrylic acid may be the metabolites responsible for toxicity. The cardiac toxicity of 1, 1, 1, 3-tetrachloropropane may be due to biotransformation of the metabolite 3, 3, 3-trichloropropionic acid by mitochondrial b-oxidation to give 3, 3-dichloroacryloyl-CoA acid. This a, b-unsaturated carbonyl compound may react with nucleophilic sites in mitochondria and may thus induce mitochondrial dysfunction and myocarditis since the heart is highly dependent on mitochondrial fatty acid oxidation for energy production.

1530 ALIPHATIC NITRILES: EFFECT OF ALCOHOL ON TOXICITY AND METABOLISM IN MALE SPRAGUE-DAWLEY RATS.

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Effect of alcohol was studied on the toxicity and metabolism of one unsaturated aliphatic nitrile acrylonitrile and was compared with the effect on one saturated nitrile acetonitrile in male Sprague-Dawley rats. The experimental rats received an oral dose of alcohol 0, 2, 3 or 4.5 g / Kg as a 50% aqueous solution and control rats received an equal volume of glucose solution isocaloric to each of the alcohol solutions of 0. 3.5, 5.3 and 7.9 g / Kg glucose respectively. Twelve hours following alcohol or glucose administration, the rats received an oral sublethal dose of 0.5 LD50 of acrylonitrile or 0.25 LD50 of acetonitrile. Animals were then observed for the typical signs of nitrile toxicity including salivation, lacrimation, convulsions. Both rat groups exhibited typical signs of nitrile toxicity except that the alcohol group was faster in exhibiting the signs. Known effects of nitriles on cyanide and thiocyanate production and glutathione metabolism were studied in organs and biological fluids of rats from both groups. The effect of alcohol was found to be dose dependent. Blood cyanide concentration was significantly lowered by alcohol at 12 hours after acetonitrile administration at the alcohol dose of 2 and 3 g / Kg whereas this effect was noticed at the alcohol dose of 3 and 4.5 g / Kg at 12 hour after acrylonitrile administration. Analysis of organ homognates including those of liver, spleen, brain, and kidney showed that the effect of alcohol on the nitrile induced effect on glutathione metabolism was dose and time dependent without a specific pattern. (Supported by NIH South Texas Doctoral Bridge Program Grant # 2 R25GM50080).

1531 MUTAGENICITY STUDIES OF URINARY METABOLITES FROM RATS TREATED ORALLY WITH LOCAL ANESTHETICS.

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Biotransformation in mammals of the local anesthetics, lidocaine (LD) and prilocaine (PL) may result in the formation of suspected carcinogens, 2, 6-xylidine (XD) and *o*-toluidine (TD), respectively. Metabolic activation of LD and PL could result in the excretion of mutagens in urine following administration of either compound. As part of an effort to characterize the carcinogenic potential of LD and PL, urine was collected for testing in the Ames mutagenicity assay from male F344 rats administered either LD, PL, XD, or TD at 100 mg/kg by gavage. Urine samples were filter-sterilized, incubated w/wo glucuronidase/sulfatase and Aroclor 1254-induced rat S9, and tested with *Salmonella typhimurium* strains TA98 and TA100 directly in the plate incorporation assay. Positive controls included testing for the presence of mutagens in urine of rats receiving 40 mg/kg of benzo[a]pyrene (B[a]P) by gavage. Initial experiments investigated the mutagenicity and toxicity of parent compounds LD and PL to Salmonella strains TA97, TA98, TA100, TA102, and TA104, w/wo S9 metabolic activation. No mutagenicity was observed for either compound in any of the strains at doses of 5 to 200 μmol/plate. Toxicity varied among treatment groups, but generally was not observed at doses lower than 100 μmol/plate. Urine samples (250 μl) collected 8 hours following oral administration of either LD or PL, incubated with glucuronidase/sulfatase, were not toxic or mutagenic to strains TA98 and TA100, w/wo S9 metabolic activation. In contrast, 2-5 fold increases in TA98 and TA100 revertants were observed for 0-8 hr urine from B[a]P-treated rats. Larger volumes (500 μl) of untreated urine from rats receiving either LD, PL, XD, or TD were neither toxic nor mutagenic to strain TA100. No potent mutagens have been detected in urine of LD- or PL-treated rats in this study. However, these results do not rule out the existence of either LD- or PL-derived mutagens in urine of these rats at levels below the limit of detection of the present experimental design.

1532 EFFECTS OF CALORIC INTAKE ON ETHANOL METABOLISM IN PREGNANT RATS: ROLE OF ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE.

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Urine ethanol concentration (UEC) dynamics were examined in animals infused ethanol intragastrically *via* total enteral nutrition at caloric levlels of 220, 187 or 154 $kcal^{3/4}$/kgd. When ethanol was infused into pregnant rats from gestational(GE)

d 6 as part of 220 kcal diets beginning at 10 g/kg/d and ending with constant infusion at 12 g/kg/d on GE d 12, little ethanol was found in the urine. When ethanol infusion began at 12.5 g/kg/d and ended at 15 g/kg/d, UECs peaked at 300-500 mg/dl on GE d 15-17 (d 9-10 of infusion). When pregnant rats were infused with 10-12 g/kg/d ethanol as part of 154 kcal diets, UECs peaked at 400-600 mg/dl on GE d 12-13 (d 6-7 of infusion). In a second study, when a similar protocol was utilized to compare infusion of 10-12 g/kg/d ethanol in non-pregnant rats fed 187 or 220 kcal diets with pregnant rats fed 220 kcal, UECs peaked at d 6-7 of infusion in the non-pregnant rats but only at d 9-10 of infusion in the pregnant animals and the area under the UEC-time curve was reduced in the pregnant rats ($p \leq 0.05$) compared to the other two groups. Although we have evidence that pulsatile UECs are associated with increased expression and activity of hepatic class I alcohol dehydrogenase (ADH), maternal hepatic ADH activity measured on GE d 20 was identical in non-pregnant and pregnant rats fed 220 kcal diets. However, maternal hepatic aldehyde dehydrogenase (ALDH) activity was significantly elevated on GE d 20. In contrast, under-nutrition using 154 kcal resulted in reduced hepatic maternal ADH activity on GE d 20 ($p \leq 0.05$). Thus ALDH may be responsible for increased ethanol metabolism in pregnancy and impaired ADH activity may account for increased UECs in undernourished pregnant animals. Supported in part by R01 AA12819 (MJJR).

1533 EFFECT OF POLYUNSATURATED FATTY ACIDS ON DNA ADDUCT FORMATION BY HETEROCYCLIC AROMATIC AMINES IN HCA-7 CELLS.

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Background: The metabolic activation of the heterocyclic aromatic amines (HCA) 2-amino-3-methylimidazo[4, 5-*f*]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4, 5-*b*]pyridine (PhIP) can proceed *via* both a hepatic and an extrahepatic pathway. In the extrahepatic pathway, the enzyme cyclooxygenase (COX) is believed to play a crucial role. Fatty acids, a class of compounds that is known to play a major role in carcinogenesis, are also subject to metabolic activation by COX. *Objective*: In this study, we investigated the effect of the polyunsaturated fatty acids (PUFA) arachidonic acid (AA, 20:4*n*-6) and eicosapentaneoic acid (EPA, 20:5*n*-3) on DNA adduct formation by IQ and PhIP in the human adenocarcinoma cell line HCA-7, which is known to express high levels of COX-2. Furthermore, the relative importance of both COX-2 and cytochrome P4501A2 (CYP1A2) was studied by using selective enzyme inhibitors. *Methods*: HCA-7 cells were exposed to either 25 µM IQ or 50 µM PhIP and different concentrations of AA and EPA. Indomethacin (IM), NS-398 and phenethyl isothiocyanate (PEITC) were used as inhibitors of COX, COX-2 in particular and CYP1A2, respectively. After 24 h exposure, the cells were harvested and DNA was isolated. Finally, DNA adduct levels were determined using ^{32}P-postlabeling. *Results*: Both AA and EPA exerted an increasing effect on DNA adduct formation in HCA-7 cells by both IQ and PhIP in a concentration dependent manner for concentrations up to 60 µM. All enzyme inhibitors showed a dose dependent decrease in adduct formation by both IQ and PhIP, indicating that HCA-7 cells also express CYP1A2 activity, and that both COX and CYP1A2 are involved in the metabolic activation of IQ and PhIP. *Conclusions*: These results indicate that COX, and more specifically COX-2, can play an important role in the metabolic activation of HCA. Furthermore, PUFA can stimulate the activation of HCA by COX, leading to higher amounts of HCA-DNA adducts in HCA-7 cells.

1534 SPECIES DIFFERENCES IN THE METABOLISM OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP) IN SEVERAL ORGANS FROM MOUSE, RAT AND MARMOSET.

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In order to clarify species differences in kinetics of Di(2-ethylhexyl) phthalate (DEHP), we measured the activities or expression of five enzymes, lipase, UDP-glucuronyltransferase (UGT), cytochrome P450 4A (CYP4A), alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), in several organs (livers, lungs, kidneys, and small intestines) from mice, rats and marmosets. Lipase activity, measured by a formating rate of mono(2-ethylhexyl)phthalate (MEHP) from DEHP, was ranged from 22 to 148 times: the activity was the highest in the small intestine from mice and the lowest in the lung from marmosets. UGT activity for MEHP in liver microsomes was the highest in mice, followed by rats and marmosets. The differences, however, were only marginal when compared with those of lipase activity. CYP4A was constitutively expressed in livers from rats, but not in those from mice and marmosets at the same conditions. ADH activity showed the highest in the liver from any animals either used 2-ethylhexanol or 2-phenoxyethanol as a substrate. ADH activity also represented species differences; the activity in the livers from marmosets was 1.6 to 3.9 times as high as those from rats or mice, which was quite different from the results of lipase or UGT activity. ALDH activity was the highest in the liver from any animal, similar to ADH activity. In comparison among species, the activity was higher in rats or marmosets (2 to 14 times) than in mice. These results clearly show that species differences exist in the metabolism of DEHP, and lipase activity is the most prominent.

1535 REDOX-CYCLING OF 7H-DIBENZO[C, G]CARBAZOLE-3, 4-DIONE AND SUPEROXIDE FORMATION.

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7H-dibenzo[c, g]carbazole (DBC) is a potent environmental carcinogen. We have previously reported a metabolic activation mechanism of DBC which involves the formation of reactive and redox active o-quinone of DBC (DBC-3, 4-dione, DBCQ) [Polycyclic Aromatic Compounds, 22, 295-300 (2002), Chem. Res. Toxicol. 15, 915-921 (2002)]. The DBCQ has been demonstrated to be a highly reactive Michael acceptor capable of adduction with nucleophiles and DNA/RNA constituents both *in vitro* and *in vivo*. The present study provides experimental evidence that the DBCQ is redox active. The non-enzymatic reduction rate of DBCQ by NAD(P)H was determined to be 0.22±0.05 nmol/min (n=8) for DBCQ and the oxidation of NAD(P)H was accompnied by the formation of superoxide anion radical. The superoxide formation rate was determined to be 0.13 ±0.05 nmol/min (n=4). These data indicate that in the redox-cycle for DBCQ, reactive oxygen species (ROS, i.e. hydrogen peroxide, hydroxyl radical and superoxide anion) are involved. Since ROS is related to oxidative DNA damage, DBC quinone is produced and may be responsible for the extreme cytotoxicity of DBC.

1536 METHAMPHETAMINE METHYLATION IN AQUEOUS SOLUTIONS OF FORMALDEHYDE.

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Methamphetamine is a widely abused drug in the US and is of forensic and toxicologic interest due to its potential involvement in deaths from intentional or accidental overdose. If drug involvement in a death is not suspected prior to embalming of the body, it may become necessary to measure drug levels in tissues that have been embalmed or preserved in formaldehyde-based embalming fluids. We hypothesized that formaldehyde would react with methamphetamine, producing new chemical entities, with the potential for complicating forensic and toxicologic analyses. To simulate the range of conditions tissues might be exposed following embalming or preservation, methamphetamine was reacted with 5, 10 or 20% formaldehyde solutions at pH 3.5, pH 7 and pH 9.5. Degradation profiles were obtained at day 0, 1, 7 and 30- post initiation of reaction, by HPLC with UV detection and the products characterized and identified by LC-MS. There was no significant degradation at pH 3.5. The percent methamphetamine remaining under other experimental conditions is presented in the table below.The decomposition of methamphetamine appears to be both pH and formaldehyde concentration dependent with the primary reaction product being N-methylmethamphetamine(1-phenyl-2-dimethylaminopropane). Supported under award number 2001-RC-CX-K013 from the Office of Justice Programs, National Institute of Justice, Department of Justice. Points of view in this document are those of the authors and do not necessarily represent the official position of the US Department of Justice.

%Formaldehyde	Day 1(%remaining)	Day 7(%remaining)	Day 30(%remaining)
pH 7.0			
5%	95±0.6	97±0.5	43±1.19
10%	78±2.2	40±1.3	7.6±2.64
20%	70±0.5	24±2.0	6.4±1.26
pH 9.5			
5%	56±0.4	11±4.5	5.5±1.4
10%	29±0.7	<LOD	<LOD
20%	6±0.4	≤LOD	≤LOD

1537 COMPARATIVE METABOLISM AND DRUG RESIDUES IN PHEASANTS, BOBWHITE QUAIL, PARTRIDGE AND CHICKENS.

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Currently there is interest in grouping similar avian species with regard to their metabolism of drugs and toxicants. This interest stems from needs in avian production and clinical medicine. The present studies were conducted to compare the metabolic capabilities of four Galliform species that are raised commercially for food and also found in the wild. Residues of the anthelmintic fenbendazole were quantified in edible tissues of pheasants, partridge and bobwhite quail after 6 days exposure to 100 ppm fenbendazole in feed. Liver, breast muscle, leg muscle, and skin were harvested at intervals after the final dose for HPLC measurement of fenbendazole (FBZ), fenbendazole sulfoxide (FBZ-SO) and fenbendazole sulfone (FBZ-SO2). In partridge and pheasants, only FBZ-SO and FBZ-SO2 were recovered from all tissues; in bobwhite quail, parent FBZ was also found in all tissues at all times. In partridge and pheasant the concentration of total residues was below FDA tolerances established for the fenbendazole marker residue (FBZ-SO2) in turkeys for liver (6 ppm) and muscle (2 ppm). In bobwhite quail, liver residues exceeded these tolerances, but residues in muscle were below tolerance. To further explore differences in metabolism, hepatic microsomal substrate assays are being conducted with pheasants, bobwhite quail, and chickens. A variety of substrates representing different cytochrome P450 isozymes are being tested. Preliminary results measuring constitutive ethoxyresorufin-O-deethylase activity show pheasants averaging 485 pmole product/min x mg protein with 0.44 nmole total cytochrome P450 per milligram protein. Chickens show about one-tenth of this activity at 39 pmol product/min x mg protein and 0.16 nmole total P450 per milligram protein. These studies suggest that there may be significant differences in drug/toxicant metabolism among the four game bird species. The quantitative versus qualitative nature of these differences is the focus of future studies. Supported by the USDA National Research Support Program Number 7 Minor Use Animal Drug Program.

1538 SYNTHESIS OF RETINYL PALMITATE- AND RETINOL-DERIVED PHOTOCHEMICAL, OXIDATIVE, AND DEHYDRATION PRODUCTS.

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Retinyl palmitate (RP) is a major component of numerous skin creams and lotions. In addition, significant skin surface areas are exposed to solar radiation following topical application of these products. However, the metabolic and photochemical fate of RP, particularly following chronic topical applications, has not been elucidated. We have synthesized several retinoids, using novel synthetic methods, to serve as HPLC standards; These compounds are hypothesized to be generated *in vivo*. 9Z-Retinol (9Z-ROL) was synthesized by reduction of 9Z-retinal with sodium borohydride. 9Z-ROL or 13Z-ROL were reacted with palmatoyl chloride in the presence of 1, 8-bis-(dimethylamino)naphthalene (Proton Sponge) and using 4-dimethylaminopyridine as a catalyst to yield the corresponding 9Z and 13Z geometric isomers of RP. These compounds are anticipated to be generated upon exposure to UV through excited singlet states. 5, 6-Epoxy-RP was synthesized by regiospecific oxidation of RP with monoperoxyphthalic acid or 3-chloroperoxybenzoic acid. The corresponding oxirane of ROL was synthesized by oxidation of retinyl acetate with peracid followed by saponification. These products are anticipated to be generated *in vivo* through peroxyl radical-dependent mechanisms. 4-Oxo-RP was synthesized from RP *via* regiospecific oxidation using manganese dioxide. 4-Hydroxy-RP was synthesized by reduction of the latter with sodium borohydride. 4-Hydroxy-ROL and 4-oxo-ROL were synthesized by oxidation of retinyl acetate with manganese dioxide followed by reduction with lithium aluminum hydride or by saponification, respectively. These oxidized retinoids are anticipated to be generated *in vivo* as a consequence of cytochromes P450- and alcohol dehydrogenase-dependent activities. Anhydroretinol was synthesized by reaction of retinyl acetate with Amberlyst 15 in ether. The structures of all products and intermediates were confirmed on the basis of NMR, IR, UV, and mass spectrometry. This work was supported by the National Toxicology Program.

1539 FURTHER STUDIES ON THE SELECTIVITY AND INHIBITORY MECHANISM OF AZAMULIN, A NEW CYP3A CHEMICAL INHIBITOR PROBE.

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Azamulin (14-O-(5-(2-amino-1, 3, 4-tiazolyl)thioacetyl)-dihydromutilin) is an azole derivative of the pleuromutilin class of anti-infectives. We have extended our studies on the cytochrome P450 (CYP) isoform inhibition specificity of azamulin. Previously, we have shown that inhibition specificity for CYP3A4, as determined by IC50 value, was more than three orders of magnitude greater than all enzymes examined from the CYP1 and CYP2 families. These families, along with CYP3, encompass the major CYP enzymes that metabolize xenobiotics. Selectivity was superior to another commonly used inhibitor of CYP3A, ketoconazole. We now extend our results to the CYP4 family, which may also metabolize xenobiotics. Both ketoconazole and azamulin were non-inhibitory to CYP4F3b and CYP4A11 activities at concentrations considered CYP3A-selective. However, same concentration of ketoconazole inhibited CYP4F2 activity approximately 62%, whereas inhibition by azamulin was negligible. The Ki of azamulin for CYP3A-catalyzed testosterone 6βhydroxylase activity in human liver microsomes was 0.076 μM as determined by Dixon plot. Inhibition is consistent with a mixed-type mechanism. Our data further demonstrate the suitability of azamulin as a highly potent and selective inhibitor for assessing the catalytic role of CYP3A in microsomes from liver or extrahepatic tissues.

1540 OXIDATIVE STRESS STIMULATES THE FORMATION OF CYTOCHROME P450 3A PROTEIN CONJUGATION IN A PROCESS THAT IS INHIBITED BY SUBSTRATE.

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We recently demonstrated that hepatic microsomes from nicardipine-treated rats formed cytochrome P450 3A (CYP3A)-ubiquitin conjugates when incubated at 37°C. These protein complexes apparently were the result of CYP3A cross-linking with other proteins that were already polyubiquitinated rather than direct "classical" ubiquitination of CYP3A. CYP3A substrates inhibited this cross-linking and the associated protein degradation, suggesting that the process is important in substrate-mediated stabilization of CYP3A. We now demonstrate that oxidative stress appears to be a key factor in the formation of CYP3A-ubiquitin conjugates in incubated microsomes and primary cultured rat hepatocytes. Our data further suggest that the effects of oxidative stress are mediated by lipid hydroperoxides, which are utilized by CYP3A during catalysis in a "peroxide shunt" pathway. Hydroxynonenal (HNE) is an autolysis product of lipid hydroperoxides. HNE-protein adducts were concentrated in the same high mass range as the CYP3A-ubiquitin conjugates, suggesting that HNE may be directly responsible for the protein crosslinking. Therefore, these studies suggest a new model of the molecular processes involved in substrate-mediated stabilization of CYP3A. That is, in the presence of substrate, the lipid hydroperoxides are consumed as cofactors in the CYP3A catalytic cycle. In the absence of substrate, the lipid hydroperoxides still bind CYP3A but eventually degrade to reactive products that can crosslink proteins and stimulate protein degradation. This research was supported by NIH grant DK54812.

1541 CYTOCHROME P450 CYP2E1 AND CYP 3A4 ACTIVITIES IN HEPATITIS C PATIENTS.

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Some cytochrome P450 enzymes, e.g., CYP2E1 and CYP3A4 can "leak" reactive oxygen species and contribute to hepatic oxidative stress. Because damage from hepatitis C virus (HCV) infection might involve oxidative stress, we studied CYP2E1 and CYP3A4 activities in HCV infected humans and controls. **METHODS:** Sixteen HCV subjects and 5 control subjects from this study and 5 subjects from a previous study were admitted for a day to the General Clinical Research Center. All were volunteers and informed consent was obtained. HCV infection was documented by standard clinical tests and subjects were otherwise healthy and treatment naïve. Each subject received a single 250 mg chlorzoxazone tablet (CZX) as a probe for CYP2E1 and 2 mg IV midazolam (MDZ) as a probe of CYP3A4. Blood & urine were collected at time points and clearances determined by standard methods. CZX, 6–OH–CZX were measured by HPLC and MDZ by GC with electron capture. **RESULTS:** Age, race, gender, smoking status, medications, liver biopsy results, genotype & quantity of HCV virus, ALT, ferritin, and parameters of the systemic oxidative stress (whole blood GSH & urine F2–isoprostanes) are presented. HCV subjects had evidence of increased oxidative stress. Results for MDZ systemic clearance were determined for 7 HCV patients and a group of 7 controls – MDZ systemic clearance was 6.4±2.1 ml/min/kg in the HCV group compared to 4.7±1.5 ml/min/kg in the controls. The 36% increase in the HCV subjects reached statistical significance (p=0.038). Oral CZX clearance in 16 HCV subjects and 5 controls was 5.08±1.99 and 6.8±3.51ml/min respectively. Formation clearance of 6–OH–CZX in 10 HCV subjects and 2 controls were 1.89±1.8 and 2.24±0.72 L/h respectively (Not significantly different). **CONCLUSION:** HCV patients with a relatively mild degree of liver injury and increased systemic oxidative stress show increased activities of cytochrome P450 CYP3A4, while no changes in the activities of CYP2E1 were found.

1542 CYP2E1 IS NOT SPECIFIC FOR FORMATION OF 6-HYDROXYCHLORZOXAZONE IN VIVO.

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Clinically and experimentally, the 6-hydroxylation of chlorzoxazone (CHZ) is widely used to measure levels of CYP2E1. However, expressed human forms of CYPs 2E1, 3A4 and 2D6 exhibited comparable CHZ 6-hydroxylase activities. Antibodies to CYP3A4 cause up to 47 % inhibition of this activity in human liver microsomes (Gorski et al., 1997). Furthermore, in addition to CYP2E1, expressed forms of rat CYPs 1A2, 3A1 and 2C11 catalyze CHZ 6-hydroxylase activity (Kobayashi et al., 2002). We assessed the specificity of CYP2E1 for the 6-hydroxylation of CHZ, by comparing the formation of 6-OH-CHZ in vivo in wild-type and Cyp2e1(-/-) mice. 6-OH-CHZ was measured in sera of mice, because, in humans and in rats, levels of this metabolite are reported to be greater in serum compared to urine at early times after administration of CHZ. Mice of both genders were administered a dose of 15 mg CHZ/kg. CHZ and 6-OH-CHZ were measured by HPLC in sera at different times after administration of the parent drug. 6-OH-CHZ was formed by both Cyp2e1(-/-) mice and wild-type mice. Our results indicate that CYPs other than CYP2E1 contribute to formation of this metabolite *in vivo*. This work was supported by NIH (AA12898, JS) and the Department of Veteran Affairs.

1543 VALIDATION AND AUTOMATION OF AN ASSAY ENABLING RAPID SCREENING FOR POTENTIAL TOXICOLOGICAL IMPLICATIONS OF CYP2E1 INHIBITION.

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Rationale: Human CYP2E1 is an alcohol-inducible enzyme that plays an important toxicological role due to the isozyme's involvement in metabolism of solvents, toxins and environmental pollutants with implications in cancer, liver and kidney toxicity. Identification of CYP2E1 inhibitors may allow prevention of the adverse drug reactions arising from toxic metabolites formed *via* the CYP2E1 metabolic pathway. The screening is currently limited due to the absence of the systematic high throughput screening (HTS) approach. Here we describe the development of an automated fluorescence-based HTS assay for the rapid detection of compounds interfering with CYP2E1 pathway. **Method:** The CYP2E1 Vivid® assay is based on a rapid conversion of initially non-fluorescent ("blocked") Vivid® CYP2E1 Blue substrate into a highly fluorescent dye as a result of oxidation by a CYP2E1 isozyme (fluorescent reporter reaction). Measuring the inhibition of the fluorescent reporter reaction in the presence of the specific drug or compound allows detection of CYP2E1 substrates and inhibitors. Vivid® CYP2E1 Substrate is a member of the panel of the Vivid® fluorogenic substrates created by a rational design approach to enable sensitive detection in HTS format. Here we employed Vivid® CYP2E1 Blue fluorogenic substrate for the development of HTS assay for screening CYP2E1 metabolism and inhibition in automated format using the Southern Research Institute's Core HTS Rail System. **Results:** As part of testing the reliability and quality of these screening assays in HTS automated format, we assessed the assay linearity range, kinetic parameters (apparent Vmax and Km), solvent sensitivity, inhibitory potential, Z'-factor and assay dynamic range. **Conclusions:** Our results demonstrate that the established parameters of the CYP2E1 Vivid® assay make it an excellent method for the sensitive detection of compound interactions with the CYP2E1 isozyme in a HTS format.

1544 IDENTIFICATION OF P450 ENZYMES INVOLVED IN THE METABOLISM AND TOXICITY OF CARBON DISULFIDE.

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Carbon disulfide (CS_2) is a widely used, highly volatile industrial solvent, and also a widespread environmental contaminant. The objective of this study was to test the hypothesis that CS_2 interacts with induced hepatic cytochrome P450 (CYP) isozymes 1A1, 2B1, 2E1 and 3A2, and metabolizes to reactive sulfur species that may be responsible for hepatic damage. Because 3-methylcholanthrene, phenobarbital, isoniazid, and pregnenolone-16 -carbonitrile are typical selective inducers of CYP1A1, 2B1, 2E1, and 3A2, respectively, they were chosen in this study to pretreat male Sprague-Dawley rats to induce the CYP isoforms. Twenty-four h following the final dose of the inducers, rats were treated ip with a low dose (0.1 mmol/kg) or a high dose (0.5 mmol/kg) of CS_2. Half of the rats from each group were sacrificed at 3 h after treatment to identify inhibited isozymes since CS_2 is a suicide inhibitor of CYPs, and the remaining half at 24 h to determine the hepatic damage using serum ALT activity and liver histopathology as toxicity indicators. At 3 h after treatment, CYP3A2 was inhibited only by the high dose while CYP1A1, 2B1 and 2E1 were inhibited by both doses of CS_2 significantly and differentially. However, within 24 h of treatment the inhibition of CYP1A1 and 3A2 was reversed with no serious liver damage while that of CYP2B1 and 2E1 was irreversible and accompanied by severe liver damage especially in phenobarbital-pretreated rats. These results suggest that induced CYP2B1 and 2E1 play an important role in the metabolism of CS_2 yielding reactive sulfur species which may be responsible for the observed liver damage.

1545 COMPARATIVE METABOLISM OF *cis* AND *trans* CROTONONITRILE (CRN) TO CYANIDE USING CYTOCHROME P405 2E1-NULL AND WILD TYPE MICE.

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Crotononitrile (CrN), an unsaturated aliphatic nitrile, causes neurotoxicity in animals. It was reported that cis-CrN is a more potent neurotoxin than trans-CrN. CrN is metabolized to cyanide presumably *via* P450-mediated epoxidation. The metabolism of the two isomers and the enzymes involved are not well studied. Since CYP2E1 is involved in the metabolism of other nitriles, current studies were undertaken to compare the metabolism of the 2 isomers to cyanide using CYP2E1-null (KO) and wild-type (WT) mice. Male WT and KO mice received a single gavage dose of 12, 24, 48 or 96 mg/kg of cis or trans CrN. Mice were sacrificed after 1, 3, or 6 hr, and blood cyanide [CN] was measured. A dose-dependent increase in [CN] was detected in WT and KO mice, reaching a maximum 1 hr after dosing, and was significantly higher after cis vs. trans dosing. Significant decline in cyanide formation occurred in KO vs. WT treated with either isomer. The decrease in cis-CrN metabolism to [CN] in KO was more profound than that of the trans isomer. Treatment of WT or KO mice with 1-aminobenzotriazole before dosing with 48 mg/kg of either isomer resulted in a near complete inhibition of cyanide formation. Subsequent *in vitro* studies compared the reactivity of the 2 isomers and their epoxides (2, 3-epoxybutyronitriles, EBN) with 3H-glutathione (GSH). This study indicated that the reactivity of both cis and trans-CrN is negligible. In contrast, cis-EBN is significantly more reactive than cis-CrN and trans-EBN. Addition of GSH transferase to the incubation significantly enhanced the reactivity of cis- and trans-EBN. In conclusion, these data showed that 1) CYP2E1 plays an important role in the metabolism of CrN to [CN], however, other P450s are also involved 2) metabolism of cis-CrN results in greater CN- release than trans-CrN, and 3) cis-EBN is more reactive with GSH *in vitro* than the trans-EBN. Reaction of EBNs with GSH is a likely mechanism leading to cyanide formation and may explain the differences in cyanide release from cis and trans CrN *in vivo*.

1546 INHIBITION OF URETHANE METABOLISM AND BIOACCUMULATION IN CYTOCHROME P450 2E1-NULL MICE.

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Urethane is a well-established animal carcinogen and was classified as reasonably anticipated to be a human carcinogen. Urethane is formed as a fermentation by-product and therefore, the primary source of human exposure is through consumption of fermented foods and beverages. Past studies suggested that although CYP2E1 is involved, esterase plays a primary role in urethane metabolism. To define the role of CYP2E1, urethane metabolism was compared in wild-type (WT) and CYP2E1-null (KO) mice. 14C-Ethyl-labeled urethane was administered as a single 10 or 100 mg/kg gavage dose or at 100mg/kg/day for 5 consecutive days. Mice were placed in metabolism cages that allowed for the quantitation of expired 14CO2, organic volatiles, urine, and feces. In WT mice, 78-88% of dose was exhaled as 14CO2/day by animals given single or multiple doses. In contrast 30-38% of dose was eliminated as 14CO2 in 24 hr after a single dose in KO mice. After the 2nd day of dosing, 43% of dose was exhaled in 24 hr as 14CO2. 14CO2 exhalation reached a plateau at approximately 52% of dose/day after dose 3, 4, or 5. An average of 28 and 13% of the cumulative doses was eliminated as urethane-derived 14C in the urine and organic volatiles of KO mice, respectively. WT mice, however, eliminated approximately 10 and 3.5% of dose *via* these routes. Current work clearly shows that 14CO2 is the primary metabolite of CYP2E1-mediated oxidation of 14C-ethyl labeled-urethane. The concentrations of urethane-derived 14C in blood and tissues were dependent on dose and number of doses given, and significantly higher in the KO vs. WT mice. Using HPLC analysis, urethane constituted >90% of urethane-derived 14C in plasma of KO mice receiving single or multiple

doses of urethane. In conclusion, present work shows that KO mice experience a significant inhibition in urethane metabolism, leading to significant accumulation of urethane in the tissues of KO mice. Whether accumulation of urethane and/or metabolites formed *via* pathways other than CYP2E1 affect toxicity and carcinogenicity remains unclear.

1547 RELIABILITY OF CHLORZOXAZONE AS AN *IN VIVO* PROBE OF CYP2E1 ACTIVITY IN HUMANS.

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Chlorzoxazone (CZX) is metabolised by cytochrome P450 2E1 (CYP2E1) to a single oxidised metabolite, 6-hydroxychlorzoxazone (OH-CZX). The aim of the study was to test the reliability of CZX as an *in vivo* probe of CYP2E1 activity in humans. The OH-CZX/CX ratio was measured in plasma from 20 male volunteers (age 27 to 53) at 2 h following a single oral dose of 500 mg CZX. The experiment was repeated about 1.5 years later in 13 subjects. In 7 subjects the experiment was also repeated during three consecutive weeks and at three different doses of 250, 500, and 1000 mg. In addition, the effect of moderate ethanol intake (0.5 g ethanol/ kg body weight) the preceding evening was evaluated. The study was approved by the Regional Ethics Committee. The OH-CZX/CX ratio at 2 h ranged from 0.12 to 0.61 and the relative standard deviation was 42 % (n= 20). A positive correlation with body weight (r= 0.7, p= 0.0003) was seen in linear regression analysis, which might indicate that the dose was too high. In the test performed with 1.5 year interval, the inter-individual variability in metabolic ratio was 66 % and the intra-individual variability was 32 % (expressed as relative standard deviation, obtained from repeated measures ANOVA, n=13). The inter- and intra-individual variabilities in the test performed during three consecutive weeks were 60 % and 15 %, respectively (n= 7). The low intra-individual variability (15 %) indicates a relatively small method error. The OH-CZX/CX ratio decreased with increasing CZX dose (n = 7, p = 0.01, repeated measures ANOVA), suggesting metabolic saturation at the higher doses. There was no significant influence of ethanol intake on the ratio (n= 7, p= 0.8, paired t test). In conclusion, the OH-CZX/CX ratio in plasma at 2 h appears to be a reliable indicator of CYP2E1 activity. However, a lower dose than the commonly used 500 mg should be used to prevent metabolic saturation. Financial support was offered from the Swedish Council for Working Life and Social Research.

1548 COMPARATIVE METABOLISM OF ACRYLONITRILE AND METHACRYLONITRILE TO CYANIDE: STUDIES USING CYTOCHROME P4502E1 (CYP2E1)- AND MICROSOMAL EPOXIDE HYDROLASE (MEH)-NULL MICE.

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Acrylonitrile (AN) and methacrylonitrile (MAN) are structurally related aliphatic nitriles. Metabolism of these nitriles occurs *via* 2 competing pathways: glutathione conjugation, and epoxidation by cytochromes P450 (P450s). We have recently shown that CYP2E1 is essential for the metabolism of AN to cyanide and the ensuing acute toxicity. Current studies were designed to compare the metabolism of AN and MAN to cyanide and to assess the roles of CYP2E1 and EH using wild-type (WT), CYP2E1- and mEH-null male mice. Mice receiving a single gavage dose of 0.047, 0.095, 0.19, or 0.38 mmoles/kg of MAN or AN, were sacrificed 1 or 3 hr after dosing, blood was collected, and cyanide was measured. Expressions of CYP2E1, mEH, and soluble EH (sEH) in mice tissues were assessed using Western blot analyses. Current data showed that blood cyanide levels in WT mice increased in a dose-dependent manner, reached a maximum at 1 hr, and were significantly higher after MAN vs. AN administration. Further, while significant reduction in blood cyanide levels was detected in MAN-treated CYP2E1-null vs. WT mice, AN metabolism to cyanide was totally abolished in CYP2E1-null mice. Pretreatment of CYP2E1-null and WT mice with 1-aminobenzotriazole (P450s inhibitor) resulted in near complete inhibition of the metabolism of MAN to cyanide. Blood cyanide levels in AN-treated mEH-null mice were significantly lower relative to levels in WT mice. Similar reduction in blood cyanide was also observed in mEH-null mice treated with * 0.09 mmoles MAN/kg. However, no significant difference was observed at the highest MAN dose (0.18 mmoles/kg). Similar expressions of mEH and sEH in CYP2E1-null and WT mice were established. CYP2E1 and sEH levels were comparable in mEH-null and WT mice. In conclusion, these data showed that: 1) CYP2E1 is the only P450 responsible for AN metabolism to cyanide, 2) while CYP2E1 plays a major role in MAN metabolism to cyanide, other P450 enzymes are also involved, and 3) mEH plays a significant role in the *in vivo* formation of cyanide from aliphatic nitriles.

1549 THE EFFECT OF ETHYL-*TERT.*-BUTYL ETHER INHALATION ON CYTOCHROME P450 ISOZYMES AND *IN VITRO* MEASUREMENT OF ENZYME ACTIVITY IN RAT LIVER, LUNG, AND NASAL MUCOSA.

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The most widely used and studied fuel oxygenate is methyl-tert-butyl ether (MTBE), but there is strong support to replace MTBE with ethyl-tert-butyl ether (ETBE)due to reported health complaints suggestive of respiratory effects in areas where gasoline contains MTBE.The possible adverse effects of ETBE in humans is a public concern, and little is known regarding specific CYP 450 isozymes involved in its metabolism.The respiratory tract is the first tissue encountered with ETBE, and subsequently exposed to the highest concentrations relative to other organs.The purpose of this study was to investigate alterations in rat liver, lung, and nasal mucosa cytochrome P450s following ETBE inhalation exposure. ETBE(250ppm or 500ppm, 6 hours)resulted in dose dependent inhibition ofCYP1A1(47%and50%), CYP1A2(82%and61%), CYP2B1(66%and29%), CYP2E1(57%and36%), CYP2A3(75%and58%), CYP3A1(73%and37%)and CYP4B1(73%and 62%)activities in nasal mucosa microsomes. There were no changes in pulmonary CYP450 activity, however hepatic CYP2B1significantly increased (126%and363%) following exposure to ETBE. *In vitro* metabolism of ETBE was determined to investigate the nature of the inhibition of cytochrome P450 isozymes in the nasal mucosa.Nasal mucosa isozymes CYP1A1, CYP1A2, CYP2B1, CYP2E1, CYP2A3 were inhibited by ETBE(IC50 concentration). Eadie-Hofstee plots for the aforementioned isozymes show non-competitive inhibition by ETBE. Oxidative deethylation of ETBE yields a primary metabolite, tert-butyl alcohol(TBA), as well as acetaldehyde, a known respiratory irritant.Metabolic enzyme activity following *in vitro* incubations in pooled liver, lung, and nasal mucosa microsomes were determined based on the formation of the two metabolites.All three tissues were active in metabolizing ETBE to TBA and acetaldehyde, and followed Michaelis-Menten kinetics with the highest metabolic activity in the nasal mucosa.The cytochrome P540 changes in respiratory tissues as a result of ETBE exposure may lead to changes in metabolic profiles in coexposure scenarios to other xenobiotics.

1550 INACTIVATION OF CYP2F1 IMPARTS DOSE-DEPENDENT DECREASE IN SUSCEPTIBILITY OF LUNG CELLS TO 3-METHYLINDOLE.

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Cytochrome P450 (CYP) enzymes 2F1 and 3A4 have been shown to bioactivate 3-methylindole (3MI) to a reactive intermediate that can induce cellular necrosis in human bronchial epithelial cell lines that overexpress these proteins. The overexpression of CYP2F1 in BEAS-2B cells (B-CMV2F1) increased the susceptibility of these cells to 3MI-induced apoptosis and cytotoxicity. Inhibition of CYP by the suicide substrate, 1-aminobenzotriazole, protected cells from cytotoxic injury and apoptosis. Low doses of 3MI from 10-50 micromolar induced DNA fragmentation and increased Annexin-V binding in B-CMV2F1 cells compared to control BEAS-2B cells. Higher doses of 3MI from 500-1000 micromolar resulted in a decreased susceptibility of cells to 3MI in the B-CMV2F1 cells but not in control BEAS-2B cells. These observations are consistent with kinetic studies of the CYP2F1 enzyme that demonstrated an inhibition of the enzyme at higher substrate concentrations of 3MI. Several experiments using higher concentrations of 3MI in human lung cell lines suggest that a reactive intermediate(s) of this pulmonary toxicant may inactivate the CYP2F1 enzyme in target cells. Since the CYP2F1 enzyme is unique in its ability to selectively produce a reactive intermediate through the dehydrogenation pathway, compared to other pulmonary specific CYP enzymes, the susceptibility of lung cells to toxic injury may be dependent upon the relative ratio of active/inactivated CYP2F1 enzyme. (supported by NHLBI #HL13645)

1551 *IN VITRO* METABOLISM OF MYRISTICIN BY RAT AND HUMAN LIVER.

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Myristicin biotransformation by cytochrome P450 (CYP) activity was assessed by incubating myristicin (100 μM) with microsomal preparations from livers of either rats or humans in the presence of an NADPH-generating system. For both species, microsomes pooled from several donors were compared with those from donors that had been exposed to phenobarbital (PB) to induce the synthesis of certain CYP isoenzymes. The products of the incubations were analyzed by HPLC with (a) UV absorption and (b) triple quadrupole tandem MS/MS detectors in series. Two predominant metabolites were found, one with a negative ion of molecular weight

179, and the other with a positive ion of molecular weight 209, which were identified as 5-allyl-1-methoxy-2, 3-dihydroxybenzene (the catechol metabolite) and 1'-hydroxymyristicin, respectively. Samples from the rat liver microsomal incubations were further analyzed by GC/MS to confirm the identity of the these two metabolites. UV absorption data were used to compare relative amounts of metabolites between samples. In all cases the catechol metabolite was the principal product of CYP activity, and in both species its formation was more rapid using microsomes from PB-induced livers. After 1 hour at 37° C with 1 mg/ml microsomal protein, the catechol metabolite accounted for 89.9 ± 4.5 % of the metabolites formed by PB-induced rat microsomes, and 84.1 ± 3.1 % of metabolites formed by pooled rat microsomes. Under these same conditions with human liver microsomes, the catechol metabolite was 73.8 ± 0.7 % of the total metabolites from PB-induced liver microsomes and 83.0 ± 2.5 % of the total from pooled human liver microsomes. PB induction of 1'-hydroxymyristicn formation was more pronounced in human microsomes than in rat microsomes. Preliminary studies incubating myristicin with rat and human primary hepatocytes attached to collagen-coated plates produced no evidence for formation of either of these two metabolites by intact cells. (*Supported by NIEHS Contract No. N01-ES-95437*).

1552 EXTRAHEPATIC METABOLISM OF BIOCHANIN A AND FORMONONETIN AND METABOLITE INHIBITION OF CYTOCHROME P450 1B1.

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Biochanin A (BA) and formononetin (FOR), are the 4′-O-methyl derivatives of the more potent phytoestrogens genistein (GEN) and daidzein (DA), respectively, and are the predominant isoflavones in red clover and chick peas. Previously, we demonstrated that human liver microsomes converted BA and FOR to GEN and DA. Since epidemiologic studies for breast cancer risk associate exposure to endogeneous estrogens with increased risk and soy diets high in DA and GEN with reduced risk, we investigated the extrahepatic metabolism of BA and FOR. Metabolic activation of estradiol (E2) to estrogen catechols is a prerequisite for its genotoxic activity with 4-hydroxylation leading to carcinogenicity and 2-hydroxylation associated with anticarcinogenicity. In humans, cytochrome P450 1B1 (1B1) is an extrahepatic E2 4-hydroxylase that activates procarcinogens, and elevated levels have been associated with estrogen carcinogenesis. We demonstrated 1B1-catalyzed O-demethylation of BA and FOR producing GEN and DA, which feedback to inhibit 1B1. Recombinant human 1B1 was incubated with BA or FOR in reactions containing NADPH regenerating systems and products were analyzed by HPLC with UV and coulometric detection. FOR was converted to DA and several hydroxylated metabolites and BA was converted to GEN and a different set of hydroxylated metabolites. DA and GEN were identified by their retention times and electrochemical oxidation profiles and confirmed by LC-MS. Inhibition of 1B1 7-ethoxyresorufin O-deethylase (EROD) activity by DA and GEN was determined using a 96-well plate assay. Inhibition of 1B1 EROD activity by GEN was primarily noncompetitive (Ki 1.9 μM). DA exhibited mixed, but predominantly noncompetitive inhibition of 1B1 EROD activity (Ki 3.7 μM). The data suggest that BA or FOR may exert anticarcinogenic effects directly by acting as competitive substrates for 1B1 or indirectly through their metabolites DA and GEN, which are both 1B1 inhibitors and more potent phytoestrogens than their methylated precusors.

1553 P450 INHIBITION BY METHYLENEDIOXYPHENYL COMPOUNDS PRESENT IN GOLDENSEAL.

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With the widespread use of nutritional supplements, pharmacokinetic interactions between botanicals and prescription or OTC drugs are of increasing concern. Goldenseal (Hydrastis canadensis) is a popular immunostimulant botanical that is widely available, either alone or in combination, with Echinacea. Earlier studies indicated that all three of the major methylenedioxyphenyl (MDP) compounds contained in goldenseal are able to inhibit *in vitro* cytochrome P450 catalyzed diclofenac-4'-hydroxylation (CYP2C9), bufuralol-1'-hydroxylation (CYP2D6), and testosterone-6β-hydroxylation (CYP3A4) activities, in human liver microsomes. MDP compounds are known for their ability to inhibit *via* the formation of metabolic-intermediate (MI) complexes with cytochrome P450. Therefore, a study was initiated to investigate the degree to which the inhibition of human cytochrome P450s by MDP compounds of goldenseal correlated with the formation of such complexes. Despite inhibiting CYP2C9, CYP2D6 and CYP3A4 activities, neither berberine nor hydrastinine were able to form MI complexes in human liver microsomes. Both the (+) and (-) isomers of hydrastine formed an MI complex. To delineate the CYP(s) responsible, MI complex formation was further investigated using supersomes (Gentest) containing a single CYP isoform. (+)-Hydrastine formed complexes with CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 but not CYP2B6. (-)-Hydrastine formed complexes only with CYP1A2, CYP2C9, CYP2D6, and CYP3A4. Co-expression of cytochrome b5 with CYP3A4 and CYP2C9 in the supersomes significantly enhanced the rate of MI complex formation. Therefore, inhibition of multiple human P450s by MDP components present in goldenseal may arise from the quasi-irreversible inhibition by MI complex formation, but other mechanisms also contribute to the inhibition.

1554 HYDROXYL RADICAL FORMATION BY HETEROLOGOUSLY EXPRESSED MICROSOMAL ENZYMES.

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It has been shown that the hydroxylation of terephthalate (TPT) to the highly fluorescent product 2-hydroxyterephthalate (2-OH TPT), is the result of reaction with hydroxyl radicals (●OH). Using a new method (Mishin and Thomas, 2002), we observed the formation of 2-OH TPT from TPT by cDNA-expressed: NADPH-cytochrome P450 oxidoreductase (OR) coexpressed with cytochrome b5 and rat cytochrome P450 enzymes (CYPs) coexpressed with OR and cytochrome b5. The formation of 2-OH TPT totally depended on the presence of NADPH and Fe/EDTA (or Fe/citrate) complexes. The 2-OH TPT generation was profoundly inhibited by DMSO a ●OH scavenger, catalase and glutathione peroxidase (+GSH), but superoxide dismutase did not have a significant effect. Several water-soluble substrates, specific for different CYP enzymes, (zoxazolamine, benzphetamine, chlorzoxazone, midazolam and buprenorphine) were without inhibitory effect on the formation of 2-OH TPT from TPT. A comparison of the relative rates of 2-OH TPT generation (nmoles 2-OH TPT/nmole P450/min) revealed CYP2A2 (23.4) and CYP2C6 (23.4) to have the highest rates and CYP2C12 (7.5) and CYP3A2 (8.1) to have the lowest rates. The other 12 CYPs-containing preparations, including CYP2E1, had similar rates that were intermediate. There was no correlation between OR levels in these expressed microsomal preparations and formation of 2-OH TPT even though OR alone (+NADPH) is sufficient for modest production of 2-OH TPT. We suggest that the generation of reactive hydroxyl radicals by microsomal enzymes proceeds *via* metal-catalysed Haber-Weiss reactions and is controlled by the rate of hydrogen peroxide formation by cytochrome P450 enzymes and redox cycling of iron ions.

1555 LUMINESCENT CYTOCHROME P450 ASSAYS THAT UTILIZE D-LUCIFERIN DERIVATIVES AS PROBE SUBSTRATES.

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Cytochrome p450s (cyp450) are the main catalyst of the oxidative metabolism of drugs and other xenobiotics. Understanding how potential new drugs interact as substrates or inhibitors of cyp450s is an essential component of drug development. To facilitate the rapid screening of multiple compounds we tested derivatives of (4S)-4, 5-dihydro-2-(6-hydroxybenzothiazolyl)-4-thiazolecarboxylic acid (D-luciferin) as probe cyp450 substrates. The D-luciferin derivatives were not substrates for luciferase in light-generating reactions but were metabolized by cyp450s to D-luciferin, which in turn reacted with firefly luciferase to produce light. The reactions were formulated using a homogenous luciferase mixture that was added directly to a conventional cyp450 reaction with D-luciferin derivatives as substrates to produce a glow-style luminescent signal that was stable for several hours. The amount of D-luciferin produced by a cyp450 was proportional to the light output of the luciferase reaction. Light was therefore used as a measure of cyp450 activity. In accord with this scheme 6′-deoxyluciferin was hydroxylated by human cyp2C9 to form D-luciferin that was readily detected by bioluminescence. 6′-deoxyluciferin was highly selective for cyp2C9 over other cyp450s. Other reactions included the dealkylation of luciferin 6′ alkyl and substituted alkyl ethers to form D-luciferin by human cyp1A1, 1A2, 2C8, 2C9 and 3A4 with varying degrees of cyp450 isoform selectivity. The luminescent cyp450 assays detected known cyp450 inhibitors with IC50s similar to those reported using conventional substrates. The assays displayed exquisite sensitivity detecting the activity of as little as 0.01 pmoles recombinant cyp450 against a minimal or immeasurable background signal and were easily configured in multi-well plate formats. We conclude that certain D-luciferin derivatives can be used as probe cyp450 substrates in simple and robust luminescent assay formats for rapid screening of multiple compounds against cyp450 activities.

1555a RAT PULMONARY CYP1A1 INDUCTION IS INHIBITED BY RESPIRABLE COAL DUST EXPOSURE.

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Cytochrome P450 1A1 (CYP1A1) metabolizes polycyclic aromatic hydrocarbons in cigarette smoke to reactive intermediates that can initiate lung cancer. We hypothesized that coal dust (CD) exposure might modify pulmonary carcinogenesis

by altering pulmonary CYP1A1 induction. To test this hypothesis, we examined the ability of respirable CD particles (<5 microns) to inhibit pulmonary CYP1A1 induction. Male, Sprague-Dawley rats (220-270g) were intratracheally exposed to 0, 2.5, 10, 20, 40 mg coal dust/rat or vehicle (saline). After 11 days, rats were injected intraperitoneally (IP) with the CYP1A1 inducer β-naphthoflavone (BNF: 50mg/kg IP). Three days later, rats were sacrificed and CYP1A1 activity in the lungs was measured as 7-ethoxyresorufin-O-deethylase (EROD) activity. CYP1A1 protein was determined by Western blot using polyclonal rabbit anti-rat CYP1A1 antibodies. Pulmonary inflammation was assessed by determining bronchoalveolar lavage polymorphonuclear (PMN) cell counts, alveolar macrophage (AM) chemiluminescence (CL), and nitric oxide (NO)-dependent AM chemiluminescence. EROD activity was suppressed by CD exposure in a dose-dependent fashion (R2= 0.932, p=0.008). Western blot showed a significant reduction of CYP1A1 protein in rats treated with 40 mg CD and BNF when compared with rats treated with BNF alone ($p<0.05$). CD exposed rats had a dose-dependent increase of PMN (R2=0.974, p= 0.002). AM count was significantly higher in all rats exposed to CD and BNF compared with rats treated with BNF alone. NO-dependent CL was also significantly increased in rats treated with 40 mg CD and BNF compared to rats treated with BNF alone (p= 0.004). These results suggest that coal dust exposure inhibits induction of CYP1A1 activity by BNF and enhances pulmonary inflammation, in a dose-dependent manner.

1556 METABOLITE OF PNU-142721 FORMS SELECTIVE ADDUCT WITH CYS125 OF RAT BETA-GLOBIN.

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PNU-142721E ((-)-6-chloro-2-[(1-furo[2, 3-c]pyridin-5-ylethyl)-thio]-4- pyrimidinamine) is a potent inhibitor of HIV-1 reverse transcriptase. Single dose excretion studies of [14C]PNU-142721 in the Sprague-Dawley rat revealed unusually long lived radioactivity associated with whole blood. Seven days post-dose, whole blood radioactivity was approximately 3 uM eq., more than 70-fold greater than corresponding plasma levels. In order to investigate the nature of the observed binding, rats were treated with increasing doses (10 to 100 mg/kg) or multiple doses (5 days) of [14C]PNU-142721 and blood samples were analyzed for radioactivity distribution. Results showed drug residues strongly associated with red blood cells; >90% of circulating radioactivity at 72 h post-dose was associated with red blood cells. Moreover, erythrocyte associated radioactivity increased additively with increasing dose or multiple doses. Fractionation and chromatographic analysis of protein from lysed erythrocytes showed distinct association of radioactivity with beta-globin. ESI-MS analysis of beta-globin isolated from a rat that had been treated with [14C]PNU-142721 showed the presence of a protein with a mass of 15976.1 Da, roughly 128 Da larger than the major form of rat beta-globin, suggesting addition of the chloropyrimidinamine functionality of PNU-142721. Amino acid sequence analysis of peptides generated from a tryptic digestion of the modified protein suggested that Cys125 of rat beta-globin was the site of modification. Further analysis of the radiolabeled peptides by MALDI-MS confirmed the sequence analysis and showed the chloropyrimidinamine adduct on cysteine. Comparison of rat and human hemoglobin sequences suggests that this reaction will not occur in humans because human beta-globin lacks a Cys residue at this position. However, it is unknown whether reactivity with protein thiols is a general feature of PNU-142721 metabolism.

1557 EQUINE CATECHOL ESTROGEN 4-HYDROXYEQUILENIN IS A SUBSTRATE AND AN INHIBITOR OF CATECHOL-O-METHYLTRANSFERASE.

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Redox and/or electrophilic metabolites formed from estrogen metabolism may play a role in estrogen carcinogenesis. 4-Hydroxyequilenin (4-OHEN) is the major phase I catechol metabolite of the equine estrogens equilenin and equilin, which are components of the most widely prescribed estrogen replacement formulation, Premarin®. Previously, we have found that 4-OHEN rapidly autoxidizes to an *o*-quinone *in vitro* and causes toxic effects including inactivation of human detoxification enzymes. 4-OHEN has also been shown to be a substrate for catechol-*O*-methyltransferase (COMT) in human breast cancer cells. In the present study, we demonstrated that 4-OHEN was not only a substrate of recombinant human soluble COMT *in vitro* with a K_m of 2.2 μM and k_{cat} of 4.7 min^{-1}, but it also inhibited its own methylation by COMT at higher concentrations. In addition, 4-OHEN was found to be an irreversible inhibitor of COMT-catalyzed methylation of the endogenous catechol estrogen 4-hydroxyestradiol (4-OHE2). 4-OHEN *in vitro* not only caused the formation of intra- and inter-molecular disulfide bonds as demonstrated by gel electrophoresis, but matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) also showed that 4-OHEN alkylated multiple residues of COMT. Peptide mapping experiments further indicated that Cys32 in recombinant human soluble COMT was the residue most likely modified by 4-OHEN *in vitro*. These data may suggest that inhibition of COMT methylation by 4-OHEN might reduce endogenous catechol estrogen clearance *in vivo* and further enhance toxicity.

1558 INHIBITION OF BAX TRANSLOCATION AND NECROSIS WITH BCL-XL OVEREXPRESSION IN A WELL CHARACTERIZED CELL CULTURE MODEL FOR TETRAFLUOROETHYLCYSTEINE-INDUCED NEPHROTOXICITY.

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Tetrafluoroethylcysteine (TFEC)-induced nephrotoxicity is initiated by the covalent modification of a relatively small number of defined mitochondrial proteins (1). The murine TAMH cell line has been characterized and faithfully reproduces TFEC-mediated renal injury and necrotic cytotoxicity with dose- and time-dependent inhibitions of both mitochondrial aconitase and α-ketoglutarate dehydrogenase target proteins (2). An early cytosolic to mitochondrial translocation of BAX (a proapoptotic BCL-2 family member) was also observed in TAMH cultures dosed with toxicologically relevant concentrations of TFEC (e.g. 250 μM). BAX translocations were confirmed immunocytochemically using confocal microscopy and with complementary immunoblot techniques. Effective and significant cytoprotection to supra-toxicological concentrations of TFEC (≤600 μM) were evident using TAMH stable transfectants overexpressing the BAX heterodimerization partner BCL-xL (c.f. vector control transfectants or parental cell line). Furthermore, BCL-xL overexpression limited the extent of BAX translocation to mitochondria in agreement with the cytoprotection observed in BCL-xL transfectants. In conclusion, effective cytoprotection to TFEC-induced necrosis is evident with BCL-xL overexpression and this appears to correlate well with the prevention of BAX subcellular relocation to mitochondria. Further data will also be presented which confirm our previous and related microarray studies (3) regarding the genomic responses of TAMH cells to TFEC-mediated intramitochondrial damage. 1. Cooper et. al., Biochem. Pharmacology 64, 553-564 (2002). 2. James et. al., Biochem. 41, 6789-6797 (2002). 3. Z-H Hu et. al., Toxicologist 66, LB66 (2002). Supported by NIH grants GM51916 (SAB), GM25418 (SDN), CA74131 (NF), American Cancer Society RPG-00-222-01-CDD (DMH) and NIEHS Center Grant P30ES07033.

1559 THE PROTECTIVE EFFECT OF FLAVONOIDS AGAINST OXIDATIVE DAMAGE INDUCED BY OCHRATOXIN A IN PROXIMAL TUBULAR CELLS.

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Flavonoids are present in many food commodities and have gained increasing interest in relation to disease prevention because of their antioxidant activity. Hence, oxidative stress leads to a variety of patho-physiological events. Worldwide, the mycotoxin ochratoxin A is a frequently found contaminant in human food and animal feeds and has been detected among others in cereals, beans and coffee. In humans, long-term exposure to ochratoxin A has been linked to a chronic kidney disease, denoted as Balkan Endemic Nephropathy (BEN). Induction of oxidative damage is one of the mechanisms involved in the renal toxicity of ochratoxin A. In the present study, GERP and LLC-PK1 cells were used as a model for proximal tubule cells, the target cells of ochratoxin A, to study the antioxidant properties of selected flavonoids. Following exposure to ochratoxin A (100 μM for 24 hours), the reactive oxygen species (ROS) production was measured using the fluorescent probe 2′, 7′-dichlorodihydrofluorescein diacetate (H2DCF-DA). The ROS scavenging ability of the tested compounds (in a concentration range of 0.1-100 μM) could be ranked in the following order: eriodictyol> quercetin > esculetin >> ebselen / naringin. The results showed that eriodictyol, quercetin and esculetin have antioxidant properties, efficient in the protection against the oxidative stress induced by the mycotoxin ochratoxin A. Therefore, the protective antioxidant properties of these compounds should be investigated further with respect to their ability to reduce ochratoxin A pathologies *in vivo*.

1560 ELUCIDATION OF REACTIVE METABOLITES OF 4-IPOMEANOL BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS).

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4-Ipomeanol, [1-(3-furyl)-4-hydroxypentanone], is a natural cytotoxin and a promising chemotherapeutic agent bioactivated in target organs by P450 enzymes into electrophilic metabolites that conjugate with glutathione (GSH). However, the structures of its reactive metabolites and the pathway of bioactivation are unknown. We propose them here. Methods: a) *In vivo*: bile of rats dosed with a mixture of d_0-4-ipomeanol/$d_6$4-ipomeanol was collected and analyzed by LC-MS/MS in order to investigate the structure of its reactive metabolites. b) *In vitro*: LC-MS/MS analysis of hepatic microsomal samples of rats incubated with 4-ipomeanol and GSH. Results: we detected 4 metabolites of 4-ipomeanol conjugated with GSH in rat bile samples. Multiple reaction monitoring scans showed that these four conjugates lose neutral fragments of 129 Da, which is characteristic of glutathione conjugates, and have a mass of 492 Da $(MH)^+$. The tracer d_6-4-ipomeanol confirmed that these conjugates derived from 4-ipomeanol. The mass of the deuterated glutathione conjugates was 498 Da (M+7)+. Since deuterium was incorporated in the alkyl chain of 4-ipomeanol, and the glutathione conjugates retain all deuterium atoms, it indicates that metabolism takes place on the furan moiety. One glutathione conjugate derived from 4-ipomeanol was also observed in hepatic microsomal samples of rats. No conjugates were detected in control samples. Conclusions: supported by the LC-MS/MS data, we propose a pathway of 4-ipomeanol bioactivation. Three reactive intermediates of 4-ipomeanol are proposed, two epoxides and an α, β-unsaturated dialdehyde, which conjugate with GSH forming 6 possible conjugates, two of which re-arrange into more stable structures. The masses of these proposed glutathione conjugates of 4-ipomeanol match well with observed masses. These data facilitate a further understanding of the mechanism of 4-ipomeanol induced cytotoxycity.

1561 HYDROQUINONE AND CATECHOL METABOLITES OF BENZENE INCREASE ENDOTHELIAL IL8 PRODUCTION BY HUMAN BONE MARROW ENDOTHELIAL CELLS.

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Benzene and the antipsychotic agent remoxipride have each been associated with induction of aplastic anemia. Both compounds also generate catechol and hydroquinone metabolites, which induce pro-apoptotic effects in bone marrow cells. Gene array analysis of remoxipride-derived catechol in HL60 cells revealed a consistent and time-dependent increase in IL8 expression and several IL8-related genes. An increase in the levels of IL8 protein in media as indicated by ELISA was also observed in HL60 cells exposed to remoxipride-derived catechol. HL60 cells secrete the endothelial form of IL8 (eIL8), which differs from the monocytic form by 5 amino acids at the amino terminus and has been reported to have proapoptotic activity. We have used human bone marrow endothelial cells as a model system to determine whether eIL8 production by bone marrow endothelial cells is affected by exposure to catechol (CAT) and hydroquinone (HQ) metabolites of benzene. The human bone marrow endothelial cell line, HBMEC60, was treated with increasing concentrations of CAT (1-100 μM) or HQ (0.1-100 μM). IL8 in media was quantified 24, 48, and 72 h after initial treatments. Treatment with either HQ or CAT resulted in a dose-dependent increase in IL8 at 48 and 72h. Phenol (100 μM), a benzene metabolite which does not induce apoptosis in human bone marrow progenitor cells, failed to increase IL8 levels in media at any time point. Because the ELISA assay used to quantify IL8 did not distinguish between the two forms of IL8, gel electrophoresis was conducted to determine which form was produced by HBMEC60s. Endothelial IL8 was the only form of IL8 detected in HBMEC60 media. In conclusion, the benzene metabolites, HQ and CAT, but not phenol, increased production of eIL8 by HBMEC60s in a dose-dependent manner. This increase in eIL8 may have relevance for benzene-induced disruption of hematopoiesis (Supported by ES09554 and Astra Zeneca).

1562 HEMATOPOIETIC STEM CELLS AS TARGETS FOR TRANSPLACENTAL TOXICANTS.

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Human fetal liver hematopoietic stem cells (HSC) comprise a significant percentage of human fetal hepatic cell populations and are capable of initiating long term hematopoiesis. Because of their hematopoietic function, injury to these cells may have potential ramifications with regards to the etiology of hematopoietic malignancy. In the current study, we examined the sensitivity of HSC to 4-hydroxynonenal (4HNE) an extremely mutagenic aldehyde and a primary toxic metabolite of alcohol. 4HNE can be formed *in utero* as a result of maternal alcohol consumption or *via* transplacental transfer of pro-oxidant drugs and chemicals. Soluble fractions prepared from HSC rapidly metabolized 4HNE through glutathione *S*-transferases and aldehyde dehydrogenases, but did not exhibit significant reductive capacity for 4HNE through aldehyde reductase or alcohol dehydrogenase pathways. Culture of HSC in the presence of 5 and 50 uM 4HNE resulted in a significant loss of cell viability and the formation of one or more high molecular weight 4HNE-protein adduct(s). In contrast, incubation of HSC with aflatoxin B_1 (AFB_1), a transplacental pro-carcinogen that requires cytochrome P450-mediated activation to form the reactive AFB_1-8, 9-*exo*-epoxide (AFBO), did not affect HSC viability or AFBO-DNA binding. Western blotting studies further demonstrated that HSC expressed comparatively low levels of CYP3A4/5-reactive protein(s) and did not express CYP1A2-reactive protein, isozymes important in AFB_1 bioactivation. Collectively, our results suggest that inefficient enzymatic reduction of 4HNE may underlie a susceptibility of HSC to cell injury. However, HSC may not be a target of the procarcinogen AFB_1 at physiologcally relevent doses. Supported by NIH ES09427 and USEPA STAR R827441.

1563 EFFECTS OF DIALLYL SULFIDE ON THIOACETAMIDE-INDUCED HEPATOTOXICITY: A POSSIBLE ROLE OF CYTOCHROME P450 2E1.

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Effects of diallyl sulfide (DAS) on thioacetamide-induced hepatotoxicity were investigated in male ICR mice. When mice were treated subcutaneously with 100, 200 and 400 mg/kg of DAS in corn oil for three consecutive days, the activity of cytochrome P450 (P450) 2E1-selective p-nitrophenol hydroxylase was dose-dependently suppressed. In addition, the activities of P450 2B-selective benzyloxyresorufin O-debenzylase and pentoxyresorufin O-dependentylase were dose-dependently induced by the treatment with DAS. To investigate a possible role of metabolic activation by P450 enzymes in thioacetamide-induced hepatotoxicity, mice were pretreated with 400 mg/kg of DAS for 3 days, followed by a single intraperitoneal treatment with 100 and 200 mg/kg of thioacetamide in saline for 24 hr. The activities of serum alanine aminotransferase and aspartate aminotransferase greatly increased by thioacetamide were recovered in DAS-pretreaed animals. Taken together, our present results indicated that thioacetamide might be activated to its hepatotoxic metabolite(s) by P450 2E1, not by P450 2B, in male ICR mice. (Supported by a grant of the Korea Health 21 R&D Project, 01-PJ2-PG3-21605-0002, Ministry of Health & Welfare, Republic of Korea.)

1564 DICHLOROACETYLATED PROTEIN ADDUCTS IN LUNG AND LIVER OF MICE TREATED WITH TRICHLOROETHYLENE.

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Trichloroethylene (TCE) exposure elicits lung and liver toxicities. We have used an immunochemical approach to investigate formation of protein adducts in mice treated with TCE. We have used a polyclonal antibody that recognizes the dichloroacetylated proteins (DCA). In immunohistochemical studies, mice were treated with TCE (1000 mg/kg, i.p.) and sacrificed 4 h later. Lung and liver tissues were fixed with 4% paraformaldehyde. Tissues were stained using the anti-DCA antibody and the avidin-biotin complex procedure. Protein immunoblotting was performed with lung and liver microsomal proteins from mice treated with TCE (250, 500, 750 and 1000 mg/kg, i.p.). Proteins were separated and transferred to a nitrocellulose membrane. In time-course studies, mice were treated with TCE (1000 mg/kg, i.p.), and were sacrificed at 2 to 6 h after treatment. The immunohistochemical studies revealed staining for dichloroacetylated proteins that was highly localized in the bronchiolar epithelium of TCE-treated mice. Staining in the liver was localized in centrilobular hepatocytes. Protein blots prepared with the anti-DCA antibody showed a major protein band with a molecular weight of 51 kDa. Immunoreactivity of this band was dose-dependent in both lung and liver. Time-course studies showed that immunoreactivity was maximal at 4 to 6 h for lung and liver microsomal proteins. When the nitrocellulose membrane reacted previously with the anti-DCA antibody was stripped and re-probed with a CYP2E1 antibody, the same 51 kDa band immunoreactive for dichloroacetylated proteins was also immunoreactive for CYP2E1. However, CYP2E1 immunoreactivity in lung microsomal proteins from TCE-treated mice was decreased, compared with that in untreated mice. This TCE-induced effect was not as marked in blots prepared with liver microsomal proteins. These findings supported the proposal that TCE metabolism results in the formation of reactive metabolites that bind to proteins of which the CYP2E1 enzyme may be a major target. Supported by EPA.

1565 BIOTRANSFORMATION OF ZAFIRLUKAST BY CYTOCHROME P450 3A4.

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Zafirlukast, (4, 5-cyclopentoxycarbonylamino-3-[(2-methoxy-4, 2-methylphenylsolfonylaminocarbonyl)phenylmethyl]-1-methylindole), is a leukotriene D4 receptor antagonist that is utilized clinically for the management of mild to moderate asthma. The drug is primarily metabolized by cytochrome P450 2C9, and was recently demonstrated to inhibit the cytochromes P450 2C9, 2C19, 3A4, and 1A2. Therefore, its clinical use requires drug monitoring due to potential interaction, particularly with the 3A4 substrate theophylline. Zafirlukast has a structural nucleus that is similar to the pneumotoxin 3-methylindole, which is bioactivated by P450 enzymes to a reactive, electrophilic methylene imine intermediate, by dehydrogenation. Kassahun et al proposed that zafirlukast is susceptible to bioactivation *via* a similar P450-mediated dehydrogenation pathway, forming an electrophilic iminium intermediate. The biotransformation of zafirlukast by recombinant cytochrome P450 3A4 has been evaluated in our laboratory. As confirmed by LC/MS, 3A4 catalyzed the formation of an electrophilic intermediate that was trapped with glutathione. The adduct was detected as partially separable diastereomers, which is consistent with glutathione addition at the methylene position of the indole nucleus, generating a chiral center at this position. Three additional hydroxylated metabolites were also formed. One of these metabolites may be a carbinol that forms at the methylene position of the indole, by hydroxyl rebound following initial hydrogen atom abstraction by the P450 enzyme. This hydroxylation reaction could compete with the dehydrogenation pathway. Participation of the proposed electrophilic iminium species as the ultimate inhibitor of cytochrome P450 3A4 is currently under investigation. Zafirlukast may therefore be a novel mechanism-based inhibitor of certain cytochrome P450 enzymes. (Supported by HL16345, Nation Heart, Lung and Blood Institute, NIH.)

1566 DETERMINATION OF TRACE LEVELS OF DICHLOROACETIC ACID (DCA) IN RAT LIVER BY LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY (LC/MS).

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DCA is a chlorination disinfection byproduct commonly found in drinking water. In addition, DCA is a metabolite of trichloroethylene (TCE), a metal degreaser that is also present in many drinking water sources. Results of studies of DCA in rodents suggest that exposure to even small quantities of the chemical in drinking water may be associated with increased cancer risks to humans. Methods currently utilized for the analysis of DCA require a derivitizing reagent that results in an artificially high level of DCA in samples, due to conversion of trichloroacetic acid (TCA) to DCA. The method developed in this study involves no derivitizing reagents, yet still achieves trace-level determination of DCA. Liver samples for this study were obtained from male Sprague-Dawley rats. Homogenized liver samples were spiked with appropriate amounts of DCA to yield 200-uL samples with concentrations of 0, 1, 5, 10, 50, 100, 500, 1000, 5000, and 10000 ng/mL DCA. Protein precipitation was accomplished by adding 400 uL of ice-cold acetonitrile to each sample. The supernatant was removed and diluted to a total volume of 1 mL with 60:40 acetonitrile:water. Solid phase extraction was then performed on each sample. The sample eluate was dried under nitrogen and reconstituted in 100 uL of 60:40 acetonitrile:water prior to LC/MS analysis. Good linearity was observed for samples across the entire concentration range, as a R^2 of .99 was achieved. The limit of detection was found to be 1 ng/mL (ppb). With this new method, it should be possible to better define TCE metabolic pathways leading to DCA and to quantify yields of DCA from TCE in different species. (Supported by SERDP Grant CU1073DOE and DOE Cooperative Agreement DE-FC02-02CH11109.)

1567 CHLOROETHANE METABOLISM COMPARED WITH TOXIC THRESHOLD ACTIVITIES.

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High chloroethane (15, 000 ppm CE) causes the highest incidence (86%) of any carcinogen in the NTP database producing an uncommon B6C3F1 mouse tumor type, endometrial carcinomas, which aggressively metastasize to 15 organs killing the ♀. F344 rats do not respond with carcinogenesis. The lowest CE noncancer effect is a low incidence of foramina in BF-1 mouse skulls at 5, 000 ppm, but none below. Other tox studies show no significant pathology < 5, 000 ppm. This analysis examines the metabolic bases for this apparent threshold of CE toxic activity. CE is oxidized by microsomal CyIIE1 and is conjugated by cytosolic GSH/GST (glutathione transferase). Oxidation proceeds *via* CypIIE1 by Michaelis-Menton elimination becoming saturated at 469 ppm CE. GSH (liver ≈5mM) effectively conjugates CE linearly up to about 5, 000 ppm CE. Above this, all rodent GSH organ levels significantly decrease, a metabolic state that likely does not maintain adequate electrophile xenobiotic protection. (1) B6C3F1 mice possess only about 1/3–1/2 of F344 rat GST levels, and (2) CE-caused GSH decreases are typically greater in the mouse than the rat. One exposure cycle shows GSH decreasing during 6 hrs CE exposure, recovery within 4 hrs, and over-compensation in some organs providing higher-than-normal levels at the end of the 18 hr resting portion just prior to the next exposure. (3) These low-to-high oscillations of GSH pools seem to occur most in murine liver, lung, and uterus—the cancer target organ. These 3 observations suggest the mouse is more at risk than the rat: this is consistent with observed toxicity. GSH oscillations week-after-week would place chronic metabolic stress on those organ systems most affected and could relate to the bioassay exposures causing foramina at 5, 000 ppm and overt uterine cancer at a persistent 15, 000 ppm CE. Notably CE toxic occurrences only take place at, or above, the CE exposures where oxidation and conjugation are both saturated. Persistent CE exposures < 469 ppm seem to produce steady-state metabolism. ∴ Low environmental CE exposures may not cause significant toxicity including chemical carcinogenesis especially since CE has low genotoxicity.

1568 HEXACHLOROBENZENE INCREASES UROPORPHYRIA IN MICE WITHOUT INCREASING CYP1A2.

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Previous work has demonstrated that hepatic uroporphyrin (URO) accumulation caused by halogenated aromatic compounds such as TCDD is prevented in CYP1A2 knockout mice of the C57BL/6 strain. This includes the uroporphyria caused by hexachlorobenzene (HCB) which, unlike TCDD, causes only a small increase in CYP1A2. Constantin et al. have reported that treatment of iron-loaded SWR mice with HCB and 5-aminolevulinate (the porphyrin precursor) produces a rapid and large accumulation of hepatic URO [Biochem Pharmacology 52, 1407-13, 1996]. SWR mice express a low affinity receptor for halogenated aromatic compounds (Ahrd) compared to the high affinity receptor (Ahrb1) in C57BL/6 mice. We have reproduced this unexpected uroporphyria in SWR mice with the additional finding that no increases in hepatic CYP1A2 were found in HCB-treated mice. Similar results were obtained with identical treatment of mice of 129 [both (S1 and S6 sublines)] background which also carry the low affinity Ahrd receptor. In the 129S6 subline, the effect of administered iron could be replaced by using mice carrying the Hfe knockout. CYP1A2 was not increased by HCB in these 129 mice. As reported previously for CYP1A2 knockout C57BL/6 mice, uroporphyria was not produced in 129 S1 mice with the CYP1A2 knockout treated with HCB, iron and ALA. These results indicate that although some level of CYP1A2 expression is required for the induction of uroporphyria, the role of HCB in producing uroporphyria includes effects other than increasing CYP1A2. This work was supported by funds from the Department of Veterans Affairs and by grants from the National Institutes of Health ES06263 (PRS) and AG14731 (GG).

1569 INTERACTION BETWEEN A BROMINATED FLAME-RETARDANT (PBBE 99) AND AN *ORTHO*-SUBSTITUTED PCB (PCB 52) ENHANCES DEVELOPMENTAL NEUROTOXIC EFFECTS.

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Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a great variety of electrical appliances, and textiles. PBDEs are persistent compounds that appear to have an environmental dispersion similar to that of PCBs and DDT. While there is a decrease for PCBs and DDT the PBDEs have been found to increase in the environment and in human mother's milk. In several reports we have shown that low-dose exposure of environmental toxic agents such as PCB, DDT, during the brain growth spurt (BGS), when the maturational processes of the developing brain are at a stage of critical vulnerability in neonatal mice, can lead to disruption of the adult brain function. Recently we have reported that different PBDEs, such as PBDE 47, PBDE 99, PBDE 153 and PBDE 209 can cause developmental neurotoxic effects when given to mice during this BGS. The neurobehavioural defects were also seen to worsen with age. In the present study a combined exposure to both PBDE 99 and PCB 52 was investigated. Ten-day-old NMRI male mice were given one single

oral dose of either PCB 52 1.4 μmol/kg bw + PBDE 99 1.4 μmol/kg bw, PCB 52 1.4 μmol/kg bw, PCB 52 14 μmol/kg bw, PBDE 99 1.4 μmol/kg bw, or PBDE 99 14 μmol/kg bw. Controls received a vehicle (20% fat emulsion). Spontaneous behaviour was studied in 4-month-old and 6-month-old mice. Animals exposed to the combined dose of PCB 52 (1.4 μmol/kg bw) + PBDE 99 (1.4 μmol/kg bw), PCB 52 (14 μmol/kg bw) and PBDE 99 (14 μmol/kg bw), showed significantly impaired spontaneous motor behaviour at the age of 4- and 6-months. In animals exposed to the combined dose of PCB 52 + PBDE 99, and the high dose of PCB 52, the defects worsen with age as the habituation capability was significantly worse in 6-month-old compared to 4-month-old. The present study shows that PBDEs and PCBs can interact and enhance developmental neurotoxic effects when the exposure occurs during a critical stage of neonatal brain development.

1570 COMPARISON OF PCB SPECIFIC CONGENER PROFILES IN SKIN AND EAR OF MICE EXPOSED TO AN ENVIRONMENTAL PCB/PCDF MIXTURE.

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PCBs are ubiquitous environmental contaminants and bioaccumulate in a congener- specific manner in several tissues. Composition of PCBs is important for assessing exposure source and possible health effects. Our objective was to determine and compare the PCB specific congener profiles in surface-cleaned skin and ears of mice exposed to an environmental PCB/PCDF mixture. Ear notches (for identification) had been previously saved and analyzed as a possible biopsy medium. Two groups of hairless mice were placed on either PCB/PCDF contaminated soil collected from an old electric landfill in Illinois or on control soil for 4 weeks. Mice were weighed weekly. Skin and ear were washed with alcohol and collected immediately after the termination of exposure. PCB specific congener profiles were determined using a 6890N gas chromatography system equipped with DB-5 60M capillary column and a μ-electron capture detector (μ-ECD). Mice exposed to the chlorinated aromatic contaminated soil were significantly ($p<0.05$) heavier and contained significantly ($p<0.001$) higher total PCB residues than those exposed to control soil. PCB congener profiles of the skin and ear samples were similar, reflecting the profile of the soil and enhanced accumulation of the more persistent congeners. However, total PCBs in the skin and ear samples differed in that the ear showed significantly higher total PCBs ($p<0.05$) than in the skin. Skin, including the ear, is an important reservoir for PCBs and may serve as a useful biopsy medium.

1571 EXPRESSION OF CYCLOOXYGENASE-2 IN HL-60 CELLS EXPOSED TO POLYCHLORINATED BIPHENYLS.

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Polychlorinated biphenyls (PCBs) are persistent environmental contaminants that affect a number of cellular systems including neutrophils. PCBs can be divided into two classes based on the chemical structure. Non-ortho-substituted, coplanar congeners act through the aryl hydrocarbon (Ah) receptor, whereas ortho-substituted, noncoplanar congeners act through different and poorly understood mechanisms. It is these latter, noncoplanar PCBs that alter neutrophil function. The objective of these experiments was to explore further the mechanisms by which PCBs affect neutrophil function. The human promyelocytic leukemia (HL-60) cell line was differentiated with DMSO to a neutrophil-like phenotype. Treatment of differentiated HL-60 cells with 2, 2′, 4, 4′-tetrachlorobiphenyl, a noncoplanar, ortho-substituted PCB congener, caused an increase in f-Met-Leu-Phe-induced degranulation, as measured by release of myeloperoxidase (MPO). Treatment with the coplanar, non-ortho-substituted congener 3, 3′, 4, 4′, 5-pentachlorobiphenyl, had no effect on MPO release. The effect of PCBs on mRNA levels of cyclooxygenase-2 (COX-2) was examined using semiquantitative RT-PCR. COX-2 mRNA was significantly elevated in response to 2, 2′, 4, 4′-tetrachlorobiphenyl in a dose-dependent manner. COX-2 expression was maximized by 30 minutes of exposure to 2, 2′, 4, 4′-tetrachlorobiphenyl. 3, 3′, 4, 4′, 5-pentachlorobiphenyl did not increase COX-2 mRNA levels. These results demonstrate that noncoplanar, ortho-substituted PCBs alter the functional status of granulocytic HL-60 cells, causing enhanced degranulation and upregulation of COX-2, whereas coplanar, non-ortho-substituted PCBs lack this activity. These data suggest that ortho-substituted, noncoplanar PCBs alter HL-60 cell function and COX-2 expression *via* an Ah-receptor-independent mechanism. (Supported by ESO4911.)

1572 CO-ELEVATION OF HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY WITH UROPORPHYRIN CONCENTRATION IN PORPHYRIC MICE.

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Mice heterozygous for a null mutation at the uroporphyrinogen decarboxylase locus (Uro-D+/-) fed a normal diet develop modest uroporphyria with polychlorinated biphenyl (PCB) exposure. Wild type (Uro-D+/+) mice treated with PCBs did not develop uroporphyria. The glutathione S-transferase (GST) activity in PCB-treated Uro-D+/- animals is double that of similarly treated Uro-D+/+ animals. The uroporphyria elicited with PCBs in Uro-D+/- animals is exacerbated by a single injection of iron-dextran and attenuated by maintenance since weaning on an iron deficient diet. With either iron-modifying treatment, the change in GST activity paralleled the change in uroporphyrin concentration. Uroporphyria can also be produced in Uro-D+/- but not Uro-D+/+ animals by continuous delta-aminolevulinic acid supplementation of their drinking water. This treatment elevated GST activity only in Uro-D+/- animals. High GST activity accompanying the uroporphyria elicited by delta-aminolevulinic acid treatment indicates that the association is independent of GST-inducing effects of PCBs. Thus over multiple treatment regimens producing uroporphyria, there is a tight association between the hepatic GST activity and uroporphyrin concentration, suggesting that uroporphyrin might be an endogenous inducer of GSTs.

1573 PCB-INDUCED INHIBITION OF MONOAMINE TRANSPORTERS PREDICTS RAT SYNAPTOSOMAL TISSUE AND MEDIA DOPAMINE AND DOPAC CONCENTRATIONS.

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Polychlorinated biphenyls (PCBs) may alter behavior *via* changes in central dopamine (DA) function. The DA transporter (DAT) and vesicular monoamine transporter (VMAT), responsible for the uptake of extracellular DA or packaging of cytosolic DA into synaptic vesicles, respectively, aid in regulation of neurotransmitter trafficking. Although PCBs inhibit DA uptake *via* the DAT and VMAT, the consequences on DA function are not known. To address this question, we exposed purified adult rat striatal synaptosomes to PCB congeners, measured tissue and media DA and dihydroxyphenylacetic acid (DOPAC, the main intracellular metabolite of DA) concentrations and determined the relationship between these changes and DAT or VMAT inhibition. The ability of specific congeners to inhibit DAT activity correlated positively with PCB-induced reductions in tissue DA content and elevations in media DA concentrations. Structurally, the most active congeners were ortho-chlorine substituted, while coplanar congeners showed no activity. Congeners with the lowest EC50 values for VMAT inhibition elicited the greatest elevations in tissue plus media DOPAC, a recognized measure of non-vesicularly stored DA. Thus, VMAT inhibition was positively correlated with DOPAC formation, and by inference, elevations in free cytosolic DA. These data demonstrate that PCB-mediated monoamine transporter inhibition influences both media and tissue DA and DOPAC concentrations. Such alterations in media and tissue DA could potentially lead to impairment of signal transmission and/or terminal damage *via* the neurotoxic effects of free DA. Together these observations enhance the importance of DA transporters in the effects of exposure to PCBs. Supported by NIH Grant 1034004 to RFS.

1574 A DIORTHOCHLORINATED PCB CONGENER 2, 2', 4, 4'-TETRACHLOROBIPHENYL (TCB) ACTIVATES EXTRACELLULAR SIGNAL REGULATED KINASES (ERKS) INDEPENDENT OF PROTEIN KINASE C (PKC) IN JB6 MOUSE EPIDERMAL CELLS.

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Non-coplanar orhto cholorine substituted polychlorinated biphenyl (PCB) congeners may contribute significantly to the toxic effects of environmental PCB mixtures. The mechanisms of the toxic effects of the non-coplanar PCB congeners remain unclear. In this study we examined the cellular interactions of 2, 2'4, 4'-TCB on the ERKs, a sub-family of the mitogen activated protein kinases that play a central role in cell signaling, in a tumor promoter sensitive mouse epidermal cell line JB6 (JB6P+). Treatment of JB6P+ cultures for one hour with 2, 2', 4, 4'-TCB (0.1 - 20.0 μM) caused a dose dependent activation of ERK 1 and ERK 2 as assessed by changes in the phsophorylation state of these proteins. At 20.0 uM concentration the TCB caused a 10-fold increase in the phoshporylation state of these proteins. The onset of ERK activation by the TCB was seen only at 15 min post treatment

relative to the rapid (2 min) effect of the mitogenic growth factor TGF-αalpha. TCB induced ERK activation sustained for at least 24 hrs. Pre-treatment of the the cultures with University-0126 (10.0 μM) an inhibitor of the upstream kinase MEK completely inhibited ERK activation by the TCB. University-0126 also inhibited ER activation induced by TPA a potent activator of PKC. However, pre-treatment of JB6P+ cultures with bisindoloylmaleimide (5-0 μM) abrogated ERK activation by TPA but failed to inhibit that induced by the TCB. Gel-shift analysis showed that TCB induced the activation of the AP-1 transcription factor that independent of PKC but was dependent on ERK. These data suggest that diortho chlorine-substuted PCBs activate ERK associated cell signaling cascade independent of PKC. Such an activation of the ERKs may lead to the activation of transcription factors such as AP-1 to alter gene expression (supported by NIH grant ES-04911 and a Philip Morris Research grant).

1575 EFFECTS OF COMPLEX PAH MIXTURES AND 7H-BENZO(c)FLUORENE ON DNA ADDUCT FORMATION IN MICE.

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Previous research has identified *7H*-benzo[*c*]fluorene (BCF) as a potent adductor of lung DNA (Carcinogenesis 21:1601-9). The goal of the present study was to determine the contribution of BCF to the formation of lung and skin DNA adducts following acute dermal exposure to analytical grade BCF and coal tar fractions. Coal tar was separated into 7 fractions (F1-F7). Polycyclic aromatic hydrocarbons (PAHs) including BCF were quantified using gas chromatography/mass spectrometry (GC/MS). An aliquot of the F2 fraction was further separated into a "BCF fraction" and an "F2-BCF fraction". The F2 fraction contained 7, 395 ppm BCF and 40, 122 ppm carcinogenic PAHs (cPAHs, or the sum of all class B2 carcinogens based on the USEPA classification). The BCF fraction contained 35, 000 ppm BCF and 215, 609 ppm cPAHs, while the F2-BCF fraction contained 3, 900 and 45, 216 ppm respectively. The three fractions and analytical grade BCF were applied dermally to female CD-1 mice. After 24 hr the mice were sacrificed and tissues harvested. DNA adduct levels were analyzed by nuclease P1-enhanced ^{32}P-postlabeling, and following subtraction of solvent control values, were quantified as RAL x 10^9 values. In skin DNA of mice dosed with 0.48 or 3.0 mg/mouse, F2 induced total adduct levels of 266.62 and 823.04 respectively, F2-BCF induced 126.41 and 331.37, and the BCF fraction yielded 424.26 and 1062.12 respectively. The lung DNA of mice dosed with 0.48 or 3.0 mg/mouse yielded the following total adduct levels: F2, 26.47 and 79.76 respectively; F2-BCF, 17.77 and 137.55 respectively; and the BCF fraction, 22.78 and 97.48. Mice that were dosed with 100 ug/mouse of analytical grade BCF, equivalent to the dose of BCF present in 3.0 mg of the BCF fraction, showed total adduct levels of 4.22 in skin DNA and 8.95 in lung DNA. At the site of application, DNA adduct levels were appreciably greater than were observed in the lungs. In addition, the removal of the BCF isolates did not reduce the level of adducts induced by F2 in the lungs. The results indicate that pharmacokinetics play an important role in DNA adduct formation.

1576 PAH REGUALTION OF CYP1 GENE IN ZR-75-1 HUMAN BREAST CANCER CELLS.

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Recent industrial society has human widely exposed to PAHs that are comming from the incomplete combustion of organic material as widerspread environmetal contaminants. Biological activities of PAHs are not known although PAHs are considered as carcinogens. The mechanism of action of PAHs has been studied extensively, however it is not clear how PAHs turn on CYP1A1 in human breast cancer. Our labolatory have been studied the effect of PAHs in the human breast cancer cell MCF-7. In this study, we examined the ZR-75-1 human breast cancer cells as a new system to evaluate bioactivity of PAHs and to compare the PAH action with that of MCF-7 cells. ZR-75-1 human breast cancer cell line is response to estrogen and progesteron. We have been able to estabilish long term culture system of this cells then used for the study to the effect of 13 different PAHs and environmental samples. We demonstrate that PAHs induced the CYP1A1 promoter and 7-ethoxyresolufin O-deethylase(EROD) activity in a concentration-dependant manner. RT-PCR analysis indicated that PAHs significantly up-regulate the level of CYP1A1 mRNA. Some of PAHs showed stronger stimulatory effect on CYP1 gene expression than TCDD. Apparently, ZR-75-1 cells have Aryl hydrocarbon receptors, therefore it would be good experimental tool to study the cross-talk between PAHs and steroid actions. [Supported by grant from the Ministry of Environment of Korea]

1577 BINDING OF PAHS AND THEIR METABOLITES TO THE ESTROGEN RECEPTOR: A COMPARISON OF THREE.

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The goal of this study was to determine the extent to which 14 polycyclic aromatic hydrocarbons (PAHs) or their metabolites bind the estrogen receptor (ER). We examined 14 PAHs frequently detected in environmental samples. The ability of PAHs and/or their metabolites to bind the ER was tested in three assays: 1) a competitive binding assay using human recombinant ER (hrER) and [^{3}H]17β-estradiol ([^{3}H]E_2), 2) a whole-cell estrogen receptor binding assay using the ER-positive breast cancer cell line, MCF-7, and [^{3}H]E_2, and 3) a dual luciferase reporter assay using transiently transfected MCF-7 cell cultures. None of the PAHs significantly displaced [^{3}H]E_2 in the hrER competitive binding assay. In contrast, 8 of the 14 PAHs displaced [^{3}H]E_2 in the whole-cell ER binding assay when the assay was conducted at temperatures permissive of metabolism. These results suggested that PAHs were metabolized in MCF-7 cell cultures to compounds that bound the ER. To further test this hypothesis, PAHs were incubated in microsomes prepared from either 1) human livers, (Xenotech Ltd.,) or MCF-7 cell cultures. However, none of the PAH-microsomal reaction mixtures significantly displaced [^{3}H]E_2 in the hrER competitive binding assay, suggesting that the hrER competitive binding assay was not sensitive enough to detect the binding of the low concentrations of metabolites in the microsomal reaction mixtures. The dual luciferase reporter assay is similar to the whole-cell ER binding assay in that metabolism of PAHs may occur during the incubation period, and the PAHs did result in a significant induction of luciferase in this assay. The data from this study support the conclusion that some PAHs are metabolized in breast cancer cell cultures to compounds that bind the ER. (Supported by grants K02 ES11726 and R01 ES09795 from NIEHS)

1578 ARYL HYDROCARBON HYDROXYLASE (AHH) ACTIVITY AND BENZO(a)PYRENE (BaP) METABOLITE CONCENTRATIONS IN F-344 RATS SUBCHRONICALLY EXPOSED TO INHALED BaP.

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This study was conducted to measure the AHH activity and metabolism of BaP subsequent to subchronic exposure by inhalation. Fisher-344 male rats were exposed 4 hours a day, for 60 days (via nose-only inhalation) to aerosol exposure concentrations of 75 μg/m^3 of BaP adsorbed onto carbon black particles using a state-of-the-art model aerosol generation system. Control animals were either exposed to carbon black or unexposed controls. After exposure, rats were sacrificed, liver, lung, kidney, and testis were removed, and microsomes were isolated. Aryl hydrocarbon hydroxylase activities were determined by reverse-phase HPLC coupled with fluorescence detection using 3-hydroxy BaP as the standard. Tissue samples from the above mentioned organs were also analyzed for BaP and metabolites by HPLC. Our results demonstrate that liver possess a higher AHH activity than lung, testis, and kidney tissues. While lung ranked next to liver, there is no significant difference between testis, and kidney with regard to AHH activity measured. Among the metabolites detected, BaP 4, 5-7, 8-, 9, 10-dihydrodiols, 3(OH) BaP, and 9(OH) BaP were predominant. Interestingly, the most reactive metabolite of BaP, the 7, 8-diol-9, 10-epoxide (BPDE) concentrations were high in testis, compared to liver, lung, and kidney. The presence of AHH activity in testis, coupled with the detection of high concentrations of BPDE suggests the likelihood that long-term exposure to inhaled BaP results in a) elevated levels of AHH activity in target organs, b) contribute to the formation of toxic, reactive metabolites in these organs, and c) impairment of reproductive potential as exemplified by a decrease in progressive motility of stored sperm, and plasma testosterone concentrations of rats in studies conducted earlier in our laboratories (supported by MHPF/ATSDR grant #U50/ATU398948, NIGMS-SCORE grant #2SO6GMO8037-28, and NCRR-RCMI grant #G12RRO3032).

1579 CHARACTERIZATION OF GENETIC CHANGES ASSOCIATED WITH BENZO(a)PYRENE IN NORMAL HUMAN EPIDERMAL KERATINOCYTES: APPLICATION OF MICROARRAY TECHNOLOGY.

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We are interested in developing risk assessment strategies for environmentally relevant chemical mixtures. To this end, normal human epidermal keratinocytes (NHEK) were chosen as an *in vitro* model for measuring potentially carcinogenic

effects of petroleum-based hydrocarbons. Our initial studies demonstrated that benzo[a]pyrene (BaP) induces substantial alterations in NHEK in culture. Similar to the known skin carcinogen arsenic, BaP acts to inhibit differentiation, as measured by cornified envelope formation. BaP also increased the rate of proliferation in a dose-dependent manner in treated cells. Current studies utilizing microarray analysis are focused on identifying molecular markers altered during BaP treatment that may be involved in these effects. Preliminary results using the Clontech Human 3.8 II array are as follows: 1) in total, 34 genes were induced and 2 genes were suppressed by >2-fold in NHEK treated with 2μM BaP, as compared to control; 2) among genes overexpressed in BaP-exposed cells were carbonyl reductase 3 and flavin containing monooxygenase 1 (metabolizing enzymes), α integrin binding protein 63 (involved in suppressed differentiation), and IL-1α and Ras (both involved in proliferation), and; 3) the genes for cytochrome P450 XXIA and SEC14 were down regulated. These results will be confirmed using Real Time-PCR. Mechanistic studies with a subset of genes may allow us to correlate alterations in these molecular markers with the transformation process in NHEK, and to develop predictive capabilities for risk assessment of petroleum-based mixtures. Also, comparison of gene expression patterns induced by BaP with those seen in arsenic-treated keratinocytes may give clues as to the mechanisms whereby these two chemicals suppress differentiation. This research was supported by NIEHS Grant # RO1 ES09655 & Minority Supplement # RO1 ES09655-01S1.

1580 ESTIMATION OF CANCER POTENCY FOR TAMOXIFEN BASED ON STUDIES IN ADULT RATS AND NEONATAL RATS AND MICE.

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Tamoxifen is the drug of choice to delay the recurrence of breast cancer and is used as a prophylactic in high-risk women to prevent the development of this disease. Epidemiology studies have demonstrated a two- to seven-fold increased risk of developing endometrial cancer in women treated with tamoxifen. In long-term (104 weeks) studies in mature male and female rats, tamoxifen induced hepatocellular carcinoma. In short-term exposure studies in rats (2-5 days) and mice (1-5 days) observed for 152 and 67 weeks, respectively, tamoxifen induced uterine adenocarcinoma. Tumor response was essentially similar (20 percent) in neonatal rats and mice on an equivalent mg/kg-day basis. The mouse dataset is comprised of multiple tamoxifen treatment groups, while the rat dataset consists of only one treatment group. Cancer potency (slope of the dose response curve) for neonatal exposure was compared to that for adult exposure. To calculate neonatal potency, dose administered to neonatal mice on days 1-5 of life was averaged over the first 21 days of life, a sensitive period for estrogenic effects in these animals. The potency derivations take into account the less-than-lifetime study duration of the neonatal studies and body size differences between humans and experimental animals. The human cancer potency estimate based on uterine adenocarcinoma in the neonatal female mouse was 28 (mg/kg-day)-1, whereas the potency estimates based on liver tumors in adult male and female rats were 0.24 (mg/kg-day)-1 and 0.23 (mg/kg-day)-1, respectively. The greater than 100-fold difference in the cancer potency derived from neonatal exposure in mice as compared to adult exposure in rats indicates that the neonatal period is a critical window of susceptibility to the carcinogenic effects of tamoxifen. Given concern over increasing incidence of endocrine related cancer, research is needed to determine if other estrogenic compounds are more potent early in life.

1581 CROSS FOSTERING OF MINIPIGS FOR JUVENILE TOXICOLOGY STUDIES: RESULTS OF A VALIDATION STUDY.

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Most pre-clinical safety evaluation of human pharmaceuticals is performed in adult or young adult animals and therefore many drugs are not licenced for use in human paediatric populations. In order to gain regulatory approval for the use of products in pre-adult humans, it is required that appropriate toxicity studies are undertaken in juvenile animals. For non-rodent species, this presents a number of practical and logistical challenges. Examples include smaller litter sizes in comparison with rodents, long gestation periods, long pre-weaning periods, space requirements and cost. There are in effect, three possible basic approaches to study design. One is to use the whole litter as the basic group or experimental unit. Another is to all treatments represented within each litter. Thirdly, one can randomise the allocation of offspring to mothers at birth (cross-fostering) and to treat all animals allocated to one mother as a group. All three approaches have advantages and disadvantages, but the cross-fostering approach has the most potential benefits. We have performed an experiment to evaluate the possibilities of cross-fostering minipigs within our laboratory with a view to offering this study design as a service to our customers. Randomised cross-fostering of piglets was performed on Day 1 after birth, and various standard evaluations were performed during the first 4 weeks. During Week 2, the piglets were subjected to electrocardiographic and ophthalmological examination. In Weeks 1 and 4, blood and urine were collected for routine haematology, clinical chemistry and urinalysis examinations. After Day 29, the piglets were killed. Full autopsy and histopathology were performed. Experimental data obtained were compared with background data from older minipigs. It is concluded that cross fostering of minipigs for performance of juvenile toxicology studies is possible. For the researcher, there needs to be an understanding that there will be differences in expected values for some standard parameters in young animals compared to older ones.

1582 FLOW CYTOMETRY IN REPRODUCTIVE TOXICOLOGICAL STUDIES : IMMUNE SYSTEM ASSESSMENT.

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The Cynomolgus monkey (Macaca fascicularis) has been increasingly utilized in pre-clinical safety evaluation of investigational new drugs (INDs). Recently the FDA has recommended immune system testing to evaluate possible immunotoxicity. Many new therapeutic strategies utilize proteins (Biologics), which either as part of a designed approach or as a side effect result in changes to immunologic capabilities. These changes may be described utilizing flow cytometry to examine lymphocyte populations. As a tool during reproduction studies, flow cytometry allows the determination of changes evoked in the fetus as determined in cord blood (segment II studies) or from neonatal samples (segment III studies). The neonate can also be followed through early development to determine any effects on immunocompetence. Current background studies conducted at SNBLUSA, utilizing cynomolgus monkeys, demonstrate differences among blood mononuclear subsets in normal mothers as compared to the fetus at 100 days and full term healthy neonates. Cord blood as well as peripheral blood samples from mothers and infants were immunostained with antibodies against T cells (CD3), T helper lymphocytes (CD4), T cytotoxic lymphocytes (CD8), Leucocytes (CD45) and nucleated red blood cells (CD71). The data Includes comparisons of: N=5, mother and fetal comparison, N= 5, mother neonate comparison and N=5, juveniles at the ages of 3 and 6 months. The data to date demonstrates that the level of all markers except CD71 were lower in the fetus than in the neonate. In the neonate CD3 and CD4 were higher than in their mothers by 30% and 80% respectively. CD71 binding is 6 fold higher than the mothers at 100 days gestation. CD45 leucocytes tend to remain constant at day 100 of gestation through birth. Lymphocyte profiles, on the juveniles (N=5), are being collected and will be compared to the 100 day and neonate profiles. Further characterization of immune cells at various stages of development will improve the assessment of immunotoxicity and the effect during reproduction on the immunocompetence of offspring.

1583 AN ASSESSMENT OF THE WEANLING BEAGLE DOG AS A MODEL FOR SCREENING PHARMACEUTICALS INTENDED FOR INTRAVENOUS INFUSION TO PEDIATRIC POPULATIONS.

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Six weanling dogs were anesthetized and using aseptic techniques, surgically implanted *via* the femoral vein with a medical-grade silicone-based catheter at 63 days of age. These animals were then infused with physiological saline at rates of 1.5 and 5.0 mL/kg/h (i.e. rates considered well tolerated in adult beagle dogs) *via* an in-line 0.2 mm filter, for 24 hours/day for up to 13 weeks. Animals remained in good clinical condition, exhibited normal neurological behavior and electrocardiograms and demonstrated normal growth (including bone growth as measured by DXA and pQCT techniques) throughout the experiment. Dosimetry data confirmed animals received within 10% of the nominal dose volume at each infusion rate over the course of the investigation. The general lack of, or minimal, local tissue damage or inflammatory reaction at the infusion site suggests that the infusion interval could be extended beyond 13 weeks, if required. A marginal reduction in numbers of erythrocytes, slightly lower levels of hemoglobin concentration and hematocrit and markedly higher urinary volumes were noted at the higher infusion rate. The markedly higher urinary volumes produced by the 5.0 mL/kg/h animals might affect metabolism of those compounds eliminated primarily *via* the kidneys whilst the marginal alterations in red cell parameters might affect susceptibility to compounds that suppress erythrocyte production although based on the magnitude of these reductions it is considered unlikely. There were no significant alterations in any end-point measured at the lower infusion rate (1.5 mL/kg/h). Therefore, infusion rate selection requires careful consideration when using weanling dogs as the non-rodent infusion model. Selection of rates around 1.5 mL/kg/h would appear to be appropriate to assess the safety and pharmacokinetics of infused pharmaceutical products intended for use in human pediatric populations.

1584 AN ASSESSMENT OF THE EFFECTS OF HUMAN SOLUBLE IL-4 RECEPTOR ON REPRODUCTION AND NEONATAL DEVELOPMENT WHEN ADMINISTERED INTRAVENOUSLY TO PREGNANT CYNOMOLGUS MONKEYS.

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Fetal development, delivery, lactation, and functional and morphological development of offspring were evaluated in pregnant cynomolgus monkeys administered human soluble IL-4 receptor (sIL-4) intravenously twice a week during the organogenesis period (Gestation Days 20 to 50). Pregnant monkeys were divided into three groups: Vehicle control, low dose (0.2 mg/kg) and high dose (2.0 mg/kg). Each group was further divided into a Natural Delivery Group (dams delivered naturally, neonates examined up to 30 days after birth) and a Cesarean-Sectioning Group (cesareans on Day 100 of gestation, fetuses evaluated). Blood was collected from all dams, fetuses, and neonates for hematology, clinical chemistry, immunology (lymphocyte subset analysis), pharmacokinetics and antibody response to the drug. Dams were evaluated for clinical signs, body weight, food consumption, embryonic/fetal viability, and placental weight. Each fetus was examined for external development, visceral, skeletal, and histopathological changes. Neonates were evaluated for clinical signs, body weight, functional and morphological development. There were no test-article-related abnormalities in maternal clinical signs, body weight, food consumption, hematological, serum biochemistry or immunological examinations. There was an increase in abortion/embryo-fetal death in the 0.2 (9/21 or 42.9%) and 2.0 (5/19 or 26.3%) mg/kg groups compared to controls (3/17 or 17.6%). All fetuses removed at cesarean-sectioning were alive and no test article-related abnormalities were noted. There were three stillborn neonates (2.0 mg/kg group) which were determined to have died before birth - no air in the lungs (anectasis). No neonates died after birth and no test-article-related abnormalities were noted. In conclusion, the No-Observed-Adverse-Effect-Level for IV administration of sIL-4R to pregnant cynomolgus monkeys is less than 0.2 mg/kg based on increased abortion/embryonic death.

1585 CLOSER RESIDENTIAL PROXIMITY TO TRICHLOROETHYLENE-EMITTING SITES INCREASES RISK OF OFFSPRING CONGENITAL HEART DEFECTS AMONG OLDER WOMEN.

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Animal & limited human data suggest that trichloroethylene (TRI) is a selective cardiac teratogen. To assess whether maternal residential proximity to TRI-emitting sites increases the risk of congenital heart defects (CHDs), records of 245 case & 3780 control infants born from 1997-1999 to Milwaukee mothers were reviewed. Case data were obtained by actively reviewing cardiac ECHO, surgical & autopsy reports from the Children's Hospital of Wisconsin. Control & additional case information, including maternal demographics, cigarette smoking & alcohol use in pregnancy & maternal diseases during pregnancy, were obtained from birth certificate data. Cases & controls were matched for birth year; infants with Down's Syndrome were excluded. TRI-emitting sites were identified from 6 environmental databases. Using GIS software, x/y coordinates were assigned to maternal residences & TRI sites & the resulting distances calculated. Classification trees were used to determine appropriate separation of mothers into groups based on older age (≥38 y) & presence of TRI "exposure" (residence within 1.32 mi of ≥1 TRI-emitting site). The proportion of offspring with CHD was greater among older, exposed mothers (29%) compared to older, non-exposed mothers (11%), or young women, irrespective of exposure (~5%, both groups). When other CHD risk factors were considered simultaneously, CHD risk was ~3-fold greater among infants of older mothers living within 1.32 mi of a TRI-emitting site [LR: OR(95% CI)=3.2(1.2-8.7)]. Older maternal age, alcohol use, pre-existing diabetes & chronic hypertension were all associated with increased risk [OR (95% CI)=2.0 (1.1-3.5), 2.1 (1.1-4.2), 4.1 (1.5-11.2), 2.8 (1.2-6.7), respectively]. Residence close to TRI-emitting sites alone was not associated with CHDs. The mechanism by which older age & TRI exposure interact to increase CHD risk is unknown.

1586 CONTINUOUS INFUSION AS A ROUTE OF ADMINISTRATION IN EMBRYO/FETAL DEVELOPMENT STUDIES IN THE RABBIT.

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This study determined whether restraint alone or in combination with surgical catheter implantation would have a significant impact on reproductive outcome and what rate of infusion could be safely used not to confound pregnancy. The rabbit is a standard model in embryo/fetal development studies, and the infusion systems are compatible with standard caging. Groups consisted of 10 naive control rabbits, 10 rabbits fitted with tethers and jackets (but not surgically modified) and 20 tethered rabbits continuously infused with 0.9% saline *via* surgically-implanted indwelling femoral catheters. The femoral vein was isolated, an incision was made and a sterile DaVinci catheter with vascular access port was inserted. The vessel was ligated distally and the vascular access port was secured between the dorsal shoulder blades. The catheter was flushed with saline and locked with heparin. Following surgery, animals were allowed to recover for seven days, when the catheter was flushed with heparinized saline. Ten days after surgery, animals were maintained on 0.9% saline delivered at 0.3 ml/hour by calibrated infusion pumps through insemination until gestation day (GD) 7. The infusion rate was increased to 0.5 ml/kg/hour from GD 7-29. Rabbits were artificially inseminated 14 days after surgeries, observed for clinical signs of toxicity, and body weights and food consumption were recorded periodically during gestation. Laparohysterectomies and uterine and fetal examinations were performed on GD 29. One gravid female in the 0.9% saline group died on GD 26; the cause of death was not determined. No infusion- or tether-related clinical observations, internal findings, effects on intrauterine parameters, malformations or developmental variations were observed. Effects on body weights and food consumption were considered related to tethering. Based on the results of this study, catheter implantation and continuous infusion are considered appropriate for embryo/fetal development studies in rabbits.

1587 SODIUM METHYLDITHIOCARBAMATE MODULATES CYTOKINES *IN VIVO* IN B6C3F1 MICE WITH VARIOUS STIMULI.

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Sodium methyldithiocarbamate (SMD) is a fumigant-type pesticide that is used as a general biocide in agriculture. Current EPA estimates rank SMD as the third most commonly used conventional pesticide. SMD use could increase due to EPA pressures to replace methyl bromide with SMD. In previous studies we have demonstrated that SMD suppresses cytokine upregulation *in vivo* after stimulation with anti-CD3. In the current study, we changed stimuli to examine the broadness of suppression by SMD and also included a study with an antigen, KLH (keyhole limpet hemocyanin). Similar to the studies with anti-CD3 as a stimulant, SMD suppresses upregulation of IL-12 when used in combination with poly I:C or LPS. However, unlike the anti-CD3 stimulation, SMD combined with LPS or poly I:C increased IL-10 production *in vivo*. Since IL-10 production might play a role in the cytokine suppression seen with SMD, we dosed IL-10 knockout mice with SMD and poly I:C. Similar to studies in B6C3F1 mice, IL-12 was suppressed in the IL-10 KO mice dosed with SMD and poly I:C. Therefore, it is unlikely that IL-10 plays a role in cytokine suppression by SMD. To examine the effects of SMD with antigen, we dosed mice with KLH (72 hr stimulation) and a single dose of SMD either 12 hours before sampling or 1.5 hrs before sampling. Real time PCR analysis of splenic mRNA indicated that IL-2 was enhanced with SMD at 1.5 hrs but not significantly different at 12 hrs, IL-4 expression was not altered with SMD, and IL-12 was suppressed by SMD 12 hrs but not significantly different at 1.5 hrs. Lastly, we examined mRNA expression by gene chip analysis on SMD, EtOH, and corticosterone treated mice. Analysis of the splenic mRNA indicated 74 genes altered by SMD, 114 genes altered by corticosterone, and 328 genes altered by EtOH. A comparison analysis between the three stressors suggested at least 5 gene products modified in a similar pattern, possibly indicating common stress-related genes. This work was supported by NIH grant #ES09158 and #AA09505.

1588 SUPPRESSION OF INTERLEUKIN-2 GENE EXPRESSION IN EL4.IL-2 CELLS BY ALKENYLBENZENES IS PARALLELED BY AN INHIBITION OF NF-AT.

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As natural components of certain spices, essential oils or vegetables, alkenylbenzene compounds may be consumed by human. However, the potential of alkenylbenzenes for immune modulation and their mechanism have not known. Based on that T-cells play an important role in immune response through the action of cytokines they elaborate, here we investigate the effect of seven naturally occurring alkenylbenzenes on interleukin-2 (IL-2) gene expression in EL4.IL-2 murine T-cells. EL4.IL-2 cells produced IL-2 in response to phorbol-12-myristate-13-acetate

(PMA) plus ionomycin, which were inhibited by myristicin, anethole, eugenol, and isoeugenol in dose-dependent manners. No effect was observed on the treatment of safrole, 4-allylanisole, and allylbenzene. The decrease in IL-2 production was found to correlate well with a decrease in its steady state mRNA expression as demonstrated by quantitative competitive RT-PCR. Electrophoretic mobility shift assay was performed to elucidate the effect of alkenylbenzenes on the activity of NF-AT. PMA plus ionomycin treatment induced NF-AT binding activity, which was inhibited by myristicin, anethole, eugenol, and isoeugenol. These results suggest that myristicin, anethole, eugenol and isoeugenol suppress IL-2 production through the decrease of IL-2 mRNA expression and the inhibition is mediated, at least in part, through the down-regulation of NF-AT, which may explain their potentials for immune modulation. (This work was supported by grant No. R01-2001-00209-0 from the Korea Science & Engineering Foundation.)

1589 Δ^9-TETRAHYDROCANNABINOL (THC) INCREASES INTRACELLULAR CALCIUM IN A CANNABINOID RECEPTOR-DEPENDENT MANNER IN T CELLS.

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Cannabinoid compounds are widely known to alter leukocyte function. Previous studies from this laboratory have shown T cells to be a sensitive target to cannabinoid treatment. Particularly, cannabinoids inhibit the production of interleukin (IL)-2 by T cells. The regulation of IL-2 gene transcription is critically dependent on intracellular calcium ($[Ca^{2+}]_i$) and the downstream calcium-dependent transcription factor, NFAT. Therefore, the objective of the present studies was to examine the effect of THC, the primary psychoactive cannabinoid in *Cannabis sativa*, on $[Ca^{2+}]_i$ homeostasis in T cells. Both murine splenic T cells and HPB-ALL human T cell line were used to investigate the effect of THC on $[Ca^{2+}]_i$. THC (10μM) induced a rapid and robust rise in $[Ca^{2+}]_i$ in both splenic T cells (1500-2000 nM) as well as HPB-ALL cells (600-800 nM). Pretreatment of splenic T cells, which express transcripts for both CB1 and CB2 receptors, with a combination of cannabinoid receptor antagonists, SR141716A and SR144528 for CB1 and CB2 receptors respectively, antagonized the THC-mediated elevation in $[Ca^{2+}]_i$. Similarly, pretreatment of HPB-ALL cells, which only express CB2 receptor transcripts, with SR144528 also antagonized the rise in $[Ca^{2+}]_i$. Finally, studies done in the absence of extracellular calcium ($[Ca^{2+}]_e$), revealed a severe abrogation in the THC-mediated elevation in $[Ca^{2+}]_i$ in both splenic T cells as well as HPB-ALL cells. These data suggest that THC causes a rise in $[Ca^{2+}]_i$ in resting T cells in a cannabinoid receptor-dependent manner involving both release of calcium from intracellular pools as well as influx from extracellular sources. (Supported by NIH grant DA07908).

1590 INHIBITION OF INTERLEUKIN-2 (IL-2) BY THE ENDOGENOUS CANNABINOID, 2-ARACHIDONYL GLYCEROL, IS PARTLY MEDIATED THROUGH PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ (PPAR-γ).

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The putative endogenous cannabinoid, 2-arachidonyl glycerol (2-AG), is an arachidonic acid derivative that has been shown through radioligand binding studies to bind to the cannabinoid receptors, CB1 and CB2. 2-AG mimics many of the physiological effects of the plant-derived cannabinoids, such as Δ^9-THC, including hypothermia, antinociception, hypomotility, and immunosuppression. Specific to the present studies, 2-AG inhibits activation of T cells causing suppression of cytokines such as, IL-2, IL-4, and IFN-γ. We now show evidence that 2-AG is also a ligand for PPAR-γ, through which 2-AG may mediate its immunosuppressive activity. The first objective of the present studies was to determine whether 2-AG could drive 3T3-L1 preadipocytes to differentiate into adipocytes. 3T3-L1 is a murine fibroblast line that differentiates into adipocytes upon activation of PPAR-γ_2. 2-AG induced differentiation of 3T3-L1 cells at a concentration of 50 μM. The second objective was to determine whether 2-AG was able to upregulate expression of aP2. aP2 is a fatty acid carrier protein which is upregulated when activated PPAR-γ binds to the PPAR response element (PPRE). 2-AG increased expression of aP2 2-6 fold over control as measured by real-time PCR. The third objective of the present studies was to determine whether the PPAR-γ antagonist, GW9662, could antagonize the inhibition of IL-2 by 2-AG. GW9662 partially antagonized inhibition of IL-2 by 2-AG at a concentration of 1 μM, but not at higher concentrations. These data suggest that 2-AG is an endogenous ligand for PPAR-γ, which appears to contribute to the inhibitory effect of 2-AG upon IL-2 secretion by T cells. (Supported in part by NIH grant DA 12740)

1591 THE EFFECTS OF SPHINGOMYELIN, CONJUGATED LINOLEIC ACID AND BUTYRATE ALONE OR IN COMBINATIONS ON IMMUNE FUNCTION AND COLON CANCER IN RATS.

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Sphingomyelin (SPG), conjugated linoleic acid (CLA) and butyrate BYT) are naturally occurring bioactive chemicals found in higher concentration in dairy products. Studied separately, each of these compounds has been shown to have chemopreventive properties to certain cancers in animal models. This study was designed to examine the effects of each compound alone and in combinations with the other compounds to observe potential additive or synergistic effects. Parameters examined were the development of preneoplastic colon lesions and major types of terminal immune responses in male Sprague-Dawley rats. Immune responses assessed were humoral immunity (antibody production), cell-mediated immunity (delayed-type hypersensitivity) and innate immunity (natural killer cell cytotoxicity-NK) The rats were treated with either 35 mg/kg SPG, 100 mg/kg CLA or 100 mg/kg of BYT or the same concentrations of all chemicals (ALL). Aberrant crypt foci (ACF) were induced in the colon by two ip injections (10 mg/kg) of the carcinogen azoxymethane (AOM) during the first two weeks of the study.). The animals were sacrificed after 10 weeks exposure and ACF were quantified in the colon and immune responses assessed. Treatment with CLA significantly enhanced natural killer cell activity compared to controls or the ALL group. Treatment with BYT enhanced NK cytotoxic responses compared to the ALL group but not the control. There was no effect of any treatments on DTH or antibody formation. It appears that the combination of bioactive compounds used in this study were less effective than the individual chemicals in alteration of immune functions. The effects on ACF formation are in progress.

1592 *IN VITRO* AND *IN VIVO* IMMUNOLOGICAL EFFECTS OF DOK DIN DAENG (*AEGINETIA INDICA* ROXB.) AN HERBAL DRUG OF THAILAND.

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Several studies have reported *Aeginetia indica* Linn. (AIL), has potential of being a medicinal herb with antitumor activity. Seed extract of AIL has been shown by others to induce antitumor immunity in tumor-bearing mice. In Thailand, Dok Din Daeng, (DDD), a parasitic plant which grows on bamboo, has been used in Thai traditional medicine for treatment of diabetes and dermal swelling. In this study, difference parts of the plants: seed and whole plant were investigated *in vitro* and *in vivo*. DDD was obtained from seed using butanol extraction and from the plant by ethanol extraction. In *in vitro* studies DDD was observed to have immunostimulatory effects. Enhanced T cell proliferation was observed following Con A stimulation and treatment with plant DDD at concentrations from 1.25 - 500 ug/ml, no significant effect was observed from seed extracts. Enhanced B cell proliferation occurred following LPS stimulation and treatment with plant DDD at concentrations from 1.25 - 100 ug/ml, higher concentrations suppressed the B cell response. In contrast, seed extracts produced enhanced B cell responses at all concentrations evaluated (1.25-2000 ug/ml). When spleen cells were stimulated with anti-CD3 antibody, cultures treated with plant DDD at concentration of or less than 10 ug/ml did not differ from controls. A slight increase was observed at 100 ug/ml with significant decreases occurring at 500 and 1000 ug/ml concentrations. Overall, seed extracts produced slight increases (21%) in anti-CD3 proliferation. To confirm DDD potential as an immunostimulant, *in vivo* studies evaluating the effects of whole plant and seed extracts on antibody responses to T-dependent antigens, mixed lymphocytes response, natural killer cell activity and splenic populations are currently on going in female B6C3F1 mice following 28 days of daily exposure. The results of these studies should provide insight into the mechanism of action of this traditional Thai medicine.

1593 SILYMARIN INHIBITS INTERLEUKIN-1β AND PROSTAGLANDIN E2 SYNTHESIS AND PROTECTS AGAINST LIPOPOLYSACCHARIDE-INDUCED SEPSIS.

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Silymarin is a flavonoid antioxidant isolated from milk thistle and has anti-inflammatory, anticarcinogenic, and hepatoprotective effects. Here we report the inhibitory effect of silymarin on interleukin-1β (IL-1β) and prostaglandin E2 (PGE2) production in macrophages. Silymarin dose dependently suppressed the

lipopolysaccharide (LPS)-induced production of IL-1β and PGE2 in isolated mouse peritoneal macrophages. Silymarin treatment also resulted in a dose-related inhibition of IL-1β and PGE2 production in LPS-stimulated RAW264.7 cells. To further investigate whether the inhibitory effect of silymarin on IL-1β and PGE2 exerts at the transcriptional level, we assessed the effect of silymarin on mRNA expression of IL-1β and PGE2 by RT-PCR. Consistent with the previous results, the LPS-induced mRNA expression of IL-1β and cyclooxygenase-2 (COX-2) was completely blocked by silymarin in RAW 264.7 cells. Moreover, the LPS-induced DNA binding activity of NF-κB/Rel was also inhibited by silymarin in RAW 264.7 cells. Further study showed that silymarin protected mice against LPS-induced sepsis. In this model of sepsis, silymarin improved the rate of survival of LPS-treated mice from 6 % to 38 %. Taken together, these results demonstrated that silymarin has a protective effect against endotoxin-induced sepsis, and suggest that this is mediated, at least in part, by the inhibitory effect of silymarin on the production of IL-1β and PGE2.

1594 ACUTE EFFECTS OF 2-BROMOPROPANE AND 1, 2-DIBROMOPROPANE ON THE ANTIBODY RESPONSE IN FEMALE BALB/C MICE.

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2-Bromopropane (2BP) is a major component of the mixture of SPG-6AR and Solvent 5200 that is a substitute of chlorofluorocarbon. Many female workers in a Korean electronic company were found to have amenorrhea and male workers were diagnosed with oligospermia. In the present studies, immunotoxic effects of 2-BP and an analog, 1, 2-dibromopropane (1, 2-DBP), were investigated in female BALB/c mice. The mice were treated po with either 2-BP at 2000 and 4000 mg/kg or 1, 2-DBP at 300 and 600 mg/kg once. Four days before necropsy, the mice were immunized ip with sheep red blood cells (SRBCs). The spleen and thymus weights were reduced by 1, 2-DBP. In addition, the antibody response to SRBCs was suppressed by the treatment with 1, 2-DBP. Meanwhile, these parameters were not significantly changed by the treatment with 2-BP. In a subsequent study, the time course effects of 2-BP and 1, 2-DBP on the hepatotoxic parameters were compared in mice. When mice were treated po with either one of these chemicals once for 6, 12, 24 and 48 hr, the activities of serum alanine aminotransferase and aspartate aminotransferase were significantly elevated only by 1, 2-DBP 24 hr after the treatment. In addition, the hepatic content of glutathione was reduced by 1, 2-DBP. The present results suggest that 1, 2-DBP contained in the Solvent 5200 may contribute to the immunotoxicity, although 2-BP is a major component. (Supported by grant No. RO1-2000-00182 from KOSEF, Korea).

1595 ALTERATIONS IN THE IMMUNE SYSTEM AFTER EXPOSURE TO THE TRIAZINE HERBICIDE ATRAZINE.

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a triazine class herbicide, is one of the most heavily applied herbicides in the United States. A recent USGS NAWQA (United States Geological Service National Water Quality Assessment) report (1999) determined that atrazine was detectable in 44% of the wells tested making it one of the most common herbicide contaminants found in ground water in agricultural areas. However, little is known about the immunotoxicity of atrazine. Although an earlier mouse study (Fournier et al. 1992) indicated that atrazine had limited immunotoxicity, a more recent study (Vos et al. 2001) examining cellular changes in rats suggested an immunotoxic effect. Using different and perhaps more sensitive techniques, we examined the effect of atrazine on the murine immune system. C57Bl/6 mice were exposed to a single oral dose (0.0875, 8.75 and 875 milligram/kilogram of body weight (mg/kg)) of atrazine mixed in peanut oil. Seven days after exposure, significant decreases in the number of $CD4^+$ T cells and $B220^+$ B cells in the spleen were observed at the 875 mg/kg dose. In the thymus, significant decreases occurred in thymus to body weight ratios and in the $CD4^+CD8^+$, $CD4^+CD8^-$ and $CD8^+CD4^-$ thymocyte subpopulations at this dose. Preliminary data suggest that both the 875 and 8.75 mg/kg atrazine doses decrease IFNg secretion and increase IL-2 and IL-6 secretion from splenocytes stimulated with conA or LPS for 24 and 48 hr respectively. Functional changes in the immune response to heat killed Streptococcus pneumoniae after exposure to either the 875 or 8.75 mg/kg atrazine dose demonstrated an increased frequency in B cells secreting antibody to the T-independent antigen phosphorylcholine. In addition, a decrease in the frequency of B cells secreting IgG antibodies to the T-dependent antigen pneumococcal surface protein A was observed. Together these data suggest that oral exposure to atrazine has the potential to alter the immune response. Supported by a WVU School of Medicine Research Grant

1596 PHENOTYPIC DIFFERENCES IN THE HEMATOPOIETIC BONE MARROW COMPARTMENT BETWEEN ARYL HYDROCARBON RECEPTOR DEFICIENT AND CONTROL C57BL/6 MICE AS REVEALED BY FLOW CYTOMETRY.

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The aryl hydrocarbon receptor (AhR), most notably known as the mediator of TCDD toxicity, is a ubiquitous bHLH transcription factor responsible for a growing list of genes. Immune alteration, including effects on both cellular and humoral immunity, is one of the most consistently observed AhR-mediated endpoints across species. Recently, hematopoietic precursors of the bone marrow were identified as targets of TCDD. Further, *in vitro* studies with c-Kit+ Sca-1+ Lin- (KSL) bone marrow cells, a population that includes all hematopoietic progenitors, from wild-type (WT) and AhR-knockout (KO) animals revealed differential abilities to proliferate in culture. In order to ascertain whether there are basal differences in the numbers of primitive stem and progenitor cells, we isolated bone marrow from WT and KO C57Bl/6 mice and stained with appropriate combinations of fluorochrome-conjugated antibodies specific for the following hematopoietic populations: long-term hematopoietic stem cells (LT-HSC), short-term hematopoietic stem cells (ST-HSC), common lymphoid progenitors (CLP), and common myeloid progenitors (CMP). Cells were analyzed on a FACS Calibur flow cytometer. The percent of KSL cells was nearly 2-fold higher in KO animals than their WT counterparts. Within this broad population, there were a near 4-fold greater number of cells with a ST-HSC phenotype (KSL CD34+) in KO animals, but no such difference in cells with a LT-HSC phenotype (KSL CD38+). Although there was no difference in the amount of CMP present, KO animals have a nearly 2-fold greater number of CLP. Heterozygotes were identical to WT animals. These data suggest that the AhR is required to maintain normal numbers of primitive hematopoietic stem cells as well as lymphoid progenitors. Funded in part by NIH Grant ES04862, Center Grant ES01247, and Training Grant ES07026.

1597 CHARACTERIZATION OF IMMUNE CELL INFILTRATES IN LIVER AND SPLEEN BY FLOW CYTOMETRY FOLLOWING TREATMENT WITH AN ANTISENSE OLIGODEOXYNUCLEOTIDE.

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The capacity for oligodeoxynucleotides (ODN) to induce an immune stimulation in rodents is species and sequence dependent. The features of immune stimulation include cytokine production, splenomegaly, lymphoid hyperplasia and infiltration of mononuclear cells into tissues. The objective of this research was to identify and characterize the cell types that comprise the immune cell infiltrate in the liver and spleen following treatment with ISIS 2302, an inhibitor of human ICAM-1. Female CD-1 mice were administered 50 mg/kg ISIS 2302 (sc, tiw) for 3 months. Non-parenchymal liver cells and splenocytes were harvested at necropsy. ISIS 2302 treatment increased liver and spleen weight by 1.7- and 5.3-fold, respectively. Flow cytometry analysis using cell specific surface markers for monocytes/macrophages (F4/80, CD11b), B-cells (CD19), T-cells (CD3) and dendritic cells (CD11c, Dec205) were used to determine the composition of infiltrating cells. In the liver, the infiltrates were comprised primarily of monocytes/macrophages, while a smaller fraction being dendritic cells. There was a 5-20-fold increase in the percentage of cells expressing F4/80, CD11b and CD11c, while no change was observed in the population of cells expressing CD3, CD19 and Dec205. The absolute number of cells in the spleen increased following ISIS 2302 treatment, but the percentage of splenocytes expressing CD3, CD19 and CD11b was reduced 20-30%. The percentage of cells expressing F4/80, CD11c and Dec205 in the spleen only increased 2-7%. Therefore, a large percentage of cells in the spleen are unaccounted for by the surface markers analyzed. Collectively, this work suggests that histiocytes make up the majority of the immune cell infiltrates in the liver following treatment with immunostimulatory ODN, and that these cells may be the initial target cells that become activated during ODN mediated immune stimulation.

1598 UPREGULATION OF TREM-1 BY ENDOTOXIN IN MOUSE LIVER MACROPHAGES.

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Many xenobiotics induce hepatotoxicity by sensitizing liver macrophages to release excessive quantities of proinflammatory and cytotoxic mediators. In the present studies we determined if TREM-1, a newly identified receptor on macrophages of

the immunoglobulin superfamily that amplifies the inflammatory response by triggering secretion of proinflammatory mediators, was upregulated in hepatic macrophages following administration of endotoxin to C3H/HeOuJ and C3H/HeJ mice. C3H/HeJ mice have point mutations in the toll-like-4 receptor, a critical component of endotoxin-mediated cell signaling, and are resistant to endotoxin-induced toxicity when compared to C3H/HeOuJ mice. Hepatic macrophages were isolated from livers following perfusion with collagenase, centrifugal elutriation and density gradient centrifugation. Macrophages from the livers of either C3H/HeOuJ or C3H/HeJ control mice expressed very low levels of TREM-1 mRNA as determined by semi-quantitative RT-PCR. Treatment of mice with endotoxin (3 mg/kg, ip) caused a time-dependent induction of hepatic macrophage TREM-1 mRNA expression which was maximal after 20 hr. Significantly less TREM-1 mRNA was induced in C3H/HeJ mice when compared to C3H/HeOuJ mice. In macrophages from both strains of endotoxin-treated animals, tumor necrosis factor-α (20 ng/ml) or interleukin-1β treatment (50 ng/ml, 24 hr) *in vitro* caused a further 2-10 fold increase in TREM-1 mRNA expression. Lipopolysaccharide (LPS) decreased TREM-1 mRNA expression 2-3-fold in macrophages from C3H/HeOuJ mice but did not alter its expression in cells from C3H/HeJ mice. Taken together, these data demonstrate that endotoxin is a potent inducer of TREM-1 mRNA in hepatic macrophages and that expression of the protein may be an important mechanism for amplifying the inflammatory response to hepatotoxicants. Endotoxin-resistance in C3H/HeJ mice may be due to limited expression of the TREM-1 protein. Support: NIH GM34310 and ES06897.

1599 IMMUNOMODULATION BY DIETHYLSTILBESTEROL IS DOSE AND GENDER LINKED: INFLUENCE ON THYMIC APOPTOSIS AND MITOGEN-INDUCED PROLIFERATION IN CD-1 MICE.

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It is perceived, but not shown, that the immunomodulatory influences of diethylstilbesterol (DES) may vary based on dose or gender. To address this, DES was subcutaneously administered to female and male CD-1 mice as four injections over 1 week at 0, 5, 15 and 30 μg/kg bw dose with immunological and reproductive parameters evaluated 24hr post last injection. Although female thymuses were significantly larger than male thymuses, short-term DES administration neither induced thymic atrophy nor altered relative percentages of thymic subsets. However, DES treatment did induce dose-related apoptosis in the CD4+8+, CD4+8- and CD4-8+ subsets using 7-amino-actinomycin D (7-AAD). The CD4-8- showed significant apoptosis only at the highest dose (30 μg/kg bw). Mitogen-induced proliferation of splenic lymphocytes also varied with hormonal doses and gender. In the females, splenic lymphocytes from low dose DES (5 μg/kg bw)-treated mice showed an increase in proliferative response to Con A, LPS or PMA/ionomycin compared to controls. Conversely, cultures from mice treated with the higher DES doses (15 or 30 μg/kg bw) showed suppressed proliferation, especially with Con A. In the males, DES appeared to produce minimal effects with the exception of increased proliferation to Con A in the 15μg/kg bw. Interestingly, the changes in mitogen-induced proliferation were not paralleled by similar changes in relative expression of CD90+ or CD45+ cells or ratios of anti-apoptotic Bcl-2 to apoptotic Bax proteins. Con A-activated splenocytes from DES-treated mice, specifically in the females, secreted less interferon-γ compared to controls. Collectively, these findings suggest that short-term exposure to DES generates a disparity in the immunological effects depending upon the dose of hormone and sex.

1600 THE GENERATION OF REACTIVE OXYGEN SPECIES DURING EXPOSURE OF PESTICIDE MIXTURES TO IMMUNE CELLS, *IN VITRO.*

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Recent reports suggest that pesticides pose potential health risks to the humans and animals by affecting their immune system. We have observed earlier that endosulfan and permethrin cause immune cell cytotoxicity mainly *via* apoptosis. We hypothesized that these chemicals induce immunotoxicity through Reactive Oxygen Species (ROS) formation. In an attempt to test this hypothesis, we have studied the production of ROS in splenocytes of C57Bl/6 adult male mice exposed to endosulfan and permethrin, *in vitro*. The generation of intracellular hydrogen peroxide (H_2O_2) and superoxide anion was monitored using a flow cytometer in combination with dichlorofluorescin diacetate (DCFH-DA) and hydroethidine (HE) dyes, respectively. Results of these analysis revealed that individual pesticides increased the production of both H_2O_2 and superoxide anion in a dose- and time- dependent manner. The mixtures of pesticides elicited a synergistic effect on the generation of H_2O_2. However, exposure to mixtures of pesticides had little effect on the generation of superoxide anion radicals as compared to individual pesticides. These findings suggest that the pesticide-induced immunotoxicity observed earlier may, at least in part, be associated with the generation of ROS.

1601 DEOXYNIVALENOL-INDUCED APOPTOSIS MEDIATED BY P38 MAPK-DEPENDENT P53 GENE INDUCTION IN RAW 264.7 MACROPHAGES.

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Deoxynivalenol (DON, vomitoxin) and other trichothecene mycotoxins cause immunosuppression by inducing leukocyte apoptosis. Upstream signaling transduction mechanisms contributing to DON-mediated apoptosis were investigated in RAW 264.7 cells, a murine macrophage model. PP1, a Src-family-tyrosine kinase inhibitor selective for Hck, and 2-AP (2-aminopurine), the chemical inhibitor of dsRNA-dependent protein kinase (PKR), additively inhibited DON-induced caspase-3 activity and apoptosis as well as phosphorylation of the mitogen activated protein kinases p38, ERK and JNK. PP1 and 2-AP also inhibited DON-induced p53 binding activity and subsequent phosphorylation of its substrate p21. Pretreatment with PFTα, an inhibitor of p53, abrogated DON-induced caspase-3 and apoptosis. The p38 inhibitor, SB 203580, abrogated DON-induced p21 phosphorylation as well as reduced DON-induced p53 binding activity, whereas ERK and JNK inhibitors were partially inhibitory. Finally, p38 inhibition blocked DON-induced apoptosis, ERK inhibition promoted DON-induced apoptosis, and JNK inhibition had no effect. The results suggest that the principal pathway for DON-induced apoptosis in the macrophage involves the sequential activation of Hck/PKR, p38, p53, caspase-3. (Supported by NIEH Grants ES-09521 and ES-03358).

1602 JP-8 JET FUEL DOES NOT ALTER SERUM CYTOKINE LEVELS IN B6C3F1 MICE FOLLOWING 7-DAY ORAL OR DERMAL EXPOSURE.

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The US Air Force uses approximately 2.5 billion gallons of jet propellant-8 fuel per year. As a result, a considerable number of military personnel are exposed to JP-8 fuel during refueling and servicing of aircrafts, and maintenance of fuel storage tanks. Those exposed have increased incidence of headaches, blocked nasal passages, ear infections, skin irritation, or fatigue. In rodent studies, we have previously reported that 7-day dermal or oral exposure to JP-8 suppresses humoral immunity and decreases thymus weight and cellularity. Additionally, other published studies have demonstrated that a single, dermal exposure to JP-8 modulated serum cytokine levels in mice, thereby accounting for a possible mechanism of immunosuppression. To determine if alterations in serum cytokines occurred after 7 days of exposure to JP-8, the following study was performed. B6C3F1 female mice aged 7-10 weeks of age were exposed to JP-8 dermally (50 uL applied to the clipped dorsal thorax of mice with an average weight of 20 g) or orally (2000 mg/kg/day) for a duration of 7 days. Serum was collected 24 hours after the last exposure to JP-8. Using ELISA and Cytometric Bead Array methods, it was determined that levels of IL-2, IL-4, IL-5, IL-6, IL-10, and TNF-alpha were not significantly altered after exposure to JP-8 *via* the oral or dermal route. It was also learned that, when used as a negative control in the dermal study, acetone as compared to olive oil induced a suppressive effect on IL-4 and IL-6 serum cytokine levels. Consequently, interpretation of immunological dermal studies utilizing acetone should be made with caution. Overall, these findings indicate that serum cytokine levels were not elevated after a 7-day exposure to JP-8, regardless of the route of administration. These observations indicate that serum cytokine levels are not sustained above normal levels following repeat exposure to JP-8.

1603 HEPATIC PHASE I AND II ENZYME PROFILES AFTER 7-DAY DERMAL OR ORAL EXPOSURE TO JP-8 JET FUEL.

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Many military and industrial personnel are exposed to JP-8 jet fuel during service and refueling of aircrafts, and maintenance of storage tanks. In toxicological studies using mice, it has been demonstrated that JP-8 can induce immunosuppression fol-

lowing dermal, oral, or inhalation exposures. After dermal or oral exposure to JP-8, we have previously demonstrated that thymus weight and cellularity are significantly diminished and specific IgM antibody production is suppressed by 50% or more as compared to controls. To further evaluate the effects of JP-8, this study presents comparative metabolic enzyme profiles obtained after oral or dermal exposure to JP-8. Western blotting was performed to determine the protein expression of Phase I and Phase II hepatic enzymes at 24 hours or 7 days after a 7-day exposure to JP-8. Female B6C3F1 mice were exposed to JP-8 either orally (2000 mg/kg/day) or dermally (50uL neat application). Twenty-four hours post-exposure, protein expression of CYP2E1, 2B1, GSTmu, and GSTpi, but not CYP1A1, were significantly increased in mice exposed orally to JP-8. Following a week recovery period, these enzymes returned to constitutive levels. In dermal studies, despite the presence of immunosuppression comparable to orally exposed mice, there was minimal to no induction of CYP2B1, GSTmu, and GSTpi. Current studies are underway to confirm the dermal exposure profile for CYP2E1 and CYP1A1. Additionally, histological examination of the livers from these same mice exposed orally or dermally, indicated that there was no increase in the amount of fat, hydropic degeneration or necrosis in the liver. These data suggest that the metabolism of JP-8 may not be required for immunotoxicity.

1604 EFFECT OF CYCLOOXYGENASE (COX) INHIBITORS ON HUMAN LEUKOCYTE MIGRATION THROUGH ENDOTHELIAL CELL MONOLAYERS.

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Previous studies have shown that traditional NSAIDs such as ibuprofen inhibit human leukocyte migration through endothelial cell monolayers. Leukocyte-endothelial cell interactions play a central role in various inflammatory processes. Upon signaling from chemokines, cytokines and adhesion molecules during inflammation, leukocytes first attach, roll and migrate through the vascular endothelium to the inflammation site. The current study using an *in vitro* double chamber cell culture system, compared the effect of an *in vitro* non-selective COX inhibitor (ibuprofen) and *in vitro* selective COX-2 inhibitors (SC-236, -791, -872 and -236) on human leukocyte migration through endothelial cell monolayers. Human umbilical vein endothelial cells were cultured on fibronectin coated 3m microporous membranes until an endothelial cell monolayer formed. Monolayers were treated with 10ng/ml recombinant human TNF for 4 hours before the migration assay began. 1x106 freshly isolated human leukocytes and/or cultured endothelial cell monolayers were preincubated with test agents at 1x, 10x and 50x multiples of their therapeutically relevant concentrations. Migration assays were carried out for 3 hours in a 37oC 5% CO2 incubator. Leukocytes that migrated through the endothelial cell monolayer to the lower chamber were quantitated. Each set of experiments was performed with peripheral blood from ten different normal human subjects. Our results confirmed inhibitory effects of ibuprofen on human leukocyte migration starting at the therapeutically relevant concentration. No effect on leukocyte migration was seen with the *in vitro* selective COX-2 inhibitors at 1X and 10X concentrations indicating that COX-2 inhibition does not affect leukocyte migration. At extremely high concentrations (50x), leukocyte migration was decreased with *in vitro* selective COX-2 inhibitors. This may be related to loss of COX selectivity and inhibition of COX-1 at these concentrations. In conclusion, *in vitro* selective COX-2 inhibitors do not affect leukocyte-endothelial cell interactions during the inflammatory process.

1605 ROLE OF IL-1BETA IN LPS POTENTIATION OF DEOXYNIVALENOL-INDUCED LEUKOCYTE APOPTOSIS IN MICE.

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LPS and Deoxynivalenol (DON) co-exposure induces corticosterone-dependent apoptosis in thymus, Peyers patches, and bone marrow in mice after 12 hr. We investigated the role of endogenous and exogenous IL-1beta in stimulating corticosterone and in inducing leukocyte apoptosis in this model. LPS (0.1 mg/kg, ip) and DON (12.5 mg/kg, po) co-exposure induced splenic IL-1beta mRNA significantly compared to vehicle or each of the toxins alone. Mice deficient in IL-1 receptor did not exhibit LPS + DON-induced leukocyte apoptosis whereas toxin co-treatment induced apoptosis in corresponding wild-type mice. Plasma corticosterone levels in LPS + DON-treated IL-1 receptor deficient mice were significantly lower at 12 hr than wild-type mice. In B6C3F1 mice, intraperitoneal injection of IL-1 receptor antagonist (100 microg/mouse, twice at 3 hr intervals) also significantly inhibited LPS + DON-induced apoptosis in thymus, Peyers patches and bone marrow compared to LPS + DON-treated mice. Three injections of IL-1beta protein (500 ng/mouse, ip at 2 hr intervals) induced apoptosis in thymus (4.7%) and Peyers patches (16.0) whereas single injection of equivalent amount of IL-1beta (500 or1500 ng/mouse) did not induce apoptosis in any of the organs in B6C3F1 mice. In parallel to apoptosis, corticosterone levels in multiple IL-1beta injected mice were significantly higher (404.0 ng/ml) at 12 hr than control or single injected mice (90.4 ng/ml). ACTH levels in LPS + DON-treated mice were not correlated with the induction of plasma corticosterone or leukocyte apoptosis. Taken together, the results indicate that IL-1beta is a critical mediator of LPS + DON-induced leukocyte apoptosis and that it possibly acts through ACTH-independent corticosterone upregulation (supported by NIH Grants DK 58833 and ES 03358).

1606 ALTERATIONS OF MATERNAL/FETAL CYTOKINE CONCENTRATIONS IN SMOKERS AND NON SMOKERS AT CHILDBIRTH.

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Plasma cotinine concentrations are the benchmark for smoking status assessment. Smoking has been shown to elicit an inflammatory response associated with respiratory dysfunctions such as asthma and emphysema. As expected, maternal smoking during pregnancy has a deleterious effect on the fetus. Using commercially available ELISA kits, we measured cytokine concentrations in the plasma of mothers and matched cord blood samples collected at birth. Maternal/fetal pairs were placed in smoking and nonsmoking categories based on plasma cotinine concentration. Tumor Necrosis Factor-α, and the Interleukins 1β, 6, 8 and 10 were measured as markers of elevated inflammatory status. Preliminary studies show that with the exception of Il-1β, fetal cytokine concentrations were lower in the plasma of the babies whose mothers smoked. We attribute this difference to the suppression of the immune response caused by cigarette smoke exposure. These alterations may also be attributed to the stress of and the events leading up to delivery. We hypothesize that this altered immune response may account for the predisposition of children whose mothers smoked throughout pregnancy to respiratory ailments such as asthma as well as childhood allergy problems. Future studies will clarify the role of these individual cytokines in the spectrum of immune system maturity.

1607 TARGETED DELETION OF CD44V7 EXON LEADS TO DECREASED IL-2-INDUCED ENDOTHELIAL CELL TOXICITY AND VASCULAR LEAK SYNDROME.

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Endothelial cell (EC) toxicity is widely reported in a number of clinical settings including autoimmunity, transplantation, graft versus host disease and cytokine therapy. Administration of IL-2 activates cytolytic lymphocytes to mediate EC injury and trigger vascular leak syndrome (VLS). In the current study, we investigated the nature of CD44 variant isoforms involved in EC injury and the mechanism of VLS induction. Administration of IL-2 into CD44 wild-type (WT) mice led to increased gene expression of CD44 variant isoforms containing the v6 and v7 exons and to significant induction of VLS in the lungs. In contrast, CD44v6/v7 KO and CD44v7 KO mice showed markedly reduced levels of VLS. The decreased IL-2-induced VLS in CD44v7 KO mice did not result from differential activation and expansion of CD8+ T cells, NK and NK-T cells or altered degree of perivascular lymphocytic infiltration in the lungs. Interestingly, IL-2 activated cytolytic lymphocytes from CD44v7 KO mice showed a significant decrease in their ability to mediate lysis of EC *in vitro*. Furthermore, adherence and conjugate formation of cytolytic lymphocytes from CD44v7 KO mice to EC isolated from the lungs of TIE2-GFP transgenic mice was markedly reduced when compared to LAK cells from CD44 WT mice. Finally, culturing of LAK cells with Abs against CD44v7 led to a significant reduction in the adherence to and killing of TME endothelial cells. The current study demonstrates that CD44 isoforms containing v7 play a key role in the adhesion of cytolytic lymphocytes to EC leading to injury of EC. This work was supported in part by grants from National Institutes of Health (HL 10455, HL 058641, DA 0114885, and ES 09098).

1608 DIFFERENTIAL REGULATION OF IL-2 GENE TRANSCRIPTION BY TGF-β1 IN NAIVE AND EFFECTOR/MEMORY CD4$^+$ T CELLS.

S. C. Mckarns and R. H. Schwartz. *Laboratory of Cellular and Molecular Immunology, NIAID/NIH, Bethesda, MD.*

TGF-β1 signaling in T cells plays a role in the maintenance of T-cell homeostasis and self-tolerance as illustrated by autoimmune disease in CD4-promoter dnTGF-βRII transgenic mice. The cellular and molecular mechanisms underlying homeostatic control of CD4$^+$ T cell populations remain elusive, but may be mediated, in

part, by the capacity of TGF-β1 to differentially regulate cytokine production. IL-2 is of particular interest since the autoimmune phenotype of IL-$2^{-/-}$ mice is similar to that observed in mice deficient in T cell-specific TGF-β signaling. Here we demonstrate that TGF-β1 is a potent inhibitor of IL-2 promoter activity in α-CD3 + α-CD28-stimulated naive 5CC7 TCR Tg $Rag2^{-/-}$ $CD4^+$ T cells transiently transfected with a luciferase reporter construct driven by a 2.2kb fragment of the IL-2 promoter. We further examine the effect of TGFβ-1 on IL-2 gene expression at the single cell level using $CD4^+$ T cells from homozygous or heterozygous mice in which green fluorescent protein (Gfp) cDNA was "knocked-into" the first exon of the IL-2 locus (GFP^{Ki}). Our results demonstrate a >90% inhibition of GFP-positive $CD4^+$ T cells by TGF-β1 following α-CD3 + α-CD28-stimulation of naive GFP^{Ki} T cells. Strikingly, upon re-stimulation with α-CD3 + α-CD28, resting previously-activated (Ag + APC) 5CC7 TCR Tg $Rag2^{-/-}$ $CD4^+$ T cells were unresponsive to inhibition of luciferase activity or GFP expression by TGF-β1. These results suggest that TGF-β1 may differentially regulate T cell homeostasis in a manner dependent upon the whether the target population of T cells has previously encountered an activation signal.

1609 A NOVEL RIBOFLAVIN-BASED PATHOGEN REDUCTION SYSTEM DOES NOT INTRODUCE NEOANTIGENS IN HUMAN PLATELETS.

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Gambro BCT is currently developing a pathogen reduction technology that utilizes riboflavin as a photosensitizer to mediate damage to pathogen nucleic acids. Because the pathogen reduction process uses a UV light source, the risk for the formation of process related neoantigens must be evaluated. This risk was evaluated by immunizing New Zealand White rabbits with control and pathogen reduced platelets, and comparing anti-sera raised against both to determine if antibodies against neoantigens were present. Control platelets were normal apheresis platelets. Pathogen reduced platelets were apheresis platelets containing 50 μM riboflavin, which were exposed to a total energy of 7 J/cm^2 UV light. Three animals received control platelets, and three animals received PR processed platelets. Antibody titers were checked, and boosts were given on days 21 and 42. After day 42, antiserum was collected from all animals, and the possible formation of antibodies against neoantigens was evaluated using the Ouchterlony double immunodiffusion assay. By comparing the immunoreactivity of antisera from animals given PR processed platelets against PR processed antigen and control antigen, complete identity was established. Therefore, it can be concluded that the riboflavin based pathogen reduction process does not introduce potentially harmful new antigenic determinants into human platelets.

1610 BIOMARKERS OF EFFICACY OF CHEMOPREVENTIVE AGENTS IN ANIMAL MODELS AND IN HUMANS.

G. D. Stoner[2], S. S. Hecht[1], M. A. Pereira[3], J. DiGiovanni[4] and T. W. Kensler[5]. *[1]University of Minnesota Cancer Center, Minneapolis, MN, [2]School of Public Health, The Ohio State University, Columbus, OH, [3]Department of Pathology, Medical College of Ohio, Toledo, OH, [4]Department of Carcinogenesis Science Park Research Division, UT MD Anderson Cancer Center, Smithville, TX and [5]Department of Environmental Health Sciences, Johns Hopkins School of Hygiene, Baltimore, MD.*

This symposium will summarize recent developments in the use of biochemical, molecular and morphometric biomarkers to assess the efficacy of chemopreventive agents in animal models and in humans. The discussion will include efficacy biomarkers for agents that inhibit both the initiation and promotion/progression stages of carcinogenesis. This topic is significant in that the field of cancer chemoprevention is rapidly emerging as a major area of research activity in the cancer field, and significant efforts are currently being made to identify appropriate biomarkers to assess the efficacy of chemopreventive agents. This symposium will be of interest to members of the SOT who have an interest in chemoprevention, as well as members of the carcinogenesis and molecular biology specialty sections.

1611 APPLICATION OF CARCINOGEN BIOMARKERS IN STUDIES OF LUNG CANCER CHEMOPREVENTION.

S. S. Hecht. *Cancer Center, University of Minnesota, Minneapolis, MN.* Sponsor: G. Stoner.

Lung cancer is the most common cancer in the world, with the number of cases estimated at 1.2 million annually. Cigarette smoking causes 90% of lung cancer. In people who cannot stop smoking and in ex-smokers, chemoprevention is an approach to decrease lung cancer mortality. Our targets for lung cancer chemoprevention are cigarette smoke carcinogens. Among these, benzo[a]pyrene (BaP) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), may cause lung cancer in smokers. Isothiocyanates are effective chemopreventives against lung tumor induction in rodents treated with NNK, BaP, and other carcinogens. 2-Phenethyl isothiocyanate (PEITC) inhibits lung tumor induction by NNK in rats and mice while benzyl isothiocyanate (BITC) inhibits lung tumor induction by BaP and other polycyclic hydrocarbons in mice. A mixture of dietary PEITC and BITC inhibits lung tumor induction by a mixture of BaP and NNK in mice. Mechanisms of inhibition by isothiocyanates were examined by determining their effects on DNA and hemoglobin adducts of BaP and NNK, and on urinary metabolites of NNK. In mice, a mixture of dietary PEITC and BITC had little effect on levels of BaP-DNA adducts or O^6-methylguanine formed from NNK, while decreasing 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing DNA adducts of NNK. In rats, a mixture of dietary PEITC and BITC had no effect on BaP-DNA or BaP-hemoglobin adducts, while significantly decreasing levels of HPB-releasing DNA adducts of NNK in lung but not in liver. In tandem with these effects, levels of HPB-releasing hemoglobin adducts of NNK decreased and amounts of the NNK metabolites, NNAL and its glucuronides in urine increased. These results, along with the results of lung tumor inhibition experiments, demonstrate that the major inhibitory effect of dietary PEITC and BITC on lung tumor induction by a mixture of BaP and NNK results from the effects of PEITC on lung DNA adducts of NNK. Urinary metabolites of NNK are influenced by the metabolic activation of NNK in the lung, suggesting that these metabolites, measurable in humans, can be used as biomarkers of chemopreventive efficacy in smokers.

1612 BIOMARKERS OF CHEMOPREVENTION IN THE RAT ESOPHAGUS.

G. D. Stoner, R. M. Aziz, P. S. Carlton and L. A. Kresty. *Environmental Health Sciences, Ohio State University, Columbus, OH.*

Squamous cell carcinoma of the esophagus is one of the most common cancers worldwide. It has a complex etiology involving both lifestyle and dietary factors. Research in China suggests that nitrosamines such as N-nitrosomethylbenzylamine (NMBA) in the diet and N-nitrosonornicotine (NNN) in tobacco smoke are causative agents of the disease. NMBA and NNN induce esophageal tumors in rats, and we employ the rat model to identify chemopreventives for the esophagus. Isothiocyanates and polyphenols were found to be potent inhibitors of tumor initiation in rat esophagus, mainly through inhibiting the metabolic activation of NMBA or NNN. Recently, lyophilized strawberries and black raspberries were found to inhibit esophageal tumor initiation through similar mechanisms. In contrast, the chemopreventive agent, N-(4-hydroxyphenyl)retinamide (4-HPR), enhanced tumor development in the rat esophagus, by both increasing NMBA-induced DNA damage and the growth of preneoplastic cells. The detection of inhibitors of esophageal tumor development in rats pre-initiated with NMBA has been difficult. We have demonstrated the importance of H-ras activation in tumor development in rat esophagus. However, perillyl alcohol, an inhibitor of farnesylation of ras p21, enhanced tumor development in the esophagus when provided in the diet of preinitiated rats. Piroxicam, an inhibitor of cyclooxygenase 1 (COX-1), had no effect on tumor development in spite of its reduction in the levels of prostaglandin E_2 in the esophagus. Studies are underway to evaluate an inhibitor of cyclooxygenase 2 (COX-2) on esophageal tumorigenesis, since COX-2 is elevated in both preneoplastic and neoplastic lesions of the esophagus. In contrast, strawberries and black raspberries are effective inhibitors of tumor progression in the esophagus. Berries reduce the rate of cell growth in NMBA-treated esophagi. Organic extracts of berries downregulate the transcription activators, AP-1 and NF-κB, and their associated kinases, *in vitro*. Studies are underway to identify compounds in berries that exhibit chemopreventive efficacy in the esophagus.

1613 MODULATION OF THE METHYLATION OF DNA AND GENES AS SURROGATE END-POINT BIOMARKERS FOR CHEMOPREVENTION OF COLON AND LUNG CANCER.

M. A. Pereira and L. Tao. *Pathololgy, Medical College of Ohio, Toledo, OH.*

Alteration in DNA methylation is an extremely common molecular change found in cancer and includes the general hypomethylation of DNA and the hypermethylation of specific genes. Chemicals can alter DNA methylation so that some might induce cancer by changing a tissue DNA methylation pattern to that found in tumors, while others are likely to prevent cancer by reversing the methylation pattern in tumors back to the normal state. The development of drugs for the prevention of colon and lung cancer could be improved by the use of surrogate end-point biomarkers and by a better understanding of the mechanism of chemoprevention. To identify surrogate end-point biomarkers, tumors were induced in mouse lung by

vinyl carbamate and in rat colon by azoxymethane (AOM). The ability of short-term treatment of chemopreventive drugs to modulate DNA methylation in these tumors was determined. Mouse lung tumors relative to normal lung tissue had decreased methylation of DNA and of the c-myc and insulin-like growth factor II (IGF-II) genes. Seven days of treatment with Budesonide, a drug that has been shown to prevent lung tumors in mice, increased the methylation of DNA and of the two genes in the tumors. AOM-induced colon tumors relative to normal colon tissue in rats were demonstrated to have increased methylation of the estrogen receptor- gene. Five days of treatment with drugs that have been shown to prevent AOM-induced colon tumors, i.e. calcium chloride, α-difluromethylornithine, piroxicam or sulindac decreased the methylation of this gene. Interestingly, these drugs also appeared to cause a limited amount of methylation at CpG and other sites that were unmethylated in both normal colonic tissue and tumors. The ability of drugs to modulate DNA methylation was associated with their ability to decrease cell proliferation. In summary, seven days of treatment with chemopreventives led to modulation of DNA methylation in lung and colon tumors, supporting modulation of gene methylation as a surrogate end-point biomarker for assessing drug efficacy.

1614 USE OF AFLATOXIN BIOMARKERS TO EVALUATE STRATEGIES FOR PREVENTION OF LIVER CANCER.

T. W. Kensler. *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is the leading cause of cancer death in Qidong, People's Republic of China. Several nested case-control studies in nearby Shanghai have demonstrated a synergistic interaction between hepatitis B virus (HBV) and aflatoxins for risk of HCC. Thus, HBV vaccination programs and efforts to reduce aflatoxin exposures could have major impact on this disease. The extent of aflatoxin contamination in foods is a function of the ecology of molds and is not completely preventable. As such, chemoprevention may be useful. Experimentally, aflatoxin-induced HCC is inhibited by many chemopreventive agents. One agent, oltipraz, is a potent inducer of phase 2 enzymes involved in the detoxication of carcinogens including aflatoxin. To test whether enzyme induction is a useful strategy for chemoprevention in humans, a randomized, placebo-controlled, double-blind clinical trial was conducted in Qidong. Administration of oltipraz by mouth daily significantly enhanced excretion of a phase 2 product, aflatoxin-mercapturic acid, in the urine of study participants. While this trial highlighted the feasibility of inducing phase 2 enzymes in humans, additional strategies for preventing the toxicologic consequences of unavoidable exposures to aflatoxins are needed. Chlorophyllin (CHL), a mixture of water-soluble derivatives of chlorophyll used as food colorant and over-the-counter medicine, is an effective inhibitor of aflatoxin hepatocarcinogenesis in animals, apparently by impeding bioavailability of aflatoxin. In another clinical trial conducted in Qidong, we tested whether CHL could alter the disposition of aflatoxin by monitoring for levels of aflatoxin-N7-guanine excreted in urine using sequential immunoaffinity chromatography and mass spectrometry. This aflatoxin-DNA adduct excretion product serves as a biomarker of the biologically effective dose of aflatoxin and elevated levels are associated with increased risk of HCC. CHL proved to be a safe and effective agent for reducing biomarker excretion in individuals consuming dietary aflatoxins.

1615 TARGETS FOR CHEMOPREVENTION OF EPITHELIAL CANCERS IN TRANSGENIC MICE.

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Both the TRAMP and the BK5.IGF-1 transgenic mouse models are being used for studies of prostate cancer chemoprevention. In TRAMP mice, the SV40 large T antigen is expressed in prostate under control of the probasin promoter. In BK5.IGF-1 mice, human IGF-1 is expressed in prostate under control of the bovine keratin (BK) 5 promoter. Recent work has shown that IGF-1R signaling pathways as well as COX-2 are up-regulated in prostate tissues of BK5.IGF-1 mice. In this regard, both PI3K and Akt are activated and COX-2 protein levels elevated in the dorsolateral (DLP) and in prostate tumors of the DLP of BK5.IGF-1 mice. A number of Akt downstream targets are also constitutively modulated (phosphorylated) in DLP tissues from these mice. COX-2 protein is up-regulated and PGE2 levels are elevated in DLP and tumors of 17-25 week old TRAMP mice. COX-2 protein levels were also elevated in cell lines established from TRAMP tumors. In these cell lines the COX-2 inhibitor celecoxib inhibited growth and PGE2 synthesis. These results suggest that both the PI3K/Akt and COX-2 pathways may be excellent targets for the prevention of prostate cancer in these model systems. Current studies are examining chemopreventive agents that target PI3K/Akt and cyclooxygenases pathways. In other studies, another novel transgenic mouse model is being used to examine the importance of COX-2 and its upstream regulation by EGFR/erbB2 signaling in cancers of the biliary tract. In these mice, rat erbB2 is overexpressed under control of the BK5 promoter. COX-2 protein is significantly upregulated in biliary tract epithelium and gallbladder adenocarcinomas that develop in these mice. In addition, the EGFR is activated constitutively in these same tissues. Thus, COX-2 and the EGFR appear to be excellent targets for chemoprevention strategies in these mice. Ongoing studies are examining the efficacy of COX-2 and EGFR inhibitors for prevention of biliary tract carcinogenesis in this model.

1616 ENVIRONMENTAL MODULATION OF PUBERTY.

B. J. Davis[1] and S. Fenton[2]. *[1]Laboratory of Women, NIEHS, Research Triangle Park, NC and [2]USEPA, Research Triangle Park, NC.*

The onset of puberty represents the orchestration of significant physiological events conducted by both genetic and environmental factors. Emerging data suggest that chemicals in the environment may be among the factors that influence pubertal characteristics and/or age of onset. This may be a particular concern for chemicals that advance the age of puberty in girls because of the emotional and physical complications associated with early puberty and because early age of menarche is a consistent risk factor for development of breast and uterine cancers later in life. This complex issue will be addressed by reviewing current knowledge of pubertal characteristics in humans and by analyzing evidence linking chemical exposures and puberty modulation. The current knowledge of neuroendocrine controls of puberty will be detailed using transgenic animal models, and we will explore recent developments in pubertal and *in utero*/lactational protocols for initial screening and testing of environmental contaminants.

1617 THE NEUROENDOCRINE CONTROL OF FEMALE PUBERTY AS REVEALED BY TRANSGENIC AND GENOMIC APPROACHES.

S. R. Ojeda, S. Heger, V. Prevot, A. Mungenas, A. Lomniczi, H. Jung and G. Smiley. *Oregon National Primate Research Center-, Oregon Health Sciences University, Beaverton, OR.* Sponsor: B. Davis.

The initiation of mammalian puberty requires increased LHRH secretion from the hypothalamus. This increase is determined by changes in the activity of neuronal and astroglial networks connected to LHRH neurons. The main inhibitory neurotransmitter controlling LHRH release is GABA, which delays the onset of puberty *via* activation of GABA-A receptors. GABA is also produced by a subset of LHRH neurons during developmental migration, but this capacity is lost when the neurons enter the brain towards their final destination. Transgenic expression of GAD-67 in LHRH neurons increased the number of neurons mistargeted during migration, suggesting that GABA production within the LHRH neuronal network may serve to enhance positional diversity. The mutant female mice also showed disrupted estrous cyclicity, decreased fertility, and a shortened reproductive span indicating that the normalcy of these major reproductive events requires silencing of GABA production within LHRH neurons. Astrocytes regulate LHRH secretion through growth factor-mediated cell-cell signaling pathways. Genetic disruption of erbB-1 or astrocytic erbB-4 in mice delays female sexual development due to impaired erbB ligand-induced LHRH release. The combined defect exacerbates the delay, indicating that both receptors act in concert. Global analysis of the changes in gene expression that occur in the primate hypothalamus during the onset of puberty revealed a broad range of expression changes in genes encoding transcriptional regulators, as well as neuron-neuron and neuron-glia communication. Thus, the initiation of puberty may require reciprocal neuron-glia communication involving excitatory amino acids and growth factors, and changes in synaptic make-up and glia-neuron adhesiveness. (NIH grants HD-25123, RR00163 and NICHD-U54-18185).

1618 A LONGER PUBERTY IN HUMANS: WHAT IN THE WORLD WILL BECOME OF IT?

M. R. Forman. *Cancer Prevention Studies Branch, NCI, Bethesda, MD.* Sponsor: B. Davis.

Age at onset of puberty is occurring earlier in boys and girls today than in the past 30 years in the United States based on nationally representative data. It occurs earlier in members of the African American ethnic group than other ethnic groups. Obesity and being taller at younger ages are proximal factors contributing to earlier age at onset of puberty, while the potential contributions of birth weight and early life environmental factors such as diet will be reviewed. Because puberty typically occurs three years before menarche in girls, is occurring earlier than in the past but

age at menarche has not significantly changed over time, puberty is occurring for a longer time interval. While pubertal stage in boys does not include an easily measured milestone such as menarche, earlier age of onset and comparable ages at reaching later pubertal stages over time leads to a similar pattern of a longer pubertal period. Pubertal stage differences in circulating levels of insulin-like growth factor 1 (IGF-1), leptin and other hormones and their co-variation with measures of body composition by gender and ethnic group will be described. Potential long-term effects from an extended pubertal period of the life cycle will be reviewed in relation to risk of chronic disease. Finally methodologic issues in pubertal staging have been described clinically and statistically; potential new molecular markers may be on the horizon.

1619 PUBERTAL DEVELOPMENT AND EXPOSURE TO POLYBROMINATED BIPHENYLS (PBBs).

M. Marcus. *Emory University, Atlanta , GA.* Sponsor: B. Davis.

In the early 1970s the Michigan Chemical Company made two products. One was a nutritional supplement for cattle feed (Nutrimaster) and the other was a flame retardant (Firemaster). A paper shortage and other obscure circumstances led to the delivery of Firemaster to the grain mill and contamination of cattle feed with PBBs. Farmers reported reduced milk production in their dairy herds, stillborn calves and calves born with hoof deformities. It took about 10 months for the contamination to be discovered and, in the meantime, thousands of farm families and local residents consumed contaminated meat and dairy products. PBBs are lipophilic and are eliminated slowly from the body. We found that breastfed girls born to the most highly exposed women had their first menstrual period a full year earlier than unexposed girls from the same community (11.6 years vs. 12.6 years).

1620 MAMMARY GLAND DEVELOPMENT: EARLY LIFE EFFECTS FROM THE ENVIRONMENT.

S. Fenton. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

As signs of precocious puberty in girls reach the earliest mean age in history, many scientists are exploring the environment as a contributing factor. Accidental exposure to environmental toxicants and precocious use of estrogen or placental-protein containing hair care and beauty aid products have been suggested to alter the mean age at which groups of girls begin to show signs of puberty. An early sign of puberty includes breast development during those years preceding the completion of puberty. Development of the breast and the rodent mammary gland follow a similar timing in relationship to puberty. EPA scientists are developing and validating pubertal and *in utero*/lactational protocols using rodent models, for initial screening and testing of environmental contaminants. Our laboratory focuses on development of mammary tissue prior to and during puberty in rat offspring exposed to EDCs during late gestation, a critical time in the outgrowth of mammary epithelia. Rapid mammary epithelial development begins prior to the emergence of regular estrous cycles in rodents and is completed shortly after that time, a progression similar to that found in humans. Preliminary evidence indicates that the timing of mammary epithelial development can be altered by various EDCs, with compounds such as nonylphenol accelerating the pace and others (i.e., dioxin) delaying differentiation of the tissue. We have also begun to examine the mode of action(s) by which mammary gland development and other pubertal indicators are modulated by individual EDCs. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy)

1621 METHODS FOR THE IDENTIFICATION AND CHARACTERIZATION OF CHEMICAL RESPIRATORY ALLERGENS.

S. E. Loveless[2] and M. I. Gilmour[1]. [1]*DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE and* [2]*USEPA, Research Triangle Park, NC.*

Chemical respiratory allergy (CRA) is an important occupational health problem because exposure to certain chemicals in the workplace can cause long-lasting effects, the symptoms of which can range from shortness of breath to life threatening anaphylactic shock. Currently, however, the prospective identification of chemicals likely to cause CRA is hampered by a lack of validated test methods. Accompanying the better understanding of the cellular interactions in allergic responses has come the realization that an essential molecule or pattern of molecules in this process may be identified that could serve as a predictor of potential respiratory allergens. The relevancy of route of exposure has been debated, with some scientists arguing that exposure of test animals to chemicals must occur *via* the respiratory tract by intranasal, intratracheal or inhalation exposure, while others claim that contact and respiratory allergens can induce differential immune responses following dermal exposure only. In January 1999, the chemical industry, through the American Chemistry Council (ACC) and the European Chemical Industry Council (CEFIC), committed more than $100 million over five years to health and environmental research related to chemical use. Respiratory allergy was identified in a global industry-wide survey as an important concern. Therefore, Immunotoxicology and Allergy research teams in both the US and Europe committed to sponsor a 3-year research program designed to develop methods to identify and characterize potential chemical respiratory allergens. Investigators at four laboratories were selected using a peer reviewed competitive process: Michigan State, Harvard, TNO Nutrition and Food Research Institute, and Syngenta (Central Toxicology Laboratory). This workshop will bring together investigators from all four laboratories to discuss the progress of their research. In addition, the current regulatory status and issues around predicting CRA will be discussed.

1622 AIRWAY CYTOKINE GENE EXPRESSION AS A BIOMARKER OF CHEMICAL-INDUCED AIRWAY ALLERGENICITY.

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The overall objective of this workshop presentation will be to critically discuss the utility of employing cytokine gene expression in airways as a biomarker of whether an agent has the potential for inducing respiratory hypersensitivity and to contrast that to changes in serum IgE. A/J mice were employed in these studies due to the fact that they exhibit an enhanced T helper 2 phenotype and low background serum IgE. The principal source of mRNA used for the assessment of cytokine gene expression was the right lung lobes, although some measurements were also made using tracheobroncial lymph node-derived RNA. The experimental approach was to expose A/J mice *via* repeated intranasal administration to one of several model low molecular weight chemicals that are either well-established respiratory allergens (toluene diisocyanate or trimellitic anhydride) or contact allergens (2, 4 dinitrochlorobenzene or oxazolone). In order to assess the type and magnitude of airway response induced by each of the test agents evaluated, a broad range of endpoints were measured including histopathology of upper and lower airways, cellularity of bronchoalveolar lavage, changes in total serum IgE, and cytokine gene expression for IL-4, IL-5, IL-10, IL-13 and IFNgamma. Responses to each of the low molecular weight chemicals was compared to the protein allergen, ovalbumin. (Supported in part by the American Chemistry Council Grant 0051 and NIEHS Training Grant ES 07255).

1623 CAN NON-INVASIVE PLETHYSMOGRAPHY PREDICT RESPIRATORY ALLERGY TO CHEMICALS?

L. Kobzik. *Environmental Health, Harvard University, Boston, MA.* Sponsor: S. Loveless.

Non-invasive plethysmography in mice is being evaluated for use in predicting the potential of chemicals to cause respiratory allergy. This technique measures enhanced pause (Penh), an index of airway hyperresponsiveness (AHR), which is a characteristic pathophysiologic outcome in asthma. To determine the utility of noninvasive plethysmography as a predictor of respiratory allergy, toluene diisocyanate (TDI) and trimellitic anhydride (TMA) were used as prototypical respiratory sensitizers, while the well-known skin sensitizer, DNCB, was employed as a negative control. Results indicate that: 1) the vehicles used to deliver sensitizers into the lungs of mice by intranasal insufflation are without effect on AHR or pulmonary inflammation; 2) intranasal delivery of sensitizers into normal (unsensitized) mice is also without effect. These control observations are important in that they validate the specificity of any positive results observed. Also, after skin sensitization with DNCB, respiratory challenge has no effect on AHR or lung inflammation, confirming one-half of our hypothesis. Finally, TDI does cause increased airway responsiveness (AHR) when administered to skin-sensitized mice. The potential benefits and limitations of this technique for identification of respiratory sensitizers will be discussed.

1624 IDENTIFICATION AND CHARACTERIZATION OF CHEMICAL RESPIRATORY ALLERGENS IN RODENTS.

R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, Cheshire, United Kingdom.*

Chemical respiratory allergy is an important occupational health problem but there are currently available no validated methods for the prospective identification of such materials. This is due in part to the fact that the relevant cellular and molecu-

lar mechanisms are unclear, although there is evidence for the development of T helper (Th) 2 type responses and, in some cases, IgE production. Respiratory sensitizing potential has been explored as a function of induced increases in the total serum concentration of IgE in the Brown Norway (BN) rat and cytokine secretion profiles in BN rats and BALB/c strain mice. Animals have been exposed topically to the reference contact allergen 2, 4-dinitrochlorobenzene (DNCB), which lacks respiratory sensitizing potential, or to the reference respiratory allergen trimellitic anhydride (TMA). BN rats can be bled serially allowing longitudinal analyses and discrimination between transient and sustained changes in serum IgE. At concentrations of TMA and DNCB of comparable immunogenicity, only TMA treatment was associated with significant and persistent increases in serum IgE. However, there was considerable inter-experimental variation in the responsiveness of the BN rat to TMA exposure, with no response to TMA in some experiments. Furthermore, other respiratory allergens failed to provoke significant changes in serum IgE. Exposure of BALB/c strain mice or BN rats resulted in Th1- and Th2-type cytokine secretion patterns being induced by DNCB and TMA, respectively. Cytokine expression patterns by BN lymph node cells (LNC) were considerably more variable than those observed for LNC derived from BALB/c strain mice. To date, 7 contact allergens and 14 respiratory allergens from different chemical classes have been shown to stimulate type 1 and type 2 cytokine profiles, respectively, in the BALB/c strain mouse. These data suggest that the measurement of induced cytokine secretion profiles in the BALB/c strain mouse provides a robust method for hazard identification and characterization of chemical respiratory allergens.

1625 APPROACHES TO INDUCE RESPIRATORY ALLERGY IN THE RAT: IMPACT OF ROUTE, INTENSITY OF EXPOSURE, AND THE ROLE OF IRRITANCY AND IRRITANT-INDUCED INFLAMMATION.

J. H. Arts[1], N. Bloksma[3,2] and C. F. Kuper[1]. *[1]TNO Nutrition and Food Research Institute, Zeist, Netherlands, [2]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Netherlands and [3]Faculty of Biology, Utrecht University, Netherlands.* Sponsor: V. Feron.

Given the serious health problems caused by occupational respiratory allergy and the ever- increasing stream of new chemicals into workplaces, the early identification of chemical respiratory allergens is vital. Although a number of test protocols have been developed to predict respiratory allergenic potential, none of these are fully accepted. Using our test protocol, a two times topical application of trimellitic anhydride (TMA) followed by airway challenge of Brown Norway (BN) rats induced several changes when compared to non-sensitized rats. While there are good indications in laboratory animals and humans that skin exposure can act as a route for respiratory tract sensitization, less is known about the effect of the route on the type of immune reaction evoked and on dose-response relationships. Intranasal, inhalation and dermal routes were therefore compared to examine whether TMA-induced effects were dose-related or route-of-induction related. Preliminary results indicated that topically sensitized rats showed the largest effects. As to the intensity of exposure, using toluene diisocyanate (TDI), it was observed that a two-times topical application was not sufficient to increase total IgE, but the same total amount of TDI given in more applications within the same time frame did result in increased total IgE levels. Finally, as to the role of irritation and irritant-induced inflammation, allergic and irritant responses to TMA resulted in quite distinct breathing patterns; pre-exposure to irritating concentrations of SO2, however, offered some protection to TMA challenge in TMA-sensitized BN rats. In conclusion, the results of the present studies suggest that the BN rat is a promising model to predict chemical respiratory allergenic potential. This research is funded by the Dutch Ministry of SZW and CEFIC-LRI (Brussels, Belgium).

1626 CURRENT STATE OF PREDICTING THE RESPIRATORY ALLERGY POTENTIAL OF CHEMICALS: WHAT ARE THE ISSUES?

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Many chemicals are clearly capable of eliciting immune responses in sensitized individuals, which, following re-exposure, can result in adverse allergic reactions. It is also generally agreed that contact sensitivity is the most common occupational health problem, and that respiratory sensitization occurs less frequently, and in a more restricted range of chemical classes. Various *in vivo* contact hypersensitivity tests, including the Buehler patch test, the mouse ear swelling test, and the local lymph node assay, have successfully identified contact sensitizers, although respiratory sensitizers also test positive in these assays. Guinea pig inhalation studies have been utilized to identify respiratory sensitizers, but these studies are expensive and labor-intensive and typically require protein conjugation of the chemical of interest in order to elicit a response. This workshop has highlighted pulmonary function testing, cytokine profiling and measurement of IgE as new, potential approaches for identifying respiratory sensitizers. While these techniques offer many advantages over the guinea pig models, further work is needed to validate these methodologies and to consider practical issues such as: which species and strain of animal to use; whether total IgE can be used as a surrogate for antigen specific reaginic antibody; and if measurement of IL4 after *in vitro* stimulation with mitogen is a reliable technique for identifying only respiratory sensitizers. Furthermore, timing and dose response curves for both the sensitization and challenge phases of the protocol need to be established to determine threshold and irritating concentrations of chemical, and to compare cytokine and antibody responses at doses which elicit the same level of immune-mediated hypersensitivity reactions. This abstract does not reflect EPA policy.

1627 NMR EVIDENCE FOR SIMULTANEOUS BINDING OF MIDAZOLAM WITH ALPHA-NAPHTHOFLAVONE OR TESTOSTERONE WITHIN THE ACTIVE SITE OF CYP3A4.

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Recent studies have indicated that CYP3A4 exhibits non-Michaelis-Menten kinetics for numerous substrates. Both homotropic and heterotropic activation have been reported and kinetic models have been used to suggest multiple substrates within the active site. We provide the first physio-chemical data supporting the hypothesis of allosteric binding within the CYP3A4 active site. Midazolam, a commonly used surgical anesthetic, is metabolized by CYP3A4 to two hydroxylated metabolites, 1'-OH- and 4-OH-midazolam. Incubations using purified CYP3A4 and midazolam showed that both α-naphthoflavone (α-NF) and testosterone affect the ratio of 1'-OH and 4-OH-midazolam. Similar to previous reports, α-NF was found to stimulate the formation of 1'-OH-midazolam while testosterone stimulated formation of 4-OH-midazolam. NMR was used to measure the closest approach of individual midazolam protons to the paramegnetic heme iron of CYP3A4 using paramagnetic T_1 relaxation times. Midazolam was ideally suited for these studies because the proton signals corresponding to both sites of oxidation were easily monitored along with two other protons, 2-H and one of the flourophenyl group protons. Incubations of 0.25 μM CYP3A4 and 500 μM midazolam resulted in calculated distances between 6.9 and 7.1 Å for all four monitored midazolam protons, consistent with free rotation within the active site or sliding parallel to the heme plane. Inclusion of 50 μM α-NF resulted in shortened proton-heme iron distances ranging from 5.6 to 6.7 Å. Consistent with kinetics of activation, the 1'-position was situated closest to the heme while the flourophenyl proton was the furthest. Proton-heme iron distances for midazolam with CYP3A4 and 50 μM testosterone ranged from 6.7 to 7.5 Å with the fluorophenyl proton furthest from the heme iron. Additionally, testosterone and α-NF experience paramagnetic relaxation when incubated with midazolam and CYP3A4, demonstrating their proximity to the heme iron in the active site. (Supported by NIH grant No. GM-32165, to SDN)

1628 THE EFFECT OF CHRONIC ETHANOL FEEDING ON THE PROTEIN LEVEL AND ACTIVITIES OF CYP3A ENZYMES IN THE LIVER OF MALE AND FEMALE SPRAGUE-DAWLEY RATS.

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Recent studies indicate that rat CYP3A subfamily is represented by at least four structural genes CYP3A1, CYP3A2, CYP3A9 and CYP3A18. In this study we investigated the protein levels and activities of CYP3A subfamily members in liver of control and ethanol-fed male and female rats. Chronic ethanol feeding (Lieber-DeCarli diet) of rats for 14 days results in the appearance of CYP3A1 protein in hepatic microsomes of female rats but not in male rats. Chronic ethanol feeding of rats of both sexes did not induce CYP3A2, an enzyme present in normal males but not females. Ethanol feeding did not effect the content of hepatic microsomal CYP3A9 and CYP3A18 in rats of either sex. We found that the enzymatic activities specific for CYP3A enzymes follow the change in immunodetecable CYP3A1. Thus, in hepatic microsomes from ethanol-treated female rats, there is an increase in quinine 3OH hydroxylation, a reaction that is more actively catalyzed by recombinant CYP3A1 compared to recombinant CYP3A2. Alprazolam hydroxylation changed little in hepatic microsomes of both untreated and treated rats. Thus, we found that ethanol has selective effect on the induction of catalytically competent CYP3A1 in the liver of female rats but not in males. Therefore, the induction of some CYP3A isozymes when large amounts of ethanol are consumed might have toxicological and pharmacological importance. (Supported by ES 05022)

1629 4-IPOMEANOL: MECHANISM-BASED INACTIVATOR OF CYP3A4.

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4-Ipomeanol, [1-(3-furyl)-4-hydroxypentanone], is a natural lung cytotoxin in animals bioactivated by cytochrome P450 enzymes into reactive metabolites. It has been investigated as a potential chemotherapeutic pro-drug. However, clinical studies reported unexpected dose-limiting liver toxicity, with no other side effects. The biochemical mechanism of its selective liver toxicity in humans is unknown. We tested 4-ipomeanol as a possible mechanism-based inactivator (MBI) of cytochrome P450 3A4 (CYP3A4). Methods: Incubations mixtures containing human recombinant CYP3A4, P450 oxido-reductase and increasing concentrations of 4-ipomeanol were prepared in microplates. The reactions were initiated by the addition of the cofactor nicotinamide adenine dinucleotide phosphate (NADPH). At 0, 4, 8 and 12 minutes, aliquots were withdrawn and assayed for CYP3A4 activity using the dibenzylfluorescein (DFB) / fluorescein assay. Some reaction mixtures included glutathione, catalase / superoxide dismutase (SOD), or DBF. Results: 4-ipomeanol accomplished all the criteria used to classify a compound as an MBI; **1.** 4-Ipomeanol produced a time- and concentration-dependent loss of the activity of CYP3A4. More than 80 % of the CYP3A4 activity was lost after its incubation with 4-ipomeanol for 12 minutes. Saturation kinetic was observed for concentrations of 4-ipomeanol higher than 75 μM. **2.** The inactivation of CYP3A4 was irreversible and NADPH- dependent. **3.** Glutathione and scavengers of reactive oxygen species, catalase/SOD, failed to protect CYP3A4 from inactivation by 4-ipomeanol. **4.** DBF substrate protected CYP3A4 for a limited time. In contrast, 4-ipomeanol metabolism had no effect in the activity of human CYP1A2, CYP2D6 or CYP2C9. Conclusion: 4-ipomeanol was found to be a potent and selective MBI of human CYP3A4 (K_{inact} = 0.07 min^{-1}, and K_I.of 20 μM). The association of CYP3A4 inactivation with hepatotoxicity is under investigation.

1630 QUANTIFICATION OF ROS PRODUCTION AND ENZYME INACTIVATION OF CYP1A BY CHLORINATED BIPHENYLS AND DIBENZO-*p*-DIOXINS.

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The mechanisms by which planar (non-*ortho*-substituted) polychlorinated biphenyls (pPCBs) and polychlorinated dibenzo-*p*-dioxins exert toxicity are not well known. These compounds elicit oxidative stress, and prior work has shown microsomal cytochrome P450 1As (CYP 1A1) to be irreversibly inactivated by pPCBs and tetrachlorodibenzo-*p*-dioxin in an O_2-, NADPH-, and time-dependent process. This process is accompanied by the release of reactive oxygen species (ROS) but the quantitative relationship between inactivation and ROS release and the identity of the inactivating species are unknown. We have fluorometrically quantified the production of superoxide (O_2^-) by liver microsomal CYP 1A using dihydroethidium and an external standard consisting of the xanthine/xanthine oxidase system. NADPH oxidation was followed spectrophotometrically to examine the kinetic rates of pPCB-dependent enzyme inactivation and associated kinetic parameters of the pPCB-NADPH-cytochrome P450 reductase-CYP 1A system. With 3, 3′, 4, 4′-tetrachlorobiphenyl (PCB-77), O_2^- production rates were found to be as much as 90% of NADPH oxidation rates, suggesting that uncoupling by PCB-77 leads to ROS release after the addition of the first electron. The rate of inactivation of CYP1A (k_i) was decreased by the addition of increasing concentrations of DMSO. As DMSO is a small-molecule radical scavenger, these results, in addition to the increase in k_i observed when HOOH is added to CYP 1A, suggest that hydroxyl radicals generated in the active site could be responsible for the pPCB-dependent enzyme inactivation. A novel fluorescamine-derivatized nitroxide spin trap has been employed to characterize the radical production. The release of ROS resulting from the uncoupling of CYP 1As by pPCBs represent a possible mechanism of toxicity of these aryl hydrocarbon receptor agonists. (EPA R 827102-01-0 and NIH P42-ES07381)

1631 FUNCTIONAL ANALYSIS OF POLYMORPHISMS IN THE PROMOTER OF HUMAN CYTOCHROMES P450 1A1 AND 1B1.

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Cytochromes P450 1A1 (CYP1A1) and 1B1 (CYP1B1) are major phase I PAH and estrogen metabolizing enzymes expressed in human lung. Inter-individual variation in the expression of both enzyme transcripts and activity has been observed in human. We have evaluated the functional consequences of SNPs, singly and in combination, in the constitutive and inducible expression of these genes. METHOD: Genomic DNA from peripheral blood mononuclear cells of twenty individuals was PCRed in a multiple overlapping fragment strategy for 1.5kb of high GC content promoter region of CYP1A1 and CYP1B1, and then direct cycle sequenced in duplicate. We found 12 CYP1A1 and 7 CYP1B1 polymorphisms, existing singly or in combination, that occur commonly in our subjects. We then cloned 1.5 kb insert sequence of each gene into a PGL3-basic luciferase reporter construct, and used iterative site-directed mutagenesis (Stratagene) to replicate the 12 CYP1A1 variants and 7 CYP1B1 variants. The constructs with wild-type and mono- or polycistronic variant insert were then subcloned into virgin PGL3 vector, to affirm that regulatory features were indeed a result of the 1.5kb insert sequence, and not due to the site-directed mutagenesis procedure itself. The subclones were then transfected into normal human bronchial epithelial cells (NHBE, Clonetics), and exposed to cigarette smoke extract (CSE), B[a]P and estradiol, each in three different doses. Additional cotransfections with estrogen receptor alpha were performed. RESULTS: To date, we have found no major expression differences between the wild-type and variant constructs for any haplotype, for reporter gene expression in response to CSE, B[a]P, nor on the repression effects of the chemopreventive agent resveretrol. Co-transfection of the NHBE cells with ER-alpha does appear to have additive effects with the inducing agents, but this appears independent of estradiol exposure. We are in the process of further evaluating carcinogen-hormonal interaction in estrogen receptor expressing lung cells.

1632 LACK OF A ROLE FOR CYP1A2 IN THE ACTIVATION OF 4-AMINOBIPHENYL INTO DNA-REACTIVE METABOLITES IN MICE.

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4-Aminobiphenyl (ABP) is a prototypic aromatic amine that causes liver cancer in mice and bladder cancer in mice and humans. Metabolic activation of ABP is believed to involve N-hydroxylation, and CYP1A2 is the most active human or rodent CYP to catalyze this reaction. To assess the role of CYP1A2 in ABP activation to DNA-binding metabolites *in vivo*, we treated wild-type [Cyp1a2(+/+)] and Cyp1a2 knockout [Cyp1a2(-/-)] mice with ABP (50 umol/kg topically). Some mice had prior induction of CYP1 by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD; 5 ug/kg, i.p.). Histopathology of liver and urinary bladder after this acute dose (24 h) showed an insignificant ABP effect, but untreated Cyp1a2(-/-) showed more fat deposits in the liver than untreated Cyp1a2(+/+), and TCDD increased fat deposits >2-fold in both genotypes. After 24 h, both male and female Cyp1a2(-/-) mice had similar levels of ABP-DNA adducts in liver and bladder as wild-type, with the exception that female Cyp1a2(-/-) actually had 3 to 4-fold higher levels of hepatic adducts than wild-type. In both genders and genotypes, TCDD pretreatment lowered both liver and bladder adducts. Interestingly, female mice of both genotypes displayed higher levels of liver DNA adducts compared to males, whereas males had higher levels of bladder DNA adducts compared to females. These results show clear sex differences in the pattern of liver and bladder DNA adducts produced by ABP. Furthermore, the results suggest that CYP1A2 does not appear to be a major contributor to the pathway responsible for ABP activation to DNA binding metabolites in mice. (Supported by NIH grants P30 ES06096, RO1 ES10133, RO1 ES06321, R01 ES08147)

1633 P450-MEDIATED METABOLISM OF CAPSAICIN DECREASES CYTOTOXICITY TO LUNG AND LIVER CELLS.

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Capsaicin, a common dietary constituent, homeopathic remedy, and component of pepper spray self-defense weaponry, has been shown to be a potent respiratory irritant and toxicant. Capsaicin can cause acute respiratory dysfunction and failure with an LD50 of 1.6 and 190 mg/kg for intratracheal and oral doses in mice. We investigated the metabolism of capsaicin by liver and lung microsomes and recombinant P450 enzymes to ascertain the role of metabolism in the apparent lung-selective toxicity of capsaicin. LC/MS/MS and 1H-NMR were used to identify several metabolites of capsaicin produced by aromatic and alkyl hydroxylation, O-demethylation, N- and alkyl dehydrogenation, and macrocycle formation. The rates of metabolism were 11.0 and 0.6 nmol/min/mg for human liver and lung microsomes, respectively. Metabolism was limited to CYP1A1, 1A2, 3A4, 2B6, 2C8, 2C9, 2C19, 2D6, and 2E1. Microsomal metabolism of capsaicin was inhibited by 1-aminobenzotriazole (1-ABT) and stimulated by glutathione. Studies with recombinant CYP2E1 demonstrated that capsaicin was a potent inhibitor of this enzyme. Addition of 1-ABT to human lung bronchiolar epithelial and hepatoma cell cultures treated with capsaicin showed that inhibition of metabolism increased cytotoxicity. These data suggest that P450-mediated metabolism of capsaicin represents

a detoxification mechanism for capsaicin rather than bioactivation, despite evidence that potentially cytotoxic reactive metabolites are produced. Additional studies have identified the capsaicin receptor (TRPV1 or VR1) as a key mediator for the cell type- and tissue-selective cytotoxicity of capsaicin. Supported by NIST (Contract# 60NANBOD0006) and the Colgate-Palmolive Post Doctoral Fellowship in *In Vitro* Toxicology.

1634 EFFECTS OF NATURAL AND SYNTHETIC FLAVONOIDS ON AROMATASE (CYP19) ACTIVITY IN H295R HUMAN ADRENOCORTICAL CARCINOMA CELLS.

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Flavonoids and related structures (flavones, isoflavones, flavanones, catechins) have various biological properties, including anticarcinogenic, estrogenic and anti-estrogenic effects; they also modulate sex hormone homeostasis. A key enzyme in the synthesis of estrogens from androgens is aromatase (CYP19). We investigated the effects of various naturally occurring and synthetically derived flavonoids on the synthesis of estrogens by the enzyme aromatase in H295R human adrenocortical carcinoma cells. Among the natural flavonoids (in order of descending potency) chrysin, apigenin and narengenin were relatively weak inhibitors of aromatase activity (IC_{50}≥10 μM). Flavone (base structure) appeared to be an inducer, increasing aromatase activity just under 2-fold at 30 μM. Rotenone, a synthetic flavone-derivative and fish poison, was a relatively potent inhibitor of aromatase with an IC_{50} of 0.5 μM; cytotoxicity occurred at concentrations of 3 μM and above. The steroidal aromatase inhibitor 4-hydroxyandrostenedione, used as positive control, had an IC_{50} of about 20 nM. Several other synthetic flavonoid and structurally related quinolone analogs were screened and found to have various effects on aromatase activity, including both inhibition or induction of the enzyme activity at concentrations in the high nanomolar to lower micromolar range. Currently, studies are underway to determine structure-activity relationships for induction or inhibition of aromatase activity and mRNA expression by flavonoid structures in H295R cells.

1635 A MOUSE MODEL WITH LIVER-SPECIFIC DELETION OF THE NADPH-CYTOCHROME P450 REDUCTASE GENE.

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To determine the relative roles of liver and extrahepatic tissues in the metabolic activation and tissue-selective toxicity of xenobiotic compounds, we have generated a mouse model for tissue-selective deletion of the NADPH-cytochrome P450 reductase (CPR) gene. The consequent loss of CPR expression will lead to a suppression of the activities of all microsomal P450s. The recombinant Cpr allele (named CPR^{lox}) contains loxP sequences in introns 2 and 15, respectively, which did not cause any changes in CPR expression. In the presence of Cre recombinase (Cre), the intervening exons would be deleted, leading to a null mutation. This is demonstrated by crossing $CPR^{lox+/+}$ mice with transgenic mice having liver-specific Cre expression (named Alb-Cre). Liver microsomal CPR level was about 50% lower in $CPR^{lox+/-}$/Alb-$Cre^{+/-}$ mice than in $CPR^{lox+/-}$/Alb-$Cre^{-/-}$ littermates at three weeks of age, and CPR levels in other tissues were not affected. Thus, the modified CPR gene can be efficiently and specifically deleted from the liver. The decreased level of CPR protein was accompanied by significant decreases in liver microsomal activities in the metabolic activation of two known P450 substrates, acetaminophen and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Homozygous $CPR^{lox+/+}$/Alb-Cre^{+} mice, which are viable, have also been obtained and are being characterized. (Supported in part by NIH grant ES07462)

1636 GENE EXPRESSION PROFILING OF SKIN CARCINOGENESIS IN MICE USING CDNA MICROARRAYS.

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The purpose of this study was to use cDNA microarrays to investigate the complex alterations in gene expression that occur during the promotion phase of the classic two-stage DMBA/TPA skin carcinogenesis model. The shaved dorsal skin of FVB/N female mice (3-5 weeks old) was topically painted with a single dose of DMBA. TPA promotion commenced one week later and continued twice weekly. Histology and microarray analysis were performed using skin excised after promotion for 2, 4, 6 and 15 weeks. cDNA microarrays were made in-house and consisted of 4147 mouse ESTs, including genes involved in apoptosis, oxidative stress, cell signalling and skin homeostasis. Mild diffuse epidermal hyperplasia was evident after 2 weeks of promotion with TPA and persisted for 15 weeks of TPA promotion. This hyperplasia was also evident in mice that had received TPA alone. Squamous papillomas were evident in 100% of the mice following 9 weeks of TPA promotion. No papillomas were seen in mice treated with DMBA or TPA alone. The maximum tumour burden was seen following 12 weeks of TPA promotion (16 ± 7.6), between 12 and 15 weeks some tumours coalesced reducing the number of individual papillomas. All papillomas were keratinised well-differentiated squamous neoplasms without evidence of invasive growth. Genomic analysis of the 15-week skin samples containing papillomas revealed an increase in the expression of the keratin gene family members. This was supported by histological data showing abundant keratin within the papillomas. Additionally members of the cornified envelope, indicative of epidermal cell differentiation (*loricrin, desmoglein 2* and *filaggrin*) were also increased in expression. Gene expression data will be presented in full for all time points analysed and will show how it has been possible to relate changes in gene expression to the pathology of skin carcinogenesis. The data provides further insight into the molecular events underlying the pathological changes in the promotion stage of this model.

1637 DMBA INDUCED DISRUPTION OF BONE MARROW HYPOCELLULARITY IS TNF-α DEPENDENT.

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Polycyclic aromatic hydrocarbons (PAHs) are potent carcinogens and immunotoxicants. Using the prototypical PAH, 7, 12-dimethlybenz[a]anthracene (DMBA), we have shown previously that DMBA administration depletes the bone marrow of progenitor B cells and granulocytes *in vivo* and DMBA metabolites cause apoptosis of progenitor B cells *in vitro*. The goal of this study was to determine a mechanism of action for the effects of DMBA on the B cell population in the bone marrow. Our hypothesis was that DMBA exerts its adverse effects on the bone marrow, at least in part, by stimulating TNF-α production by bone marrow stromal cells. TNF-α can then up-regulate PKR (dsRNA dependent protein kinase), which in turn can activate p53, leading to apoptosis in the susceptible bone marrow cell populations. In support of this hypothesis, we have demonstrated that bone marrow cells produce TNF-α in response to DMBA *ex vivo*, and that TNF receptor gene deleted mice (null for both p55 and p75) are resistant to DMBA induced bone marrow cell depletion. We have observed PKR up-regulation in 70Z/3 cells (a murine preB cell line) after DMBA treatment, and in whole bone marrow cell lysates after i.p. injection of DMBA in C57BL/6 mice. Furthermore, p53 gene deleted mice are resistant to DMBA mediated bone marrow cell depletion, thus indicating that the process is p53 dependent. These studies demonstrate that TNF-α and its down-stream signaling pathways may play an important role in the mechanism of DMBA mediated bone marrow cell depletion.

1638 NATURALLY OCCURRING COUMARINS INHIBIT HUMAN CYTOCHROMES P450 AND BLOCK BENZO(a)PYRENE AND 7, 12-DIMETHYLBENZ[A]ANTHRACENE DNA ADDUCT FORMATION IN MCF-7 CELLS.

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Naturally-occurring coumarins (NOCs) inhibit skin tumor initiation in mice by blocking cytochrome P450 (CYP)-mediated bioactivation of benzo[a]pyrene (B[a]P) and 7, 12-dimethylbenz[a]anthracene (DMBA). Bergamottin selectively inhibits tumor initiation by B[a]P, whereas imperatorin and isopimpinellin inhibit tumor initiation with both carcinogens. The goals of the current study were to examine the whether NOCs, which are anticarcinogenic in mice, can block carcinogen bioactivation in cultured human cells. In these experiments we examined the effects of bergamottin, imperatorin, and isopimpinellin on DMBA and B[a]P DNA adduct formation in the human breast adenocarcinoma cell line, MCF-7. Co-incubation of cells with the three different NOCs significantly inhibited DMBA DNA adduct formation by 29% to 82% at doses ranging from 2 to 10 microM; and significantly inhibited B[a]P DNA adduct formation by 37% to 80% at doses ranging from 20 to 80 microM. HPLC analysis of the DNA hydrolysates demonstrated that inhibition of DNA adducts corresponded to inhibition of the major B[a]P and DMBA diol-epoxide derived adducts. Furthermore, these NOCs

significantly inhibited CYP mediated ethoxyresorufin O-dealkylase activities in MCF-7 cells treated with acetone, B[a]P, and DMBA. Bergamottin, which was not effective at blocking DMBA bioactivation in the mouse skin model, had effects similar to imperatorin and isopimpinellin in MCF-7 cells. These results demonstrate that NOCs, which are present in citrus fruits and other components of the human diet, are capable of inhibiting carcinogen metabolizing enzymes and blocking bioactivation of both B[a]P and DMBA in MCF-7 cells. (CA 79442, CA 16672, ES07783).

1639 SUPPRESSION OF FERTILITY AND ITS REGULATORY HORMONES BY INHALED BENZO(a)PYRENE (BaP).

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Two experiments were conducted where F-344 rats were assigned randomly to a treatment and a control group, to evaluate the effect of BaP on fertility and the hormones that regulate this process. In experiment 1, treatment consisted of sub-acute exposure of rats *via* inhalation to 100 $\mu g/m^3$, 4 hr/day for 10 days. From the 4th day of exposure, animals in the control (unexposed) and treatment group were synchronized with subcutaneous progesterone (P_4) injections (1 mg P_4/animal /day for 4 days). Two days were allowed for unstimulated folliculogenesis to occur, which corresponded with the last day of BaP exposure. Blood samples were collected daily for plasma, beginning from 24 hr post P_4 administrations until the cessation of exposures, by sinus retro-orbital puncture. Plasma samples were assayed for ovarian steroids. In experiment 2, pregnant rats were similarly exposed from day 8 of gestation through day 21. Blood samples were similarly collected on day 17 only for the determination of ovarian steroid and placental lactogen (PL) concentrations in plasma. Postpartum litter size was determined for exposed rats and compared to those of controls with unpaired T-test. Hormone levels for animals in experiment 1 were analyzed by ANOVA with repeated measures while those for animals in experiment 2 were analyzed with a one-way ANOVA. The differences among means for hormone data in the 2 experiments were tested for significance by orthogonal contrasts. Exposure to BaP resulted in a reduction of circulating ovarian steroid concentrations in non-pregnant and pregnant rats. Similarly, litter size as well as pups characteristics were affected by BaP exposure. A concomitant decrease in PL was observed among BaP-exposed rats. These data suggest that inhaled BaP compromised fertility and the hormones necessary for the regulation of this process. (Supported by MHPF/ATSDR grant #U50/ATU398948, NCRR-RCMI grant #G12RR03032, NIGMS-SCORE grant # 2S06GM08037-28.

1640 BENZO(a)PYRENE DIONES PREVENT EGF WITHDRAWAL-INDUCED APOPTOSIS IN HUMAN MAMMARY EPITHELIAL CELLS THROUGH THE REACTIVE OXYGEN SPECIES (ROS)-DEPENDENT ACTIVATION OF AKT.

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Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP), are known mammary carcinogens in rodents and may be involved in the development of human breast cancer. Previous work in this lab has shown that BaP can mimic growth factor-like signaling in human mammary epithelial cells (HMECs). We have hypothesized that the mitogenic response to BaP may be due to the metabolic conversion of BaP into BaP-diones (i.e. BaP-quinones, BPQs) which redox-cycle to produce reactive oxygen species (ROS) and activate growth factor receptor pathways. Here, we demonstrate by electron paramagnetic spectroscopy (EPR) and gene expression analysis that 1, 6- and 3, 6-BPQ produce ROS in a growth factor dependent, spontaneously immortalized HMEC cell line, MCF-10A. Similar to BaP, treatment of MCF-10A cells with 1, 6- and 3, 6-BPQ increased cell number despite the absence of EGF in the media. This effect is likely due to a BPQ-induced protection from EGF withdrawal-induced apoptosis, since BPQs reduced Annexin-V staining as determined by FACS analysis. BPQs also produced a concentration-dependent increase in Akt phosphorylation occurring between 12 and 24 h of BPQ treatment. Co-treatment of MCF-10A cells with PI3-K inhibitors and N-acetylcysteine attenuated Akt phosphorylation and appeared to re-establish EGF withdrawal-induced apoptosis. Therefore, the ROS-dependent activation of PI 3-K/Akt by BPQs may represent one mechanism by which BaP increases HMEC cell proliferation in the absence of exogenously added growth factors. This work was supported by NIEHS R01-ES-07259 and ADB by the US Army Medical Research Grant BC-010026.

1641 BROMODICHLOROMETHANE TOXICOKINETICS: LINKING METABOLISM TO EFFECT.

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Bromodichloromethane (BDCM), a trihalomethane (THM), is among the most prevalent disinfection byproducts found in chlorinated drinking water. Weak associations between THM exposure and cancers of the bladder and lower GI tract have been suggested by positive epidemiological studies of chlorinated drinking water exposures in humans. Chronic high-dose administration of BDCM to rodents induced kidney and colon carcinomas. A series of *in vivo* and *in vitro* studies have been undertaken to evaluate the toxicokinetics and effects of BDCM. BDCM is metabolized by cytochrome P450 isozymes and by cytosolic glutathione *S*-transferase theta 1-1 (GST T1-1). CYP2E1 is the principal metabolizing enzyme in both humans and rats, and the *in vitro* metabolic parameters for recombinant preparations of human and rat CYP2E1 were almost identical. We have found that CYP1A2 metabolizes BDCM, and can now report a lower Km for the human enzyme compared to its rat counterpart. BDCM is also metabolized to a mutagenic intermediate(s) *via* GST T1-1. The relative mutagenic potency of the four THMs correlates well with their relative potency as inducers of preneoplastic aberrant crypt foci in the rat large intestine. GST T1-1 is the primary catalyst for BDCM-glutathione conjugation, leading to product(s) that covalently modify DNA and form deoxyguanosine adducts *in vitro*, consistent with previous observations of GST T1-1-dependent mutagenicity of BDCM. Additional metabolites of this pathway were identified, including S-formyl-GSH and formate. Comparison of the catalytic efficiencies of CYP2E1- and GST T1-1-mediated BDCM metabolism indicate that GST-dependent metabolism is a quantitatively minor pathway, but glutathione conjugation may give rise to extremely reactive intermediates that induce disproportionately toxic responses. Tissues (kidney and colon) that have higher ratios of GST T1-1:CYP2E1 activities are targets for BDCM-induced carcinoma in rats. (MK Ross and G Zhao were supported by UNC/EPA Cooperative Training Agreement CT827206. This abstract does not necessarily reflect EPA policy.)

1642 UPTAKE AND DISPOSITION OF 1, 1, 1-TRIFLUOROETHANE IN MAN.

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Hydrofluorocarbons (HFC) are used as substitutes for the ozone depleting chlorofluorocarbons, e.g. in refrigeration. There are scarce human data on the toxicokinetics and toxicology of HFCs. The aim of the study was to determine the toxicokinetics of 1, 1, 1-trifluoroethane (HFC-143a) in humans. Nine occupationally exposed male volunteers were exposed to 500 ppm trifluoroethane for 2 hours during light physical exercise (50W) in an exposure chamber. Blood, urine and exhaled air were collected before, during and up to 2 days after exposure and analysed for trifluoroethane by headspace gas chromatography. Trifluoroacetic acid and fluoride in urine were analysed using high performance liquid chromatography and a selective ion electrode, respectively. The electrocardiogram was monitored during and until 20 hours after exposure. Irritative and central nervous system symptoms (e.g. discomfort in the eyes, headache and dizziness) were rated in a questionnaire prior to, during, and after exposure. The study was approved by the Regional Ethical Committee. Preliminary analyses suggest no increases in symptom ratings during or after exposure. The uptake of trifluoroethane in the human body was only a few per cent. As expected, trifluoroethane in blood increased rapidly during exposure and decreased rapidly after exposure. A plateau of about 1.4 ppm trifluoroethane was reached. Two elimination phases were observed with half times of about 4 and 300 minutes, respectively. The toxicokinetic behaviour is explained by the low metabolic rate and low solubility in blood. Increased fluoride concentration in urine was seen in two of nine volunteers. To our knowledge, this is the first human toxicokinetic study of trifluoroethane.

1643 STANDARDIZATION OF THE TAPE-STRIP SAMPLE BY DETERMINATION OF KERATIN IN THE SAMPLE AFTER EXPOSURE TO JET FUEL.

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Chemicals may bind to and react with keratin proteins in the skin. We have evaluated a non-invasive tape-stripping method for the extraction and quantification of napthalene and keratin from the skin for normalization and exposure assessment. Samples from 20 human volunteers were examined before and after exposure to 25 µl of jet fuel (JP-8). Due to variable amounts of squame recovered with each tape-stripped sample, we investigated the need to normalize extracted chemical to the amount of tissue stripped using keratin as a biomarker. Keratin proteins were ex-

tracted in a buffer and quantified using a modified Bradford Method (AmrescoTM). Confirmation of the extraction of keratin was verified by western blotting using a monoclonal mouse anti-human cytokeratin antibody (DakoTM Corporation). Tape stripping removed a consistent amount of keratin from the skin, which decreased with sequential tape strippings, and naphthalene was effectively quantified during the 25-min exposure from individuals with normal skin. The mean mass of keratin proteins for sequential tape strips varied from 154 ± 75.3 μg for the first tape strip to 52.8 ± 17.3 μg for the fifth tape strip for the unexposed sites and from 128 ± 63.8 μg for the first tape strip to 58.4 ± 21.6 μg for the fifth tape strip for the exposed sites. Jet fuel exposure did not affect the amount of keratin recovered from the tape strips. There was no significant difference in the removal of keratin per tape strip from unexposed and exposed sites between sex, age, ethnicity, or skin pigmentation. We conclude that the actual concentration of naphthalene, as a marker for jet fuel exposure, per unit of keratin can be determined using this method, which may prove to be required when measuring occupational exposures under occupational field settings. The technique developed may be a powerful tool to measure concentration of a compound in the skin and to determine dermal exposure. Supported by NIEHS P42 ES05948, USAF *via* Texas Tech University 1331/0489-01, & NIOSH 5-52678.

1644 IDENTIFICATION OF BIOMARKERS AND MECHANISMS OF THE ACUTE PHASE RESPONSE IN LIVER USING A CANINE MICROARRAY.

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A greater understanding of mechanisms of toxicity and better markers to measure adverse effects in the dog would improve our ability to extrapolate risk to humans. However, the tools to find these markers and mechanisms are lacking in the dog compared to other commonly used pre-clinical species, such as the rat and mouse. For this reason, we developed an Affymetrix-based oligonucleotide microarray capable of monitoring the expression of thousands of canine genes simultaneously. The array is composed of 22, 774 probe sets, with 13, 675 canine sequences, 115 normalization controls, and standard non-eukaryotic Affymetrix hybridization controls. An additional 8, 945 probe sets, designed to the terminal 600 bases of the coding region of full length human cDNAs to improve cross-species hybridization, were tiled on the array. To assess the ability of this genomic tool to detect differential gene expression, the array was used to profile the acute phase response in male beagle dogs after a single dose (0.2mg/kg) of lipopolysaccharide (LPS). Hepatic gene expression 4 and 24 hours post-LPS administration was compared to vehicle-treated animals (3 per treatment group). Further validating the array, a strong correlation was shown between gene expression data and alterations in clinical chemistry parameters such as serum amyloid A (SAA), alkaline phosphatase, and aspartate aminotransferase. Microarray data illustrated early aspects of an acute inflammatory response, with multiple cytokines and acute phase proteins induced 4 hours post treatment. The most robust transcriptional change was observed with SAA; transcript levels were induced 30-fold by 4 hours and steadily increased thereafter. In contrast, the most profound changes observed only at 24 hours involved genes associated with glucose and cholesterol metabolism, and biotransformation. In addition to these mechanistic findings, this new genomic tool identified several potential biomarkers that could serve as indicators of inflammation in human patients.

1645 SEPARATING GENES BETWEEN CHEMICAL SPECIFIC RESPONSES AND GENERAL STRESS RESPONSES BASED ON EXPRESSION PROFILES IN RAT HEPATOCYTES EXPOSED TO CADMIUM AND HYDRAZINE.

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DNA microarray analysis is a useful technique for studying the molecular basis for chemical toxicity from a global prospective. However, chemical exposure results in changed expression of a large number of genes. Some changes may be related to metabolic activity reflecting adaptive response, while others may be related to toxicity with adverse outcome. Besides affecting genes involved in general stress response, toxicants also have effects on genes responsive to specific stress/damage imposed by the toxic insults. We have identified that the expression levels of 91 and 133 genes were changed in primary cultures of rat hepatocytes exposed to cadmium and hydrazine, respectively. Unexpectedly, changes in expression levels of 209 genes were observed in untreated controls during a 24-hour period, which are probably resulted from dedifferentiation and adaptation to the *in vitro* culture environment of rat hepatocytes. Analysis of these gene lists revealed that 65 of 91 cadmium-related genes and 84 of 133 hydrazine-related genes are likely related to general stress response. Consistent with this notion, there is a significant overlap between the stress-related genes affected by these chemicals (i.e., 52 genes were identical). In contrast, 26 of 91 cadmium-related genes and 49 of 133 hydrazine-related genes appeared to be chemical specific. Between these two groups, only 11 genes were identical. The general stress genes were further characterized using Self Organization Map (SOM) Clustering. By comparing the clustering patterns of cadmium and hydrazine treatments with that of the controls, we were able to further divide genes into subgroups that are likely co-regulated under these experimental conditions. This result provides some insight into the molecular events associated with cadmium and hydrazine exposure.

1646 ALTERED PROTEIN / DNA INTERACTIONS OF LIVER ENRICHED TRANSCRIPTION FACTORS AS PREDICTORS FOR TOXICITY - IMPLICATIONS FOR TOXICOGENOMIC RESEARCH.

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New platform technologies offer unique opportunities to explore simultaneously an expression of thousands of genes and proteins and in conjunction with other molecular endpoints, reliable predictions of drug safety may now be feasible, at early stages of drug development. Drug induced alterations in gene and protein expression are also considered to be of critical importance in the onset and progression of organ toxicity and major efforts are on the way to determine the relevance of individual expression profiles in response to drug exposure. The observed changes in target gene/protein expression may be traced back to modulation of transcription factor protein/DNA interactions. Examples will therefore be presented that demonstrate, on the one hand, the severe limitations in the use of tumor cell lines and other badly validated cell culture models for the prediction of tissue specific toxicity and, on the other hand, how properly designed and validated *in vitro* models hold promise for reliable predictions of hepatotoxicity. Results from studies with hepatotoxins, such as Aroclor1254, are given to provide the experimental evidence for the principality of this approach. (1) Schrem, H., Klempnauer, J. and Borlak, J. Liver enriched transcription factors in liver function and development. Pharmacological Reviews 2002, 54: 129 -158 (2) Borlak, J., Dangers, M. and Thum, T. Aroclor 1254 modulates gene expression of nuclear transcription factors: Implications for albumin gene transcription and protein synthesis in rat hepatocyte cultures. Toxicology and Applied Pharmacology 2002, 181, 79-88 (3) Borlak, J. and Thum, T. Induction of nuclear transcription factors, CYP monooxygenases and GSTA2 gene expression in Aroclor 1254 treated rat hepatocyte cultures. Biochemical Pharmacology 2001, 61: 145-153 This work is supported by Grants from the Lower Saxony Ministry of Science and Culture and the German Ministry of Science and Education (BMBF).

1647 TOXICOGENOMICS OF BROMOBENZENE-INDUCED HEPATOTOXICITY.

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Toxicogenomics combines the expression analysis of thousands of genes (transcriptomics) or proteins (proteomics) with classical methods in toxicology, enabling detailed comparison of mechanisms and early detection and prediction of toxicity. The bromobenzene-induced hepatotoxicity in rats was studied at the gene expression level using cDNA microarrays 6, 24 and 48 hours after an oral dosing (3 levels). Moreover, liver samples were subjected to proteomics analyses to identify proteins with a (putative) role in bromobenzene hepatotoxicity. Urine and plasma metabolite patterns were recorded by NMR and subjected to pattern recognition analysis. Profiles of gene and protein expression and metabolite levels from treated rats were clearly distinct from controls as was shown by principal component analysis, while the greatest difference from controls was observed in high dosed groups 24 hours after dosage. Apparent signs of hepatotoxicity were found only in high dosed rats after 24 and 48 hours as determined with clinical chemistry and histopathology. The physiological symptoms coincided with many changes of the hepatic mRNA and protein content. Transcriptomics analysis confirmed involvement of glutathione-S-transferase isozymes and epoxide hydrolase in bromobenzene metabolism. Observed glutathione depletion coincided with induction of key enzymes in glutathione biosynthesis. Oxidative stress was apparent from strong up-regulation of heme oxygenase, peroxiredoxin 1 and other genes. An acute phase response was characterized by both up- and downregulation of specific genes. Clusters of genes commonly regulated by a specific transcription factor were found to be expressed in a similar manner.

1648 GENE EXPRESSION CHANGES IN F344 RATS FOLLOWING A PHARMACOLOGICAL DOSE OF ACETAMINOPHEN.

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Acetaminophen (APAP) is a commonly used over-the-counter analgesic and antipyretic that is hepatotoxic in high doses. Centrilobular necrosis occurs in the liver following APAP exposure after glutathione stores are depleted, allowing its toxic metabolite N-acetyl-p-benoquinone imine to react with cellular macromolecules causing mitochondrial dysfunction and hepatotoxicity. We treated ad libitum fed male Fisher 344 rats orally with APAP at doses that may be experienced during normal therapeutic regimes, 50 and 150 mg/Kg and examined gene expression changes in the liver after 6 hours. Although no clinical signs of toxicity were observed by histopathologic examination or clinical chemistry measurements, gene expression changes were observed consistent with energy metabolism disruption. The adaptive response by the liver included down regulation of fatty acid synthesis and cholesterol metabolism, pathways that are energy intensive. Upregulation of stress related genes such as heme oxygenase and metallothionein 1 were also observed. Several of the changes were consistent with previous reports but additional novel gene changes were observed. By 48 hours after dosing only a small number of genes are differentially expressed and these appear to be associated with a return to energy homeostasis. These early changes in gene expression indicate a disruption of energy metabolism and of normal metabolic processes at pharmacologic doses of APAP. These data suggest that differential gene expression analysis may provide a more sensitive indicator of toxicity than routine histology and clinical chemistry evaluations.

1649 IDENTIFICATION OF LIVER TOXICITY USING CLINICAL CHEMISTRY *VERSUS* GENE EXPRESSION MICROARRAY FACTORS.

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Clinical chemistry parameters assay, at most, a few dozen factors in even the most comprehensive of safety studies, and usually only a subset of these measures can be assayed due to cost and workload concerns. Toxicogenomic studies are capable of assaying tens of thousands of genes in a single experiment. However, the advantages of transcriptome-wide observations over those of classical measures have only recently begun to be estimated. In this study, we characterize the ability of a modeling approach to define liver toxicity by using the Affymetrix RGU34 series of microarrays and, alternatively, by using 20 clinical chemistry measures, including serum concentrations of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. The training set for each model consisted of > 1000 liver samples extracted from rats treated with known hepatotoxicants, non-hepatotoxicants, or various vehicles from a large reference database. The gene expression data-based model exhibited > 80% true positive rate along with < 5% false positive rate on a sample-by-sample basis. The clinical chemistry-based model produced a large decrease in true positive rate with a comparable rate of false positives. In addition, the gene expression data-based model identified human-specific toxicity in the absence of clinical chemistry indications. This study indicates there is a significant advantage in the use of gene expression data to characterize the toxic response for not only mechanistic pursuits, but also to predict and classify toxicity.

1650 PPAR ALPHA-DEPENDENT ALTERATIONS IN CHEMICAL-INDUCED STRESS AND LONGEVITY CORRELATES WITH INCREASED EXPRESSION OF HEAT SHOCK PROTEINS.

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A number of mouse mutants that exhibit decreased levels of reactive oxygen species (ROS) and increases in longevity carry mutations in growth hormone signaling genes. In gene expression profiling experiments we observed in the livers of Snell dwarf mice an increase in the expression of genes under control of the nuclear receptor peroxisome proliferator (PP)-activated receptor alpha (PPAR) as well as PPAR itself. PPAR mediates the carcinogenic effects of PP and is a known or experimental therapeutic target for PP drugs in diseases associated with aging in which oxidative stress plays a major role. Transcript profiling experiments in wild-type mice treated with a PPAR agonist, revealed coordinated increases in the expression of members of heat shock protein (HSP) and anti-oxidant gene families. HSPs are required for the correct folding of nacent polypeptides and repair of misfolded proteins after damage from chemicals that induce ROS. We examined the extent of the overlap in regulated genes between a PP and a classical heat stress by exposing wild-type or PPAR-null mice to a 25 or 42 degree C heat stress for 40 min. Transcript profile experiments using Affymetrix chips containing ~9, 000 mouse genes were used to assess gene expression in the livers of the mice. We found that PPAR-null mice 1) exhibit defects in HSP gene product induction after heat shock or PP treatment, 2) are more sensitive to hepatocellular damage from treatments that increase oxidative stress, and 3) show decreased longevity. Our results are consistent with the hypothesis that PPAR alpha controls stress resistance and longevity through coordinated regulation of genes that mediate responses to ROS including members of the HSP gene family.

1651 IDENTIFICATION OF GENE EXPRESSION PROFILES PREDICTIVE OF SPECIFIC MODES OF HEPATOTOXICITY *IN VIVO*.

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Liver toxicity is a major cause of drug failure during preclinical safety evaluation as well as in post-marketing. Recently, gene expression technologies have been integrated into toxicology studies. These technologies provide a new tool that can potentially be more efficient and sensitive than the current methods of evaluating drug-induced toxicity by providing an insight into the mechanisms of action that underlie specific toxicities at the molecular and cellular levels. The goal of this study was to establish a marker set of expressed genes that are associated and predictive of eleven specific subtypes of rat hepatopathologies: hypertrophy, cholestasis, apoptosis, zone 3 necrosis, multifocal necrosis, general necrosis, genotoxic and nongenotoxic carcinogenesis, steatosis, reactive inflammation and Kupffer cell aggregation. To achieve this, male Wistar rats were dosed with over 100 hepatotoxic and non-toxic compounds belonging to different chemical classes but with previously known toxic effects on rat liver. Rats were dosed daily with either a toxic dose or a low dose of each compound and euthanized at 4 specific time points. Transcriptional profiling of these liver samples was completed, and statistical analysis was implemented to select the final marker set using Kruskal-Wallis filtering and various statistical models including Logistic Regression, Classification Trees and Multivariate Analysis. A marker gene set has been selected that is predictive of all eleven histopathology modes *in vivo*, with an average cross-validated true positive rate of 89.2% and an average cross-validated true negative rate of 99.3%.

1652 PPAR ALPHA PLAYS A MAJOR ROLE IN DETERMINING THE GENE EXPRESSION PROFILE ALTERED BY CALORIC RESTRICTION.

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Caloric restriction (CR) in diverse species results in increased resistance to chemical-induced stress and extension of lifespan. CR has been shown to have a major impact on the results of 2 year chemical bioassays. This is likely due to alteration in genes that increase resistance to damage from reactive oxygen species (ROS) and/or allow increased repair of cellular components. CR mimics some aspects of exposure to peroxisome proliferators (PP) including hypolipidemic effects and protection of the liver from cytotoxicants. As the nuclear receptor for PP (PP-activated receptor alpha (PPAR)) mediates some of the adaptive responses to fasting, we hypothesized that PPAR also plays an important role in mediating the effects of CR in the mouse liver. Male wild-type and PPAR-null mice were given an ad libitum diet or a diet consisting of 65% of ad libitum for 5 weeks. Both wild-type and PPAR-null mice fed the CR diet had significantly lower body weights than ad libitum controls. Transcript profile experiments using Affymetrix chips containing ~9, 000 mouse genes were used to assess gene expression in the livers of the mice. Almost 1000 genes were identified that were significantly different between two of the four groups. In both mouse strains CR down-regulated genes involved in cholesterol synthesis, bile acid synthesis, and male-specific responses and both up- and down-regulated Phase I and II metabolism genes. A large percentage of the altered genes (~35%) depended on PPAR for expression changes. These included the up-regulation of fatty acid catabolism genes and surprisingly, genes involved in nucleotide excision repair (NER). Preliminary western blotting experiments confirmed alterations in the lipid metabolism proteins. These results indicate that PPAR alpha plays an important role in regulating large numbers of genes that mediate the effects of CR in the liver and allows us to predict the abrogation of CR-mediated resistance to chemical carcinogens in the livers of PPAR-null mice.

1653 DIMETHYLSULFOXIDE ANTAGONIZES THE CLEFT PALATE-INDUCING EFFECT OF SECALONIC ACID D IN MICE BY NEGATING ITS EFFECTS ON THE EMBRYONIC PALATAL CYCLIC AMP PATHWAY.

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Secalonic Acid-D (SAD) is a teratogenic mycotoxin causing cleft palate (CP) in the offspring of the exposed mice by reducing the proliferation of the palatal mesenchymal (PM) cells leading to smaller palatal shelf size. Coadministration of dimethylsulfoxide (DMSO) reversed the CP-inducing effect of SAD. Although SAD has been shown to affect both protein kinases A (PKA) and C (PKC) pathways, the effect on which of these pathways is relevant to its CP induction is unknown. The present studies were designed to further investigate the effect of SAD on the events of the PKC and cAMP pathways and to assess whether the protective effect of DMSO is mediated by its specific reversal of the effect(s) of SAD on one of the two pathways. Secalonic acid D was shown to inhibit the activity of PKC and the catalytic subunit of PKA (PKAc) but not the holoenzymes of PKA. Dimethyl sulfoxide specifically reversed the effect of SAD only on PKAc. Within the PKA pathway, SAD reduced the amount of PKAc migration into the nuclei of the PM cells; elevated phosphorylated cAMP response element (CRE) binding protein (pCREB) levels on GD 12 and reduced them on GD 14; reduced binding of CREB to the CRE but not of AP1 to 12-O-tetradecanoate-13 phorbol acetate-response element (TRE); downregulated the expression of the CRE-containing gene, proliferating cell nuclear antigen (PCNA); and reduced PM cell growth. Dimethyl sulfoxide reversed all of these effects with the exception of the elevation in pCREB levels on GD 12. These results suggest a greater significance of the PKA pathway in normal palate development than the PKC pathway and the relevance of its perturbation in the pathogenesis of CP by SAD.

1654 DEVELOPMENTAL TOXICITY OF THIODIGLYCOL IN RATS.

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Thiodiglycol (TG), a hydrolysis product of sulfur mustard (HD), is a potential contaminant of soil and water at certain military sites. In order to establish developmental criteria for TG to facilitate remediation, an oral developmental toxicity study was conducted in pregnant Sprague-Dawley rats. Neat thiodiglycol (99.9 %) was administered to mated female rats from day five through day nineteen of gestation. Three groups of rats (25/group) received TG by gavage at a rate of either 430, 1290 or 3870 mg/kg/day. A fourth group served as a sham control. On day twenty of gestation, all females were euthanized and a Caesarian section performed. Litters were examined for soft tissue and skeletal alterations. Maternal toxicity was limited to dams receiving TG at 3870 mg/kg/day. At this dose, body weights and food consumption were reduced during certain periods of gestation. Fetuses derived from those dams exhibited an increased incidence of variations when compared to controls, but were not statistically significant. Fetal body weights in the 3870 mg/kg/day group were significantly lower than controls. There was no increased incidence of anomalies when thiodiglycol-treated fetuses were compared to controls. It was concluded that TG is not teratogenic, but is a developmental toxicant at high dose levels that produced maternal toxicity. The No Observed Adverse Effect Level (NOAEL) of 1290 mg/kg/day was determined for oral developmental toxicity during the major period of organogenesis. (Abstract does not reflect US Army policy).

1655 DEVELOPMENTAL TOXICITY EVALUATION OF EMODIN IN RATS AND MICE.

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Emodin (EMOD), a widely available over-the-counter herbal remedy, was evaluated for potential effects on pregnancy outcome. EMOD was administered in feed to timed-mated Sprague-Dawley (CD®) rats (0, 425, 850, and 1700 ppm; gestational day [gd] 6 to 20), and Swiss albino (CD-1®) mice (0, 600, 2500 or 6000 ppm; gd 6 to 17). Ingested dose was 0, 31, 57, and 80-144 mg EMOD/kg/day (rats) and 0, 94, 391, and 1005 mg EMOD/kg/day (mice). Timed-mated animals (25/group) were monitored periodically throughout gestation for body weight (wt), feed/water consumption and clinical signs. At termination (rats: gd 20; mice: gd 17), confirmed pregnant dams (21-25/group) were evaluated for clinical signs; body, liver, kidney and gravid uterine wts (GUW); pregnancy status; uterine contents; and number of corpora lutea. Live fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations/variations. There were no maternal deaths; discolored, soft or loose feces were observed at the high dose in mice. In rats, maternal body wt decreased at the high dose (gd 9, 12); wt gain was reduced at the mid (gd 6-9) and high dose (gd 6-9; 6-20). Maternal body wt (gestation) and wt gain (corrected for GUW) exhibited a decreasing trend. In mice, maternal body wt and wt gain decreased at the high dose. Prenatal mortality, live litter size, fetal sex ratio, and morphological development were unaffected in both rats and mice. At the high dose, rat average fetal body wt/litter was unaffected, but was significantly reduced in mice. The rat maternal LOAEL was 1700 ppm based on maternal body weight and weight gain; the NOAEL was 850 ppm. The rat developmental toxicity NOAEL was >1700 ppm; a LOAEL for rat developmental toxicity was not established. In mice, the maternal toxicity LOAEL was 6000 ppm based on reduction of maternal body wt and wt gain; the NOAEL was 2500 ppm. The developmental toxicity LOAEL was 6000 ppm, based on reduction of fetal body wt; the NOAEL was 2500 ppm. Supported by NIEHS/NTP Contract N01-ES-65405.

1656 DEVELOPMENTAL TOXICITY EVALUATION OF GOLDENSEAL ROOT POWDER (GRP) IN MICE.

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Goldenseal (Hydrastis Canadensis) ranks among the top herbal supplements in the world market. Thus, potential effects during pregnancy warranted investigation. In this study, GRP was administered to timed-mated Swiss albino (CD-1®) mice in ground NIH-07 diet (0, 3125, 12, 500 or 50, 000 ppm) from gestational day (gd) 6 to 17. Average ingested doses were 0, 514, 2048 and 7738 mg GRP/kg/day. Alkaloid content included 5% berberine, 4.5% hydrastine and 0.1% canadine. Timed mated mice (25/group) were monitored throughout gestation for body weight (wt), feed/water intake and clinical signs. At termination (gd 17), confirmed pregnant dams (23-25/group) were evaluated for clinical signs; body, liver and gravid uterine wts (GUW); uterine contents; and number of corpora lutea. Maternal livers (10/group) were fixed, sectioned, stained (hematoxylin/eosin), and examined for histopathology. Live fetuses were weighed, sexed, and examined for external (100%), visceral (50%), or skeletal (50%) malformations/variations. No maternal deaths or remarkable clinical signs were noted, except yellow discoloration of fur due to contact with high-dose feed. At 50, 000 ppm, maternal feed intake decreased (gd 6 to 9 and 15 to 17), possibly due to altered palatability. Water intake increased at 3125 ppm (gd 6 to 9 and 9 to 12) and 50, 000 ppm (gd 6 to 9, 9 to 12 and 12 to 15). Maternal body wt, wt gain (treatment and gestation), GUW, and corrected wt gain were not affected. Maternal liver wt (absolute and relative) was increased at >12, 500 ppm in the absence of treatment-related histopathology. Prenatal mortality, live litter size, and fetal sex ratio were unaffected. At 50, 000 ppm, cleft palate, to which this species/strain is predisposed under stress, was slightly increased, and exencephaly was slightly above historical range (1% vs. 0.7%). Fetal body wt was significantly reduced (~8%) at 50, 000 ppm. The developmental toxicity NOAEL was 12, 500 ppm and the LOAEL was 50, 000 ppm (~300 times higher than estimated human intake). Support: NIEHS/NTP Contract N01-ES-65405.

1657 EFFECTS OF CALORIC INTAKE AND ETHANOL METABOLISM ON FETAL ETHANOL TOXICITY IN RATS.

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Urine ethanol concentrations (UEC) are significantly lower in pregnant than non-pregnant Sprague-Dawley rats infused with the same ethanol dose *via* total enteral nutrition. Under-nutrition appears to abolish this effect. Increased ethanol metabolism in pregnancy may be a protective mechanism that could account for the low penetrance of fetal alcohol syndrome (FAS) among the children of alcoholic mothers. In contrast, under-nutrition may be a risk factor for FAS. Groups of 3-15 time-impregnated female Sprague-Dawley rats were intragastrically infused TEN diets containing an optimal 220 kcal/kg$^{3/4}$/d for nutritional support during pregnancy from gestational (GE) d 6 until GE d 20. Ethanol was substituted isocalorically for carbohydrate calories at levels of 8-14 g/kg/d. UECs were measured and averaged from 69 ± 6 to 327 ± 22 mg/dl. No full litter resorptions occurred at ethanol doses below 11.8 g/kg/d and no significant reductions in birth weight were observed at ethanol doses lower than 13 g/kg/d. At 14 g/kg/d ethanol, 33% of litters were resorbed and those that survived had significant fetal wastage ($p \leq 0.05$) and reduced birth weight (3.7 ± 0.3 g, control vs. 1.4 g ± 0.2 g, ethanol). When groups of N = 7-8 dams were infused with 13 g/kg ethanol and fed TEN diets containing either

optimal 220 kcal/kg$^{3/4}$/d or restricted to 154 kcal/kg$^{3/4}$/d, full litter resorptions were noted in 63% of the 154 kcal rats and none of the 220 kcal rats. Decreased birth weights ($p \leq 0.05$) were observed in pups from the 154 kcal animals accompanied by significantly increased blood and urine ethanol levels (average UEC, 233 ± 34 vs. 157 ± 20 mg/dl) . Therefore, increased ethanol metabolism in pregnancy protects the fetus against ethanol toxicity and this toxicity is increased by underfeeding as a result of impaired ethanol clearance and increased fetal exposure. Supported in part by R01 AA12819 (MJJR).

1658 MATERNAL AND DEVELOPMENTAL TOXICITY OF PERFLUOROOCTANE SULFONATE (PFOS) IN THE MOUSE.

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The maternal and developmental toxicity of PFOS, an environmentally persistent compound that has been used in the manufacture of surfactants and insecticides, were evaluated. Timed-pregnant CD1 mice were gavaged with 1, 5, 10, 15, or 20 mg/kg/day PFOS/K+ from GD 2 to term. Controls received an equivalent volume of 0.5% Tween-20 vehicle (10 ml/kg). Some dams were killed on GD 18 for teratological examination, while those remaining were allowed to deliver to monitor the postnatal growth and development of their offspring. PFOS levels in maternal serum and liver were determined at term. Maternal weight gain was suppressed by 20 mg/kg PFOS, indicating the general maternal toxicity of the chemical. Serum triglycerides and thyroxine in dams treated with greater than 5 mg/kg PFOS were significantly lower than controls. Dose-dependent maternal liver weight increases were observed at term. PFOS did not alter the number of implantations, live fetuses, or fetal weight at term. Cleft palate, sternal defects, cardiac ventricular septal defect, and enlargement of the right atrium were detected, primarily in the 20 mg/kg group. Live birth was observed in all groups; however, neonates in the 20 mg/kg group were moribund and died within 4-6 h. While newborns in the 15 mg/kg group appeared viable, all were found dead within 24 h. Postnatal viability was greater in the lower dose groups and surviving neonates appeared to thrive, but significant delays in eye opening were observed. These dose-dependent adverse effects will be compared to the body burdens of PFOS. These results are similar to the maternal and developmental toxicity of PFOS previously described in the rat, although the mouse appears to be a less sensitive species. This abstract does not necessarily reflect EPA policy.

1659 EMBRYO-FETAL DEVELOPMENT STUDY OF HYDROXYPROPYL METHYLCELLULOSE ACETATE SUCCINATE (HPMCAS) IN RATS.

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The potential for HPMCAS to produce developmental and reproductive toxicity was evaluated in a series of studies that included, rat and rabbit teratology studies, a rat fertility study, and a rat peri- and postnatal study (Hoshi and coworkers 1985). The authors concluded that there were no compound-related findings. However, in the cesarean-section phase of the rat teratology study, clubfoot was reported for 3, 8, 19 and 16 fetuses in the control, 625, 1250 and 2500 mg/kg groups, respectively. There were no significant increases in external anomalies, but the apparent dose-related increase in clubfoot was not specifically addressed. The increase in clubfoot did not appear to be related to treatment with HPMCAS based on the following: 1) the external diagnosis of clubfoot did not have an underlying skeletal finding; 2) the incidence of clubfoot in control animals was substantially outside of historical norms; and 3) clubfoot was not noted in offspring of HPMCAS treated animals allowed to deliver. To definitively address the uncertainty regarding the potential for HPMCAS to induce developmental anomalies, a repeat of the rat teratology study was performed. Groups of 20 pregnant Sprague-Dawley rats were dosed with 0, 50, 150, 625 or 2500 mg/kg HPMCAS from GD 7-18. Fetuses were examined on GD 22 for external, visceral and skeletal development. No developmental toxicity was observed as a result of HPMCAS exposure demonstrating that maternal HPMCAS exposure during gestation does not induce developmental anomalies. There were no findings of clubfoot or other limb anomalies in this study at dose levels equivalent to those that previously demonstrated an apparent increase in clubfoot. Therefore, the conclusion of Hoshi and coworkers that the apparent dose-dependent increase in clubfoot is not related to treatment with HPMCAS was confirmed by this repeat study.

1660 EMBRYO-FETAL DEVELOPMENT STUDY OF HYDROXYPROPYL METHYLCELLULOSE ACETATE SUCCINATE (HPMCAS) IN RABBITS.

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The potential for HPMCAS to produce general and reproductive toxicity was evaluated in a series of studies that included acute toxicity studies in rats and rabbits, a 6-month rat study, rat and rabbit teratology studies, a rat fertility study, and a rat peri- and postnatal study (Hoshi and coworkers, 1985). There were no compound-related developmental findings in the rabbit. However, in the rabbit teratology study, the number of litters evaluated (12-13 per group) did not meet current regulatory guidelines. Therefore, the teratology evaluation of HPMCAS in rabbits was repeated with an increased number of does to meet regulatory guidelines. Groups of 20 pregnant New Zealand White rabbits were dosed with HPMCAS twice daily by oral gavage for a total dose of 0, 50, 150, 625 or 2500 mg/kg from GD 8-20. The does were euthanized on GD 30 and all fetuses were examined externally. Following external examination, the fetuses were examined viscerally by fresh tissue dissection. The fetuses were then stained with Alizarin Red and examined for skeletal development. There was no developmental toxicity demonstrated as a result of HPMCAS exposure. Therefore, the conclusion of the earlier study indicating that treatment with HPMCAS at doses up to and including 2500 mg/kg/day did not produce developmental toxicity was confirmed with this study.

1661 METHAMPHETAMINE ENHANCES OXIDATIVE DNA DAMAGE IN MURINE EMBRYONIC AND FETAL BRAIN AND LIVER.

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Methamphetamine (METH) decreases dopamine levels, structurally alters dopaminergic neurons and causes functional deficits in adult animal models, but its developmental effects are unclear. We investigated METH-initiated reactive oxygen species (ROS) generation and oxidative DNA damage in the embryonic and fetal periods. Pregnant CD-1 mice were treated with METH (20 or 40 mg/kg ip) or its saline vehicle at 1000 hr on gestational day (GD) 14 or 17. Dams were sacrificed 1 or 4 hr later, and conceptal brains and livers were isolated and analyzed for oxidized DNA, determined by 8-oxoguanine formation. On GDs 14 and 17 at 1 hr, METH enhanced conceptal DNA oxidation in brain and liver by at least 2-fold ($p<0.05$). Maximal DNA damage generally was not significantly increased by the higher dose. At 4 hr, DNA damage was decreased, unchanged or greater in brain, and variably decreased in liver, suggesting more active repair in the latter. Both baseline and METH-enhanced DNA damage in brain and liver were 2- to 3-fold higher on GD 14 than GD 17 ($p<0.05$), likely due in part to the lower activity of antioxidative enzymes, and possibly DNA repair enzymes, on GD 14. The sustained or increased METH-enhanced DNA oxidation in brain over 4 hr, unlike liver, at both GDs suggests that the brain may be particularly susceptible to METH-initiated structural and functional anomalies. This is the first evidence of conceptal oxidative DNA damage caused by amphetamines, and suggests potential problems with amphetamine use by women of child-bearing age. (Support: CIHR)

1662 EFFECTS OF *I.P.* MATERNAL VITAMIN E ADMINISTRATION ON FETAL DEVELOPMENT AND PHENYTOIN EMBRYOPATHIES.

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The anticonvulsant drug phenytoin (PHT) initiates the formation of reactive oxygen species (ROS), which may contribute to its embryopathic effects. Here, we examined the effect of *i.p.* maternal pretreatment with the antioxidant vitamin E (VE) on normal embryonic development and PHT teratogenicity. Pregnant CD-1 dams were treated at 0800 hr on gestational days (GD) 12 and13 with ±-α-tocopherol-acetate (125 or 250 mg/kg *i.p.*), or its corn oil vehicle, followed 3 hr later by PHT (65 mg/kg *i.p.*) or its 0.9% saline vehicle. On GD 19, dams were sacrificed and fetuses examined for anomalies. VE at either dose did not alter the spontaneous incidences of fetal resorptions, postpartum lethality or cleft palate, nor fetal birth weight. As expected, PHT increased the incidences of fetal resorptions, postpartum lethality, and cleft palate by 2.0-, 1.9-, and 2.3-fold respectively ($p<0.05$), while decreasing fetal body weight by 12% ($p<0.001$). Compared to its corn oil vehicle, VE 250mg/kg reduced PHT-initiated cleft palates by 54% ($p<0.004$). Trends were ob-

served for protection by VE compared to its corn oil vehicle against fetal resorptions and postpartum lethality, but the differences were not statistically significant ($p<0.08$, $p<0.1$). VE had no effect on PHT-initiated fetal weight loss. Compared to VE 125 mg/kg, VE at 250 mg/kg lowered spontaneous levels of fetal resorption by 58% ($p<0.05$), and appeared to lower the incidence of PHT-initiated cleft palates ($p<0.07$). Conversely, compared to higher dose of VE, VE 125 mg/kg reduced PHT-initiated postpartum lethality by 42% ($p<0.04$). These results indicate that maternal VE given *i.p.* provides dose-dependent protection against phenytoin teratogenicity, while suggesting that the optimal dose may vary for different embryopathies. Taken with other studies using dietary VE, the embryoprotective efficacy of VE against endogenous and xenobiotic-enhanced ROS depends upon the dose, route and duration of VE administration, and suggests an embryopathic role for ROS. (Support: CIHR)

1663 PROTECTION BY OXOGUANINE GLYCOSYLASE 1 (OGG1) AGAINST METHAMPHETAMINE-INITIATED DNA OXIDATION IN FETAL BRAIN IN OGG1 KNOCKOUT MICE.

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Oxidative DNA damage, in particular the lesion 8-oxoguanine (8-oxoG), has been implicated in the initiation of teratogenesis. 8-OxoG is removed by oxoguanine glycosylase 1 (ogg1) in the initiating step of the base excision repair pathway. We have previously shown that *in utero* exposure to the neurotoxic drug methamphetamine (METH) on gestational day (GD) 17 results in increased DNA damage to fetal brain and liver in CD-1 mice. Here we used OGG1 knockout mice to investigate the developmental role of ogg1 in repairing METH-initiated fetal DNA oxidation following *in utero* exposure. METH (20 mg/kg) or its saline vehicle was administered on GD 17 to pregnant heterozygous (+/-) OGG1-deficient females (mated to OGG1 +/- males). Dams were sacrificed 4 hr later, and fetal brains and livers were isolated and analyzed for oxidized DNA, determined by 8-oxoG formation. In fetal liver, loss of functional ogg1 did not affect 8-oxoG levels in either saline or METH-exposed fetuses. In fetal brain, no significant differences were observed in DNA oxidation among saline-exposed wild type (+/+) OGG1-normal, +/- or homozygous null (-/-) OGG1-deficient fetuses. However, with METH exposure, there was an 80% increase in 8-oxoG levels in +/- OGG1-deficient fetuses compared to +/+ OGG1-normal littermates, and a doubling in DNA oxidation in -/- versus +/+ fetuses ($p<0.05$). DNA damage also was elevated in fetal brain from METH-exposed -/- fetuses compared to saline-exposed controls of the same genotype ($p<0.05$). This is the first evidence of increased DNA oxidation in drug-exposed fetuses lacking ogg1, and suggests that functional DNA repair may be important in protecting against METH-initiated developmental neurotoxicity. (Support: CIHR)

1664 SINGLE INTRAMUSCULAR INJECTIONS OF BOTOX® ARE NOT DEVELOPMENTALLY TOXIC IN RATS.

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The expanding clinical use of BOTOX® for the treatment of neuromuscular disorders increases the likelihood that pregnant women may be exposed to the drug. No adverse effects on human reproductive endpoints have been reported and no direct embryo/fetal developmental effects have been observed in animal studies previously conducted to evaluate multiple gestational intramuscular injections of the drug. The objective of this study was to evaluate the effects of a single treatment of BOTOX® administered at specific points of gestation. Twenty groups of pregnant rats (18/treatment day/dosage) were treated on days 3, 6, 9, 12, or 15 of gestation with a single intramuscular injection (0.2 mL/kg) of BOTOX® at 0 (Control), 1, 4, or 16 University/kg in the left hindlimb. Dams were monitored for pharmacological effects of the toxin and clinical signs of toxicity. C-section was performed on day 21 of gestation to evaluate effects on implantation and embryo/fetal development. Pharmacological effects in the injected hindlimb (curling of the toes, lameness, and reduced grip strength) were observed in all drug-treated groups, and increased in degree and/or incidence with dosage. Decreased body weight and feed consumption occurred in dams treated with 16 University/kg, while no adverse effects were observed in dams given ≦4 University/kg. No drug-related effects on implantation or embryo/fetal development were observed. In summary, single intramuscular injections of BOTOX® given on days 3, 6, 9, 12, or 15 of gestation were not developmentally toxic in rats.

1665 LACK OF EMBRYO-FETAL TOXICITY WITH THE ANTI-INFECTIVE DB289 AND ITS ACTIVE METABOLITE DB75, A DIAMIDINE WITH DNA MINOR GROOVE BINDING ACTIVITY.

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DB289 (2, 5-Bis[4-(N-methoxyamidino)phenyl]furan monomaleate) is a dimethoxime prodrug designed for oral delivery of the active diamidine, DB75 (2, 5-Bis[4-amidinophenyl]furan). DB75 has antiprotozoal, antifungal and antimalarial activities. DB289 is currently in Phase II clinical trials for the treatment of African sleeping sickness and Pneumocystis pneumonia. The mechanism of anti-infective activity is unknown, but probably involves binding of the diamidine molecule (DB75) to the minor groove of pathogen DNA. *In vitro* and *in vivo* genotoxicity assays indicate that DB289 and DB75 are not mutagenic or clastogenic. Nonetheless, the potential interaction of DB75 with DNA warrants evaluation of the potential effects on the developing embryo and fetus. DB289 was evaluated for potential effects on embryo-fetal development when administered orally to pregnant CD rats and New Zealand White rabbits during the period of organogenesis. Dosages of DB289 free base were 5, 10 and 20 mg/kg/day in rats, and 3, 10 and 15 mg/kg/day in rabbits. These dosages were based on the results of dose range-finding studies. In rats the only effects were grossly pale livers in occasional animals at 10 and 20 mg/kg. In rabbits maternal toxicity was evident at the two highest doses. Detailed fetal examinations indicated no adverse effect of DB289 treatment on the type, incidence or distribution of visceral or skeletal abnormalities in either species. DB289 and DB75 were detected in plasma at all dose levels confirming continuous systemic exposure in both rats and rabbits. Thus, DB289 and DB75 exposure and potential binding to the minor groove of DNA are not associated with adverse effects on embryo-fetal development in rats or rabbits.

1666 CHEMICAL CHARACTERIZATION OF PINE BARK AND GRAPE SEED EXTRACTS.

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Pine bark extract (PBE) and grape seed extract (GSE) are herbal supplements that have been selected for toxicological evaluation by the NIEHS. PBE and GSE are known to contain proanthocyanidins, or condensed tannins, which are polymeric compounds consisting of chains of flaval-3-ol units, (+)-catechin and (-)-epicatechin, linked through C4-C6 and C4-C8 interflavan bonds. PBE also contains taxifolin, a flavon-3-ol. These polyphenolic compounds are known to possess potent antioxidant activities and are used in the treatment of a wide variety of medical conditions, including circulatory disorders. A strategy, which included extraction, HPLC, GC/MS, LC/MS, MALDI-TOF/MS, and GPC, was developed for overall characterization and comparison of two lots of PBE and four lots of GSE. A gradient RP-HPLC method was developed to separate catechin and epicatechin monomers, dimers, and several corresponding gallic acid esters. Catechin and epicatechin solvent standards ($\backsim$ 30 to $\backsim$ 1000 µg/mL) were linear ($r \geq 0.9999$). Extraction of six PBE and GSE components proved to be linear ($r \geq 0.999$) over an analytical concentration of $\backsim$ 0.05 to $\backsim$ 25 mg/mL. The HPLC method was transferred directly to APCI LC/MS, which was used to identify the various monomers, dimers, and their gallates. GC/MS analyses of PBE and GSE led to the identification of the ethyl esters of hexadecanoic acid, linoleic acid, and oleic acid, as well as smaller phenolic and terpene components. GPC molecular weight distribution profiles indicated the presence of PBE and GSE components ranging from $\backsim$ 162 to $\backsim$ 5500 MW. The majority of PBE components were less than 1180 MW, while the majority of GSE components ranged from $\backsim$ 1180 to $\backsim$ 5000 MW. MALDI-TOF/MS analyses, used to characterize larger MW proanthocyanidins, confirmed the presence of hexamers and heptamers as well as pentamers with multiple gallic acid esters.

1667 ANALYTICAL METHOD VALIDATION OF *GINKGO BILOBA* L. POWDER EXTRACT DOSED IN 0.5% (W/V) AQUEOUS METHYLCELLULOSE.

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Ginkgo biloba L. powder extract (GBPE) is an herbal supplement that has been selected for toxicological evaluation by the National Institute of Environmental Health Sciences (NIEHS). A method was validated for the analysis of GBPE in

0.5% (w/v) aqueous methylcellulose to cover a dose formulation range of ∽ 25 to ∽ 1000 mg/mL. The recently developed analytical method simultaneously monitors aglycones and terpenelactones using high-performance liquid chromatography (HPLC) with ultraviolet (UV) and evaporative light scattering (ELS) detection. Quercetin and bilobalide, two of the major components in GBPE, were used to prepare quantitation standards (∽ 30 to ∽ 75 µg/mL) to determine the weight percentages of quercetin glycosides and bilobalide present in GBPE solvent and spiked vehicle standards. In addition, quercetin was used as a marker compound to qualitatively monitor kaempferol and isorhamnetin (aglycones) and bilobalide was used as a marker compound to qualitatively monitor ginkgolides A, B, and C (terpenelactones). In order to simultaneously monitor aglycones and terpenelactones, standards containing GBPE were hydrolyzed at 90°C for 1 hr. The responses of quercetin and bilobalide from the quantitation standards were linear ($r \geq 0.99$) and accurate (%RE ≤ 3.3). The responses of quercetin and bilobalide from hydrolyzed GBPE solvent and spiked vehicle standards were linear ($r \geq 0.999$) and precise (%RSD ≤ 2.2). The average percent of quercetin glycosides and bilobalide in the solvent standards containing GBPE was 16.1 ± 0.4(s) and 7.0 ± 0.1(s) (n = 10), respectively. The average percent of quercetin glycosides and bilobalide in the spiked vehicle standards containing GBPE was 15.4 ± 0.7(s) and 6.8 ± 0.3(s) (n = 10), respectively. The average response recovery of marker compounds in GBPE from the spiked vehicle standards, relative to the solvent standards, was 95.4 ± 3.7(s)% for quercetin and 96.9 ± 3.9(s)% for bilobalide.

1668 A RAPID EXTRACTION AND ISOLATION METHOD FOR THE DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN COMFREY.

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Sponsor: M. Cunningham.

Preparations from comfrey (*Symphytum officinale* and *S.* x *uplandicum*) root and leaf contain varying levels of the hepatotoxic pyrrolizidine alkaloids (PAs). Reference compounds for comfrey are not commercially available, and there is currently no rapid extraction or analytical method to determine low levels in raw materials or commercially available extracts. A solid phase extraction (SPE) method was developed using an Ergosil cleanup column that specifically binds the PAs. Two comfrey root extraction methods were evaluated, and recoveries following SPE were determined for identified and proposed PAs. The simplified 1 hr extraction with basic chloroform using sonication resulted in PA yields ranging from 20 to 80 fold greater than the traditional 2 day alkaloid extraction/partition method with N-oxide reduction. With this improved method, 10 g of powdered comfrey root is extracted with 100 mL of basic chloroform. The extract is applied to the cleanup column under vacuum, washed with 2 mL of acetone:chloroform (8:2), followed by 2 mL petroleum ether to remove excess chloroform. The column is dried and the PAs are eluted to a final volume of 2 mL with methanol. Percent recoveries of the PAs following SPE had an overall average of 92.2%. The unique properties of the cleanup column allow a rapid sample cleanup and concentration of PAs from comfrey extract, and allows the eluant to be analyzed directly by HPLC, HPTLC, or GC methods.

1669 CHARACTERIZATION OF GINSENG FOR USE IN TOXICITY STUDIES.

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Ginseng, a nutritional supplement, has been selected for toxicological evaluations in 14-day, 90-day and 2-year chronic rodent studies by the NTP. This material was characterized using a variety of techniques to determine its composition for comparison to literature information prior to it use. The objectives of the work were to determine the water/volatile content; the concentrations of organophosphate pesticides, nitrosamines, total and individual amino acids, metals, selected anions, and total and ethanol soluble carbohydrate concentration; the identity and concentrations of the major simple sugars and disaccharides; the molecular weight distribution of the glycans; the identity of all possible UV detectable components using HPLC/MS; the reverse phase HPLC/UV profile of the sample including the quantitation on any detected ginsenosides; a GC/FID profile of volatile organic soluble components and the identity of all possible components by GC/MS. These analyses indicated a water/volatile content of ~5%; no detectable levels of organophosphate pesticides or nitrosamines; a fatty acid concentration of <0.014%; amino acid contents ranging from 0.02 (cystine) to 3.1 (glutamic acid)% with a total amino acid concentration of 7.0%; potassium (0.9%)and silicon (0.2%) were the major metallic components; acetate (1.3%), chloride (0.6%), nitrate (0.14% and sulfate (0.01%) were the major inorganic anionic components; a total carbohydrate content of ~70%, all of which was ethanol soluble, the major simple sugars/disaccharides were sucrose (46.8%), glucose (2.9%), fructose (1.5%), and maltose (8.3%); the molecular weights of the glycans were ~6000 and ~10-12, 000; the ginsenosides represented ~10% of the total peak areas detected by reverse-phase HPLC at 205 nm; the ten largest ginsenosides were Rg1 (2.2%), Re (0.7%), Rf (0.3%), Rb1 (1.7%), Rc (1.0%), Rb2 (0.8%), Rd2(0.7%), Rh1 (<0.1%), Rg2 (<0.1%) and Rb3 (<0.1%); and the identified volatile impurities were furfural, furfuryl alcohol, 5-methyl-2-furfural, and 5-hydroxymethyl-2-furfural.

1670 CYTOTOXICITY OF SELECTED PTERINS IN MCF-7 CELLS.

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Pterins are naturally occurring compounds, some (e.g., biopterin, folic acid) with important roles in metabolism. Xanthopterin (X, 2-amino-4, 6(3H, 5H)-pteridinedione) and isoxantopterin (IX, 2-amino-4, 7(3H, 8H)-pteridinedione) may also regulate cell replication in mammals and other species by mechanisms which are not well undersood. In clinical studies, levels of X and IX in urine from cancer patients differ significantly when compared with levels in from healthy subjects. Other studies report differing rates of pterin metabolism by tumor cells relative to controls, and variable effects and cytotoxic potencies of X in tumor cells, ranging from inhibition to proliferation. Unpublished studies suggest that oral X+IX alters growth of mammary tumors occuring spontaneously in female C3H/HeN-MTV+ mice. This in particular prompted us to examine for the first time the responsiveness of human mammary carcinoma MCF-7 cells to X and IX. Measuring cell viability using the MTS assay, X and IX were tested alone and in combinations at total pterin concentrations of 12.5-200 uM. When tested singly, the IC50s of X and IX were similar: 112±3.4 uM and 102±4.2 uM, respectively. MCF-7 cells seemed to be as sensitive as some renal cell lines (RPTC and LLC-PK1) that had been tested with X after the kidney was identified as a primary organ of toxicity. X and IX were less potent than tamoxifen, a known inhibitor of estrogen-sensitive MCF-7 cell growth. When IX:X were tested repeatedly in combination to explore interactions, IC50s showed the following order: 3:1<4:1<1:3≅1:2<2:1≅10:1<5:1<1:1≅1:4<1:5<1:10, although an ANOVA revealed no statistical differences. Up to 100 uM total pterins, the 3:1 ratio was always the most cytotoxic of the ratios tested, and the 4:1 ratio consistently ranked second. Over 100 uM, again the 3:1 ratio was the most cytotoxic, but the 1:3 ratio ranked second. Information from these experiments will be particularly useful when designing future mechanistic and efficacy studies to increase understanding of the potential for using pterins in cancer chemotherapy.

1671 EPIGALLOCATHECHIN 3-GALLATE ATTENUATES BRAIN DAMAGE INDUCED BY 3-HYDROXYKYNURENINE.

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3-hydroxykynurenine (3-HK), an endogeneous metabolite of tryptophan in the kynurenine pathway, has been known to play roles of potential neurotoxin in several neurodegenerative disorders. Epigallocathechin 3-gallate (EGCG), extracts of tea leaves, is recognized as a promising natural substance in protecting neuronal diseases. In the present study, we investigated the possible protective roles of EGCG against 3-HK induced cell injury. We found that 3-HK induced the decrease in cell viability in SH-SY5Y cell lines. The reduced cell viability produced apoptotic characteristic features in cell culture. The cells cultured in 3-HK added medium showed the increase in reactive oxygen species (ROS) as well as in caspase activity. Both of them are involved in the apoptosis. EGCG attenuated the reduced cell viability by 3-HK in a dose and time dependent manner. In microscopy, EGCG inhibited the cell morphological disorders featured in 3-HK treated cells. Furthermore, the rise of ROS and caspase activity by 3-HK was also blocked by EGCG. These results showed that EGCG had a protective effect on the 3-HK induced cell death and also abolished ROS and caspase actions leading to apoptosis. These findings suggested that EGCG may be a promising substance against the neuronal degenerative diseases.

1672 BREVETOXIN AUGMENTS NMDA RECEPTOR SIGNALING IN MURINE CEREBROCORTICAL NEURONS.

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Brevetoxins are potent allosteric enhancers of voltage-gated sodium channel function and are associated with the periodic Red Tide blooms. These neurotoxins produce acute neuronal injury and death in cerebellar granule cells following

acute exposure. In cerebrocortical neurons brevetoxin (PbTx-2) exposure enhances the amplitude of spontaneous Ca2+ oscillations. PbTx-2 produces this modulatory effect by influencing glutamatergic signaling. Activation of glutamate receptors is associated with an increase in Ca2+ influx, which triggers ERK1/2 activation. PbTx-2 exposure was similarly found to induce a concentration-dependent increase in ERK1/2 and Akt activation in cerebrocortical neurons. We have therefore explored the modulatory effect of PbTx-2 on NMDA receptor signaling using western blotting and calcium monitoring with a fluorescent plate reader (FLIPR). We found that PbTx-2 augments NMDA-induced ERK1/2 activation. Due to the involvement of Ca2+ in ERK1/2 activation, the effects of PbTx-2 on NMDA-induced Ca2+ influx were explored. PbTx-2 was found to potentiate NMDA-induced Ca2+ influx in cerebrocortical neurons. These results are consistent with studies demonstrating that a rise in intracellular sodium ion augments excitatory transmission through NMDA receptors. These findings may have important functional consequences for various physiological events in the central nervous system such as development, learning and memory.

1673 HEPATOPROTECTIVE EFFECTS OF THE RADIX OF *PLATYCODON GRANDIFLORUM* ON CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN MICE.

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The protective effects of a Platycodi Radix (Changkil: CK), the root of Platycodon grandiflorum A. DC (Campanulaceae), on carbon tetrachloride-induced hepatotoxicity and the possible mechanisms involved in this protection were investigated in mice. Pretreatment with CK prior to the administration of carbon tetrachloride significantly prevented the increased serum enzymatic activities of alanine and aspartate aminotransferase in a dose-dependent manner. In addition, pretreatment with CK also significantly prevented the elevation of hepatic malondialdehyde formation and the depletion of reduced glutathione content in the liver of carbon tetrachloride-intoxicated mice. However, hepatic reduced glutathione levels and glutathione-S-transferase activities were not affected by treatment with CK alone. Carbon tetrachloride-induced hepatotoxicity was also essentially prevented, as indicated by a liver histopathologic study. Histopathological evaluation of the rat livers revealed that CK reduced the incidence of liver lesions induced by carbon tetrachloride, including hepatocyte swelling, leukocyte infiltration, and necrosis. The effects of CK on the cytochrome P450 (P450) 2E1, the major isozyme involved in carbon tetrachloride bioactivation were also investigated. Treatment of mice with CK resulted in a significant decrease of P450 2E1-dependent p-nitrophenol and aniline hydroxylation in a dose-dependent manner. Our results suggest that the protective effects of CK against carbon tetrachloride-induced hepatotoxicity possibly involve mechanisms related to its ability to block P450-mediated carbon tetrachloride bioactivation.

1674 THE RADIX OF PLATYCODON GRANDIFLORUM REDUCES HEPATIC FIBROSIS IN RATS INDUCED BY DIMETHYLNITROSAMINE OR CARBON TETRACHLORIDE.

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Herbal medicines are increasingly being utilized to treat a wide variety of disease processes. We previously reported that aqueous extract from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK), had hepatoprotective effects against acetaminophen induced liver injury. In the present study, we assayed the preventive and therapeutic effects of CK on experimental hepatic fibrosis induced by dimethylnitrosamine or carbon tetrachloride in rats. Rats were given a single intraperitoneal injection of 20 mg/kg dimethylnitrosamine or 0.5 ml/kg carbon tetrachloride twice weekly for 4 weeks. In each model, CK was given orally at 10-200 mg/kg daily for 4 weeks. CK reduced the hepatic levels of malondialdehyde, a production of lipid peroxidation and partially prevented the marked decrease in body weight and reduced the mortality rate. The degree of fibrosis was evaluated by image analysis and also by measurements of collagen and hydroxyproline content in the liver. CK treatment significantly decreased the dimethylnitrosamine- or carbon tetrachloride-induced collagen and hydroxyproline contents. Immunohistochemical examination showed that CK reduced the deposition of type I and III collagen and the expression of a-smooth muscle actin in the liver in a dose-dependent manner. These findings indicate that CK suppress the induction of hepatic fibrosis and suggest that CK might be useful therapeutically in hepatic fibrosis/cirrhosis.

1675 EVALUATION OF THE HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF 18B-GLYCYRRHETINIC ACID.

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The protective effects of 18 b-glycyrrhetinic acid (GA), the aglycone of glycyrrhizin derived from licorice, on carbon tetrachloride-induced hepatotoxicity and the possible mechanisms involved in this protection were investigated in mice. Pretreatment with GA prior to the administration of carbon tetrachloride significantly prevented an increase in serum alanine, aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. In addition, pretreatment with GA also significantly prevented the depletion of glutathione content in the livers of carbon tetrachloride-intoxicated mice. However, reduced hepatic glutathione levels and glutathione-S-transferase activities were unaffected by treatment with GA alone. Carbon tetrachloride-induced hepatotoxicity was also prevented, as indicated by a liver histopathologic study. The effects of GA on the cytochrome P450 (P450) 2E1, the major isozyme involved in carbon tetrachloride bioactivation, were also investigated. Treatment of mice with GA resulted in a significant decrease of the P450 2E1-dependent hydroxylation of p-nitrophenol and aniline in a dose-dependent manner. Consistent with these observations, the P450 2E1 expressions were also decreased, as determined by immunoblot analysis. GA also showed anti-oxidant effects upon FeCl2-ascorbate induced lipid peroxidation in mice liver homogenate and upon superoxide radical scavenging activity. These results show that protective effects of GA against the carbon tetrachloride-induced hepatotoxicity may be due to its ability to block the bioactivation of carbon tetrachloride, primarily by inhibiting the expression and activity of P450 2E1, and its free radical scavenging effects.

1676 INHIBITORY EFFECT OF THE ROOTS OF *PLATYCODON GRANDIFLORUM* ON OXIDATIVE DAMAGE INDUCED BY BUTYL HYDROPEROXIDE IN RAT LIVER.

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Increasing evidence regarding free radical generating agents and inflammatory processes suggests that accumulation of reactive oxygen species can cause hepatotoxicity. In this study, we used t-butyl hydroperoxide (t-BHP) to induce hepatotoxicity *in vitro* and *in vivo* and determined the antioxidative bioactivity of aqueous extract from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK). CK-treated cells showed an increased resistance to oxidative challenge, as revealed by a higher percent of survival capacity in respect to control cells. Treatment with CK significantly decreased the leakage of lactate dehydrogenase and also decreased lipid peroxidation in a dose-dependent manner in primary cultured rat hepatocytes. Furthermore, CK protected from the t-BHP-induced intracellular generation of reactive oxygen species assessed by monitoring dichlorodihydrofluorescein fluorescence. An *in vivo* study in rats showed that pretreatment with CK prior to the administration of t-BHP significantly prevented the increase in serum alanine aminotransferase and aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. CK also protected the t-BHP-induced depletion of hepatic glutathione levels. Histopathological evaluation of the rat livers revealed that CK reduced the incidence of liver lesions induced by t-BHP, including hepatocyte swelling, leukocyte infiltration, and necrosis. Based on the results described above, we speculate that CK may play a hepatoprotective effects *via* reducing oxidative stress in living systems.

1677 RUBRATOXIN B INDUCED THE SECRETIONS OF M-CSF AND GM-CSF IN HEPATOCYTE-DERIVED CELL LINE HEPG2.

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Rubratoxin B is a potent hepatotoxic and teratogenic mycotoxin produced by certain Penicillium fungi. To date, biochemical, cytological and serological changes have been reported, however, the mechanism of its toxicity remains unclear. In this study, the induction of cytokine secretion by rubratoxin B was investigated using hepatocyte-derived hepatoma cell line HepG2. IL-8, M-CSF and GM-CSF were detected in the media of rubratoxin B-treated cells, and their levels peaked at about 40 mg/ml. The secretions of cytokines were enhanced by TNF-alpha; however, no synergism between the effects of rubratoxin B and TNF-alpha were observed.

While tyrosine kinase inhibitor emodin increased GM-CSF secretion, the secretions of two other cytokines were decreased, indicating that they are regulated differently in rubratoxin B-treated cells. Calcium channel blockers reduced the secretions of these three cytokines, suggesting that calcium channels play important roles in signal transduction of rubratoxin B. To our knowledge, this is the first report that exogenous stimulus induced the secretions of M- and GM-CSF in hepatocyte-derived hepatoma cells, suggesting that rubratoxin B is a superb model compound to study the mechanisms of M- and GM-CSF secretions. Furthermore, our results indicate that not only hepatocytes are the target cells for cytokines but they stimulate other types of cells by cytokines.

1678 ACTIVATION OF *PERITONEAL MACROPHAGE* FUNCTIONS AND NUCLEAR FACTOR-KB-DEPENDENT GENE EXPRESSION BY AQUEOUS EXTRACT OF PLATYCODON GRANDIFLORUM.

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Aqueous extract from the root of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK), is reported to have antitumor and immunomodulatory activities; however, the mechanism underlying its therapeutic effect is not known. The associated biological mechanisms are unclear, however, of the wide diversity of effects, it is believed that their activities may be exerted through several potent effector cells such as macrophages. Therefore, the effects of an aqueous extract from the root of Platycodon grandiflorum (Changkil: CK) on mouse peritoneal macrophage function were investigated. It was found that the CK stimulated macrophage proliferation, spreading ability, phagocytosis, cytostatic activity, and nitric oxide (NO) production in a dose-dependent manner, and that the production of cytokines such as TNF-a, IL-1b and IL-6 were similarly increased. Reverse-transcription polymerase chain reaction showed that CK increased the appropriate cytokine mRNAs. Transient expression assays with NF-kB binding sites linked to the luciferase gene revealed that CK-induced increase of inducible NO synthase mRNA and TNF-a mRNA were mediated by the NF-kB transcription factor complex. These results demonstrate that CK is a potent enhancer of macrophage function and CK stimulates NO and TNF-a release and is able to up-regulate iNOS and TNF-a expression through NF-kB transactivation.

1679 *IN VIVO* EFFECTS OF MYRIOCIN ON SPHINGOLIPID METABOLISM AND C-*MYC* EXPRESSION IN MOUSE LIVER.

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Myriocin, a fungal metabolite isolated from *Isaria sinclairii*, is a potent inhibitor of serine palmitoyltransferase (SPT), a key enzyme in *de novo* synthesis of sphingolipids. Exposure of cells in culture to myriocin decreases biosynthesis of sphinganine leading to a depletion of free sphingoid bases, and therefore myriocin has been widely used to study the biological role of sphingolipid metabolism. To evaluate the toxic effects of myriocin *in vivo*, we investigated the levels of free sphingoid bases and expression of selected genes regulating cell growth in mouse liver. Male Balb/c mice weighing 22 g were injected intraperitoneally with myriocin at 0, 0.1 0.3, and 1.0 mg/kg body weight daily for 5 consecutive days. Animals were euthanized 24 hours after the last treatment. Levels of plasma alanine aminotransferase and aspartate aminotransferase, indicators of liver injury, were not significantly altered by the treatment, suggesting no overt liver toxicity at the doses of myriocin employed. A dose-dependent decrease in free sphinganine but not sphingosine was detected by high performance liquid chromatography in both liver and kidney. The activity of SPT in liver was decreased by 80% at the highest dose of myriocin. The decrease of free sphinganine paralleled the observed decrease in SPT activity. Reverse transcriptase polymerase chain reaction analysis on liver mRNA indicated a dose-dependent increase in the expression of liver c-*myc*, but no changes in tumor necrosis factor α, transforming growth factor β, and hepatocyte growth factor. Results showed that myriocin inhibited *de novo* synthesis of sphingolipids *in vivo* by SPT inhibition and altered c-*myc* expression in liver. Long-term exposure to myriocin or the organism producing it may have adverse effects on health by interfering sphingolipid metabolism and modulating the expression of c-*myc* oncogene. (Supported in part by NIH ES09403 and TW01009).

1680 ACTIVATION OF NF-KB/REL AND P38 IN CHITOSAN-STIMULATED MACROPHAGES.

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Water-soluble chitosan stimulates murine macrophage to produce cytokines including iNOS. In the present study we investigated the molecular mechanism by which chitosan stimulate macrophages to produce iNOS. Treatment of RAW 264.7 cells with chitosan activated NF-kB/Rel in a dose-dependent manner. The involvement of NF-kB/Rel activation in chitosan-induced iNOS expression was confirmed by the experiment with the specific NF-kB/Rel inhibitors. Treatment of RAW 264.7 cells with chitosan resulted in significant activation of p38. The specific p38 kinase inhibitor SB203580 abrogated the chitosan-induced iNOS synthesis. In conclusion, we demonstrate that NF-kB/Rel and p38 kinase activation are required to transduce signals leading to iNOS expression in chitosan-stimulated murine macrophages.

1681 CDNA MICROARRAY ANALYSIS OF MATRIX METALLOPROTEINASE GENE EXPRESSION IN RAT MICROGLIA EXPOSED TO THE MARINE TOXIN DOMOIC ACID.

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Amnesic Shellfish Poisoning(ASP), one of the shellfish poisoning syndromes in the United States, is caused by the marine diatom toxin domoic acid (DOM), a glutamate analog. Our working hypothesis is that exposure to DOM may activate BMΦ and cause generation of potentially neurotoxic matrix metalloproteinases (MMP). We have recently reported that after a short-term (1-6 hours) *in vitro* treatment of rat BMΦ with DOM there was an increase in MMP-9 protein levels (Mayer et al. *BioMedCentral Pharmacology* 1:7-19, 2001). To further evaluate the role of other MMPs we investigated MMP gene expression in 4 hour-DOM [1mM]-treated BMΦ using a MMP-specific rat cDNA array, consisting of 22 MMP genes, including MMP-9 (SuperArray Inc., Bethesda, MD). Total RNA was extracted from BMΦ using TRI REAGENT®. Through side-by-side hybridization with cDNA probes prepared from RNAs of control or 4 hour DOM-treated rat BMΦ, the expression profiles of the 22 MMP genes was determined. Only 6 out of the total of 22 MMP genes investigated appeared to be constitutively expressed in untreated (Control) rat BMΦ *in vitro*. Furthermore, DOM-treated rat BMΦ showed altered transcription of 6 MMP genes as follows; **enhanced expression**: collagenase-like A, MMP-19, MMP-2, and tissue inhibitor of metalloproteinase-4 or TIMP-4; **reduced expression**: MMP-9 and TIMP-2. Our results constitute the first experimental evidence that DOM appears to alter the expression of rat BMΦ MMP genes *in vitro*. Thus cDNA microarray analysis provides potentially useful information to investigate candidate MMP genes that are most likely involved in DOM toxicity to rat BMΦand perhaps *in vivo* in ASP. Supported by NIEHS grant ES10138-01(AMM).

1682 ST. JOHN'S WORT REDUCES TRIBROMOETHANOL-INDUCED SLEEP TIMES IN MICE.

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The effects of St. John's wort (SJW) on tribromoethanol (TBE)-induced anesthesia were examined in mice. SJW is a popular herbal supplement for humans, which appears to induce cytochrome P450 3A (CYP3A) and can cause serious drug interactions. The utility of a mouse model to study this interaction was tested. TBE is a mouse anesthetic metabolized by CYP3A. It was hypothesized that SJW induces CYP3A causing increased clearance of other drugs (e.g. TBE). Mice were treated daily with SJW extract *via* gavage (1.0, 0.1, or 0.01 mg/g bodyweight/day) or water for 21 days. As a positive control for CYP3A induction, mice were treated with dexamethasone (DEX, 0.05mg/g of bodyweight, I.P.) at 48 and 24 hours prior to treatment with TBE. TBE was given at 0.4mg/g body weight, (S.C.) on days 1, 7, 14, or 21. SJW exposure (1 mg/g bodyweight) significantly decreased in sleep time compared to control mice on days 7, 14, and 21: day 7: C 45.4±2, SJW 24.18±3; day 14: C 43.77±2.87, SJW 30.92±0.44; day 21: C 41.75±5.24, SJW 30.19±1.88 ($p<0.05$; means±SE (min)). These results offer a preliminary demonstration of the utility of the mouse to study SJW-drug interactions.

1683 ALTERATIONS IN HUMAN AND PORCINE RENAL CELLS AFTER REPEATED EXPOSURE TO OTA AND OTB.

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Chronic dietary exposure to Ochratoxins induces porcine nephropathy (PMN) and has been strongly associated with the development of a similar human disease, namely Balkan Endemic Nephropathy (BEN). Both diseases are characterized by progressive fibrosis of the tubular interstitium and glomeruli. The mechanism(s)

underlying OTA's toxicity in the kidney is still unknown. Previous *in vitro* observations, have shown, that even after exposure to extremely high Ochratoxin concentrations (>10 μM), a certain percentage of renal epithelial cells always survive with an altered morphology and that fibroblastic cell types are more resistant to OTA-mediated toxicity. Thus, it is proposed that repeated exposure to ochratoxins transforms normal renal epithelial cells to a fibroblast-like phenotype. The aim of the study was to generate a pool of such altered cells and thereafter, to characterize the differences between these cells and their corresponding control cells. Thus, primary human and porcine renal epithelial cells were repeatedly exposed to acutely toxic concentrations of ochratoxin A (OTA) and its structural analogue ochratoxin B (OTB). For comparison with previous results, the human (IHKE) and porcine (LLC-PK1) continuous cell lines were tested in parallel. As shown before [Heussner et al., Toxicology. Sciences. 66 (1-S): 82 (2002)], repeated (x3) exposures of about 96 hours ensured selection and survival of the altered cell type. The morphology (microscopy), growth behavior (growth curves) and protein constitution (2D-PAGE, immunodetection of marker proteins) of these cells were subsequently characterized and compared with their respective untreated control cells. Preliminary results support the aforementioned hypothesis that renal epithelial cells attain a fibroblastic morphology and protein expression pattern.

1684 STRUCTURE-NEPHROTOXICITY RELATIONSHIPS AMONG THE CHLOROANILINES IN ISOLATED RENAL CORTICAL CELLS FROM FISCHER 344 RATS.

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Chloroanilines are widely used as chemical intermediates in the manufacture of pesticides and other compounds. Chloroanilines can appear in the environment following accidental spillage into waterways, as wastewater contaminants and through the degradation of pesticides. Mammals exposed to chloroanilines *in vivo* can develop nephrotoxicity characterized as oliguric acute renal failure. The purpose of this study was to examine the nephrotoxic potential of several mono-, di- and trichloroanilines *in vitro* using freshly isolated renal cortical cells (IRCC) from untreated male Fischer 344 rats as the kidney model. IRCC were obtained following collagenase perfusion of the kidneys in anesthetized rats. Cells (~ 3 - 4 million cells/ml) were incubated at 37o C under a 95% oxygen/5% carbon dioxide atmosphere with up to 4.0 mM of a chloroaniline for 60 or 120 min. The compounds tested were 2-; 3-; and 4-chloroaniline; 2, 3-; 2, 4-; 2, 5-; 2, 6-; 3, 4-; and 3, 5-dichloroaniline; 2, 3, 4-; 2, 4, 5-; 2, 4, 6-; and 3, 4, 5-trichloroaniline. Cytotoxicity was determined at the end of the incubation period by measuring the release of the cytosolic enzyme lactate dehydrogenase (LDH) into the medium. None of the monochloroanilines were toxic at 60 or 120 min at the concentrations tested. Among the dichloroanilines, 3, 4- and 3, 5-dichloroaniline were the most toxic. These isomers increased LDH release at bath concentrations of 1.0 mM or higher at 60 min and at bath concentrations of 0.5 mM at 120 min. Trichloroanilnes were generally intermediate in toxic potential between 3, 4- or 3, 5-dichloroaniline and the remaining mono- and dichloroanilines. These results indicate that the number of chloro groups and their position on the aromatic ring are important determinants for nephrotoxic potential among the chloroaniline compounds.

1685 PYRUVATE REDUCES MYOGLOBIN *IN VITRO* TOXICITY IN RENAL CORTICAL SLICES.

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Crush injury and drug overdose are associated with renal dysfunction due to myoglobin release. The purpose of the present study was to investigate pyruvate attenuation of myoglobin cytotoxicity using an *in vitro* renal slice model. Renal cortical slices from male Fischer 344 rats (200-250 g) were isolated, rinsed and allowed to equilibrate at 37oC for 5 min. Pyruvate treated slices (n=4/group) were co-incubated with 0 or 10 mM pyruvate along with 0, 4, 10 or 12 mg/ml myoglobin for 30-120 min. Cytotoxicity was assessed by comparison of lactate dehydrogenase (LDH) release, pyruvate stimulated gluconeogenesis and adenine nucleotide levels. Co-incubation with pyruvate prevented a rise in LDH leakage and maintained ATP levels comparable to control levels in the 4 and 12 mg/ml myoglobin treated group. Co-incubation with 1.67 mM glucose as an energy substrate did not attenuate myoglobin toxicity. A 60 and 120 min exposure to 4 and 12 mg/ml myoglobin decreased total glutathione levels. Glutathione levels following myoglobin exposure in the pyruvate treated tissue remained above control values indicating diminished oxidative stress. Glutathione disulfide levels were comparable in all pyruvate treated tissue exposed for 120 min to 4 and 12 mg/ml myoglobin while in the absence of pyruvate glutathione disulfide levels were increased 2 fold by 12 mg/ml myoglobin. These studies determined that pyruvate reduced myoglobin toxicity through a reduction in radical generation and *via* supply of an energy substrate.

1686 ADRENERGIC MODULATION OF ETHYLENE DIBROMIDE-INDUCED TOXICITY.

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Ethylene dibromide (EDB) has been used as a model compound for eliciting hepato- and nephrotoxicity. Conjugation with glutathione (GSH) or direct oxidation by the cytochrome P450 system has been shown to play a role in the bioactivation of EDB. The aim of this study was to determine whether activation of α-adrenergic receptors, which causes a decrease in cellular GSH levels, could modulate the nephrotoxicity of EDB. For this purpose, male ICR mice were administered (i.p.) EDB and/or the α-adrenergic agonist, phenylephrine (Pe), or the α-adrenergic antagonist, phentolamine (Phe). Animals treated with EDB (40 mg/kg) had a 9.3-fold increase in urinary γ-glutamyl transpeptidase (GGTP) activity and a 38% decrease in renal GSH levels; however, animals co-treated with EDB and Pe (50 mg/kg) exhibited a 27.8-fold increase in urinary GGTP activity and a 60% decrease in GSH levels. The enhanced presence of urinary GGTP and decrease in cellular levels of GSH was nearly blocked by treating animals concomitantly with EDB and Phe (10 mg/kg) or EDB, Pe, and Phe. Histopathological examination revealed the enhanced degree of tissue damage and necrosis following treatment with EDB and Pe, and the protective effect of Phe at ameliorating EDB toxicity. These results indicate that factors that can influence α-adrenergic receptors may be critical in assessing dose-response data used in the risk assessment process. (Supported in part by the Department of Defense)

1687 HEAT SHOCK PROTEINS AND URANIUM NEPHROTOXICITY.

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Military use of depleted uranium has renewed interest in uranium toxicity. Experimental evidence suggests that prior exposure to uranyl compounds can reduce nephrotoxicity of subsequent uranium exposures. The mechanism of this protection is not clear but has been attributed to heat shock protein (Hsp) induced cytoprotection. This study examined the relationship between stress proteins and acquired resistance to uranyl compounds. Male Sprague-Dawley rats were conditioned with 5 mg/kg uranyl acetate i.p. (UA) or saline (control) 10 days prior to challenge with 10 mg/kg i.p. UA. Five days after the second administration of UA, renal function, pathology and Hsp expression were evaluated. Based on histology and serum markers, a single acute dose of UA (10 mg/kg) produced acute proximal tubular necrosis. However, rats preconditioned with UA (5 mg/kg) exhibited improved kidney function and pathology. Kidney samples taken at the time of the second UA administration revealed strong induction of Hsp25, Hsp32 and Hsp70i, but not Hsc70, in preconditioned animals. Immunohistochemical staining demonstrated that UA preconditioning upregulated Hsp70i throughout the kidney while Hsp25 was highly localized in regenerating tubular epithelium. These observations demonstrate that several stress proteins are induced by UA administration and suggest that stress proteins confer resistance to UA-mediated nephrotoxicity by sparing proximal tubular epithelial cells, especially those undergoing active regeneration.

1688 CELLULAR PROFILES OF BENZO(a)PYRENE-INDUCED NEPHROPATHY.

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The nephrotoxic effects of polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (BaP), are not well understood. In agreement with data from rodent and human models of IgA nephropathy and glomerulonephritis, this laboratory has shown that repeated, weekly exposure of adult Sprague-Dawley rats to BaP (10 mg/kg *i.p.*) for up to 16 weeks is associated with glomerular hypercellularity. This response involves loss of renal function, as evidenced by increases in total urinary protein, protein/creatinine ratios, and urinary albumin, and structural deficits associated with activation of mesangial cells and disruption of visceral epithelial (i.e. podocyte) foot processes. Based on these observations we hypothesized that glomerular hypercellularity is mediated by proliferation of resident glomerular cells in response to hydrocarbon injury. To test this hypothesis, the present studies focused on the identification and quantification of glomerular cell types present in glomeruli of BaP-treated rats. Immunohistochemical markers of cellular identity

examined included Wilms' tumor suppressor protein (WT1) for podocytes, Thy1 for mesangial cells and CD68 for macrophages. Sprague-Dawley rats treated with BaP (10 mg/kg *i.p.*) for 8 weeks exhibited a significant increase in total cell number when adjusted for glomerular area [8.32 ± 0.11 ($p \leq 0.05$) compared to controls 7.86 ± 0.09]. Immunohistochemical analysis of WT-1 expression, a podocyte-specific antigen, showed modest, but significant increases in podocyte number normalized to glomerular area after 8 weeks of BaP treatment [2.4 ± 0.08 compared to controls 2.21 ± 0.06]. In contrast, CD68 and Thy1 expression was not altered following BaP exposure. These data indicate that BaP induced glomerular hypercellularity is partly mediated by podocyte proliferation. A role for other glomerular cell types in the nephropathic response remains to be established. (Supported by NIH grants ES04917, ES09106 and CA 90301).

1689 NEPHROPROTECTION FROM *S*-1, 2-DICHLOROVINYL-$_L$-CYSTEINE IN DIABETIC MICE.

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Streptozotocin (STZ)-induced diabetes has been reported to protect rats against renal injury produced by gentamycin, cisplatin and HgCl2. The aim of this work was to determine if STZ-induced diabetes protected Swiss Webster mice against a lethal dose (75 mg/kg ip) of *S*-1, 2-Dichlorovinyl-$_L$-cysteine (DCVC). Diabetes (plasma glucose > 200 mg/dL) was induced in male mice with STZ (200 mg/kg ip in 0.1 M citrate buffer) while the controls received only the citrate buffer. On day 10 the mice were then administered either DCVC (75 mg/kg ip) or vehicle alone. Ninety percent of the diabetic mice given DCVC survived in contrast to a 10% survival observed in non-diabetic mice given DCVC. Time-course measurements of plasma blood urea nitrogen (BUN) and creatinine as markers of renal injury and ^{3}H-thymidine (^{3}H-T) incorporation into renal DNA as an index of tissue repair were performed. Renal injury was also assessed by histopathology (H & E). Renal cytosolic and mitochondrial cysteine conjugate β-lyase activity, the enzyme that bioactivates DCVC, was also measured. In non-diabetic mice, DCVC produced a temporal increase in BUN and creatinine, which was associated with marked renal tubular necrosis and resulted in 90% mortality within 48 h. In contrast, in the diabetic mice, BUN and creatinine increased until 36 h after which time the renal injury declined to normal and the mice survived. An early and steady onset in nephrogenic repair in the diabetic mice, as evidenced by ^{3}H-T incorporation, may explain the recovery from ARF and survival. Renal β-lyase activity was not altered in the kidney of diabetic mice, indicating that lack of metabolic activation of DCVC could not explain the protection. These findings suggest that protection of diabetic mice from DCVC-induced mortality may be due to upregulation of tissue repair resulting in recovery from the renal injury and survival. (Supported by Kitty DeGree Endowment and the LBRS)

1690 CELLULAR AND MOLECULAR MECHANISMS UNDERLYING OCHRATOXIN A-INDUCED *IN VITRO* NEPHROTOXICITY.

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Ochratoxin A (OTA) is a mycotoxin produced by storage moulds (primarily by Aspergillus and Penicillum species) that contaminates cereals and animal feed, causing nephropathy and renal tumors to a variety of animal species. *In vitro*, OTA may induce apoptosis, inhibit protein synthesis, and form DNA adducts. With the aim to study the cellular and molecular mechanisms underlying OTA-induced toxicity, the porcine renal proximal tubule epithelial cell line LLC-PK1 was exposed for 24 h to 0-50 μM OTA. Cytotoxicity assays, such as lactate dehydrogenase (LDH), revealed significant toxic effects after exposure of the cells to 50 μM OTA. At the same concentration, we observed alterations in the cytoskeleton organization with the classical formation of stress fibers, besides mitochondrial membrane potential modifications. In addition, confocal microscopy showed DNA fragmentation, clearly indicating induction of apoptotic cell death. Interestingly, by using a cDNA macroarray, the expression of genes involved in OTA-induced nephrotoxicity has been analyzed, revealing an up-regulation of the early growth response gene EGR 1. Our data confirm that the LLC-PK1 cell line may be considered a good *in vitro* model for studying toxicity in the renal epithelium.

1691 MODULATION OF RENAL CYCLOOXYGENASE-2 (COX-2) BY DIET AND STAGE OF ESTROUS IN CD SPRAGUE-DAWLEY RATS.

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Both cox-1 and cox-2 are constituitively expressed in rodent kidney with distinct regional localizations. Gene knockout and cox inhibitor studies have indicated that cox-2 plays a significant role in development of the kidney as well as in adult kidney function. Soy-containing diets have been shown to protect the rodent kidney in strains prone to polycystic kidney disease and after treatment with renal toxins. We hypothesize that modulation of renal cyclooxygenase activity by soy may play a role in this protection. In an initial experiment to examine this question, female NCTR CD rats were maintained on soy- and alfalfa-containing NIH31 diet until weaning when half were shifted to a soy- and alfalfa-free diet (5K96). Estrous cycles were monitored by daily vaginal cytology and animals were sacrificed on postnatal day 50 ±5 in either proestrus or diestrus. Stage of cycle at necropsy was confirmed by histological examination of the vagina, and serum was collected and analyzed for estradiol and progesterone. One kidney was snap frozen and the second processed for the preparation of frozen sections for immunohistochemical analysis. Measurements of cox-2 mRNA levels (relative to glucose 6-phosphate dehydrogenase mRNA) and protein levels were made by RT-PCR and Western blots, respectively. Analysis of mRNA levels indicated a significant effect of cycle stage, with levels in proestrus significantly greater than those in diestrus in both diets. Analysis of protein levels indicated significant effects of diet (NIH31 higher) and cycle and a significant interaction of cycle with diet. Consistent with the mRNA data, cox-2 protein was found in higher amounts in proestrus than in diestrus, but this was only the case with the 5K96 diet. This may indicate the involvement of both transcriptional and post-transcriptional controls of cox-2 expression. These data provide preliminary evidence that renal cox-2 is modulated by the hormonal fluctuations of the estrous cycle and that this modulation is affected by diet.

1692 DECREASED N- AND KSP-CADHERIN EXPRESSION ASSOCIATED WITH HGCL2-INDUCED ACUTE RENAL FAILURE IS RELATED TO THE SPATIAL EXPRESSION PATTERN OF CADHERINS ALONG THE NEPHRON.

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Cadherins are cell surface molecules which play an important role in cell-cell adhesion and signal transduction in conjunction with their cytoplasmic binding partners, catenins. In the kidney, these adhesion molecules are essential for the maintenance of epithelial barriers and are critical for establishing polarity in proximal tubule epithelial cells. Multiple cadherins and catenins are expressed in the kidney and although much is known about patterns of expression during development and in renal carcinomas, the spatial pattern of cadherin and catenin co-expression has not been defined. Cadherin and catenin expression was analyzed by western blot analysis, immunohistochemistry and immunofluorescence in mouse kidney. Four cadherins were detected by Western blot analysis in the adult mouse kidney including E-, K-, Ksp- and N-cadherin. In addition, four catenins were also detected, i.e., alpha-, beta-, gamma and p120. Using immunohistochemistry and immunofluorescence, N- and Ksp-cadherin were primarily localized to the proximal tubules, while E-cadherin expression was confined to the distal tubules. K-cadherin expression was not detected by either technique using several different antibodies. Alpha- and beta-catenin were expressed in both proximal and distal tubules, while p120 was limited to the proximal tubules and gamma-catenin to the distal tubules. This pattern of expression may explain, in part, the selective decrease in Ksp- and N-cadherin, as well as alpha- and p120-catenin, expression observed during the later stages of mercuric chloride-induced ARF as injury to the proximal tubules is a hallmark of acute renal failure.

1693 INFLUENCE OF EDTA AND CITRATE ON HEMOLYSIS INDUCED BY OXALATE.

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Background: Ethylene glycol (EG) poisoning produces multiple system organ injury, including acute renal failure and CNS depression. A common observation in EG-poisoned patients is the precipitation of oxalate crystals (COM) in blood vessels and various tissues, including the renal tubular lumens. Oxalate crystals have been proposed as the etiologic agent for organ injury in EG poisoning. The hemolysis assay is a good model to screen for acute cell membrane damage induced by chemicals. In the present studies, we compared the inhibition of EDTA and citrate on the hemolytic rate induced by COM. Methods: 0.5% rat erythrocytes were exposed to COM, EDTA-treated COM and citrate-treated COM for 1 hour at 37oC. The hemolytic rate was measured at 540 nm. Results: EDTA and citrate significantly inhibited the hemolytic rate induced by COM. The effect of citrate was significantly greater than EDTA at the same dose levels, but EDTA and citrate produced different effects on the solubility of COM. COM remained in the precipitate in the presence of citrate, while EDTA produced significant amounts of soluble oxalate. Conclusion: The results indicate that EDTA and citrate have different mechanisms in prevention of the cell damage induced by COM.

1694 DEGRADATION OF AC2993 (SYNTHETIC EXENDIN-4) IN MICE, RAT, RABBIT AND MONKEY KIDNEY MEMBRANE PREPARATIONS.

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AC2993 is synthetically manufactured exendin-4, a 39-amino acid peptide. Nonclinical studies have shown that AC2993 has several potentially beneficial antidiabetic (glucose-lowering) actions. These include amplification of insulin secretion (insulinotropic effect), suppression of postprandial glucagon secretion (glucagonostatic effect), reduction in food intake, and modulation of nutrient delivery. It has been shown that the kidneys are the major route of elimination of systemic AC29931. The purpose of this study was to determine the relative rates of degradation and the major degradation products of AC2993 *in vitro* using membrane preparations of mice, rat, rabbit and monkey kidneys. Membranes were prepared from the kidneys by homogenization followed by differential centrifugation. The total protein content of each membrane preparation was determined by BCA assay; the final protein concentration was adjusted to approximately 10 mcg/mL in the assay. The rate of AC2993 degradation was determined by HPLC analysis of samples collected over a 120 min period following initiation of the reaction by the addition of 10 mcg AC2993. Relative rates of degradation were found to be rat > mouse > rabbit @ monkey. The major breakdown products are being identified using a combination of HPLC, LC/MS/MS, and protein sequencing. The first degradation product, in the mouse kidney membrane preparation, appears within one minute and has been identified as exendin?4 (1-22) using LC/MS/MS techniques. 1. Parkes D, Jodka C, Smith P, et al. Pharmacokinetic actions of exendin-4 in the rat: comparison with glucagons-like peptide-1. Drug Dev Res 2001; 53: 260-267.

1695 CADMIUM NEPHROTOXICITY IS ASSOCIATED WITH ALTERATIONS IN THE PATTERN OF CADHERIN LOCALIZATION IN THE PROXIMAL TUBULE EPITHELIUM.

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Exposure to cadmium (Cd) results in a generalized dysfunction of the proximal tubule that is characterized by an increase in the urinary excretion of glucose, amino acids and low molecular weight proteins. While these effects of Cd have been well-documented, relatively little is known regarding the mechanisms by which Cd alters proximal tubule function. Recent studies utilizing renal epithelial cell lines in culture have shown that Cd has specific damaging effects on the cadherin dependent junctions between the cells. The objective of the present study was to determine if Cd can disrupt cadherin-dependent junctions in the proximal tubule *in vivo*. Male Sprague-Dawley rats received subcutaneous injections of Cd (0.6 mg/kg in isotonic saline, 5 days per week for up to 6 weeks), while control animals received the saline vehicle alone. One day each week, 24-hour urine samples were collected and analyzed for protein and creatinine. The results showed that after 5-6 weeks, the Cd-treated animals developed significant proteinuria, with no change in creatinine excretion. Histopathologic analysis of the kidneys from Cd-treated animals showed widespread evidence of vacuolization and separation of the epithelial cells in the proximal tubule. Visualization of pan-cadherin immunoreactive materials by immunoperoxidase labeling showed that Cd caused a marked reduction in the intensity of cadherin labeling associated with the basolateral surface of the epithelial cells of the proximal tubule, but no change in the pattern of cadherin labeling in other segments of the nephron. Additional studies utilizing specific antibodies against E- and N-cadherin showed changes in the localization of both cadherins in the proximal tubule epithelium. These results indicate that Cd can disrupt cadherin dependent cell-cell junctions in the proximal tubule, and they raise the possibility that a loss of cadherin-mediated adhesion may contribute to the nephrotoxic effects of Cd. (Supported by Grant R01-ES06478).

1696 SERIALLY-AGITATED DILUTE SOLUTIONS OF CDCL2 FAIL TO PROTECT AGAINST THE ACUTE CYTOTOXIC EFFECTS OF CD2+ IN LLC-PK1 CELLS.

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Homeopathic pharmacotherapy is based on the so called "law of similars" and the premise that drug efficacy can be enhanced by the repetitive agitation and dilution of drug solutions. Recently, it has been reported that homeopathically-prepared, dilute solutions of Cd salts can protect against the acute toxic effects resulting from exposure to high doses of Cd^{2+}. The objective of the present study was to determine if serially-agitated dilute solutions (SADS) of $CdCl_2$ can protect against the toxic effects of Cd^{2+} in LLC-PK_1 cells, an immortal cell line that has been widely used as a model for Cd^{2+} toxicity studies. SADS of $CdCl_2$ in endotoxin-free distilled water were prepared by starting with a 1 mM stock solution and using a homeopathic centesimal dilution technique; the final concentrations of the SADS ranged from 10^{-2} - 10^{-60} of the original stock solution. Confluent cells on Falcon cell culture inserts were pretreated with the SADS by adding the solutions directly to the tissue culture medium (10λ SADS/ml of medium) and incubating the samples for 24 hours. The cells were then challenged with a toxic dose of $CdCl_2$ (10 μM) and monitored for evidence of cytotoxicity using 3 end points (ie. morphologic changes, a decrease in the transepithelial electrical resistance (TER) and the leakage of lactate dehydrogenase (LDH) into the medium). The results showed that in all samples, exposure to 10 μM Cd for 1-6 hours caused the cells to separate from each other without detaching from their growing surface, an effect that coincided with a marked drop in the TER. Longer periods of exposure (8-24 hours) resulted in the death of the cells as evidenced by the detachment of the cells from the growing surface and the leakage of LDH into the medium. Most significantly, there were no differences in the intensity of these responses between the control samples and the samples that had been treated with the SADS. These results do not support the previous claims that SADS of Cd salts can protect the acute toxic effects of Cd^{2+}. Supported by Grant R01-ES06478 from the NIEHS.

1697 CADMIUM ADVERSELY AFFECTS CHOLINE UPTAKE AND THE CYTOSKELETON IN OPOSSUM KIDNEY CELLS.

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Long-term cadmium exposure has been shown to be nephrotoxic. The proximal tubule is a specific target, and damage to this segment results in elevated excretion of vital nutrients such as glucose and serum proteins. However, the effects of cadmium on renal reabsorption of choline, an essential nutrient for membrane and neurotransmitter synthesis, have not been well characterized. The effects of long-term cadmium exposures on apical choline uptake were characterized in immortalized opossum kidney cells, a model for renal proximal tubule. The effects of cadmium on the cytoskeleton were also studied, as they may contribute to changes in choline uptake. Cells were grown to confluence on solid supports and then treated for 24 h with 500 nM-25 μM $CdCl_2$ without FBS. Carrier-mediated uptake of 10 μM ^{3}H-choline±750 μM hemicholinium-3 was assayed (37C, 30 min). F-actin and microtubules were examined by epi-fluorescence microscopy. Cytotoxicity and cell viability were assessed based on LDH release and WST-1 formation, respectively. At Cd concentrations ≤ 1 μM, choline uptake was similar to control. However, treatment with 5-100 μM Cd progressively decreased uptake. LDH release from cells treated with ≤ 1 μM Cd was comparable to controls; however, release increased in a dose-dependent manner with 10 μM-25 μM Cd. WST-1 data suggest a biphasic dose-response to cadmium treatment, with increased viability at concentrations ≤ 10 μM and decreased viability at concentrations > 25 μM. Epi-fluorescence images indicated that ≤ 1 μM Cd induced little change in actin filaments, whereas ≥ 5 μM Cd decreased actin filaments. Microtubules remained intact at Cd concentrations ≤ 5 μM but were disrupted at concentrations ≥ 10 μM. These data show that extended exposure to 5-10 μM Cd may decrease choline uptake and disrupt the cytoskeleton with minimal adverse affects on cytotoxicity and cell viability. Decrease in uptake may be mediated in part by cytoskeletal disruption; this remains to be established. Modulation of signaling cascades or intracellular calcium may also be involved. ES10439; NS39452; T32ES07026

1698 INITIAL CHARACTERIZATION OF THE GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT *Gclm(-/-)* KNOCKOUT MOUSE: NOVEL MODEL SYSTEM FOR A SEVERELY COMPROMISED OXIDATIVE STRESS RESPONSE.

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Reduced glutathione (GSH) is the most abundant antioxidant in the cell and exists in both prokaryotes and eukaryotes. The rate-limiting enzyme in GSH biosynthesis is glutamate-cysteine ligase (GCL). In higher eukaryotes, this enzyme is a heterodimer comprised of a 72.8-kDa subunit with catalytic activity (GCLC) and a 30.8-kDa modifier subunit (GCLM), which changes the catalytic characteristics of the holoenzyme. To define the cellular function of GCLM, we disrupted the mouse *Gclm* gene to create a null allele. *Gclm(-/-)* mice are viable, fertile and have no overt phenotype. In liver, lung, pancreas, red cells and plasma, however, GSH levels in *Gclm(-/-)* homozygous knockout mice were 9-16% of that in *Gclm(+/+)* wild-type littermates. Cysteine levels in *Gclm(-/-)* mice were 9%, 35% and 40% of that in *Gclm(+/+)* mice in kidney, pancreas and plasma, respectively, but remained unchanged in the liver and red cell. Comparing the hepatic GCL holoenzyme with GCLC in the genetic absence of GCLM, we found the latter had a ~2-fold increase in Km for glutamate and a dramatically enhanced sensitivity to GSH inhibition. The major decrease in GSH, combined with diminished GCL activity, rendered

Gclm(-/-) fetal fibroblasts strikingly more sensitive to chemical oxidants such as H_2O_2. We conclude that the *Gclm(-/-)* mouse represents a model of chronic GSH depletion that will be very useful in evaluating the role of the GCLM subunit and GSH in numerous pathophysiological conditions, as well as in environmental toxicity associated with oxidant insult (supported in part by NIH P30 ES06096).

1699 HEPATOCYTE-SPECIFIC KNOCKOUT OF GLUTAMATE-CYSTEINE LIGASE CATALYTIC SUBUNIT IN MOUSE: EARLY DEATH WITH PROGRESSIVE LIVER DEGENERATION AND RESCUE BY N-ACETYLCYSTEINE.

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GSH depletion has been associated with numerous liver diseases, but the cause-and-effect relationship has not been established. To define a role for hepatic GSH *in vivo*, we generated an hepatocyte-specific Cre recombinase-mediated disruption of the *Gclc* gene encoding the catalytic subunit of glutamate-cysteine ligase, an essential enzyme in GSH biosynthesis. By selective breeding, we recovered offspring that expressed Cre recombinase driven by the hepatocyte-specific albumin promoter and in which both *Gclc* alleles were flanked by *loxP* sites (floxed, *f*). In these mice, deletion of the *Gclc(f)* allele led to detection of the hepatocyte-specific *Gclc(h)* knockout allele as early as gestational day 16; however, *Gclc(h/h)* were born at the expected Mendelian frequencies. By day 14 after birth (d14), Southern blot analysis showed that deletion of *Gclc(f)* was complete, and hepatic GSH levels were 1-2% of normal. Surprisingly, *Gclc(h/h)* mice appeared normal until weaning, at which time they failed to gain weight and died of hepatic failure around d30. Plasma GSH levels dropped to 19%, 16%, and 2% of that in *Gclc(+/+)* wild-type at days 14, 21 and 30, respectively, but GSH depletion (20-50%) in nonhepatic tissues was not obvious until d21. Livers from d30 *Gclc(h/h)* mice showed focal necrosis, inflammation and apoptosis. Remarkably, N-acetylcysteine supplementation in the drinking water, starting at d21, rescued *Gclc(h/h)* mice. Our data suggest that GCLC is essential for hepatocyte function, but that other thiols may be able to replace some essential functions of GSH (supported in part by NIH P30 ES06096).

1700 ACETAMINOPHEN HEPATOTOXICITY IS NOT ENHANCED IN GENETICALLY ALTERED MICE (GR1A1NEU) WITH DIMINISHED GLUTATHIONE REDUCTASE ACTIVITIES.

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Glutathione reductase (GR) is a component of the glutathione-dependent antioxidant system and functions to reduce glutathione disulfide (GSSG) to the thiol form (GSH). In previous studies *in vitro*, we have demonstrated that transgene-driven enhancement of GR activities increased resistance to oxidant injuries and that antisense expression of a transgene could diminish GR activities and resistance to oxidants. To test the hypothesis that decreased GR activities would increase susceptibilities to oxidant injuries *in vivo*, we studied a genetically altered strain of mice (Gr1a1Neu) [Pretsch, Genet Res Camb, 1999] that express <10% of the hepatic GR activities of the C3H wild type animals (Neu, 247+47, C3H, 3841+207 mU/g tissue). Despite the low GR activities in the Neu mice, no marked physiological effects have been observed or reported. Hepatic GSH (Neu, 7.59+0.9, C3H, 7.18+0.3 mM) and GSSG levels (Neu, 64+9; C3H, 45+18 uM) were not different between Neu and C3H mice, and basal glutathione peroxidase activities were (Neu, 106+22; C3H, 166+6 University/g tissue). As assessed by real time PCR, the relative hepatic GR mRNA expressions in the Neu mice were 1/1000 the levels observed in the C3H wild type mice. Male and female Neu and C3H mice that had been fasted 18 h were given doses of 0, 50, 100, 200, or 300 mg/kg of acetaminophen, 6 h later the animals were sacrificed, and plasma ALT activities were measured for assessment of liver injury. Plasma ALT activities were increased modestly in the Neu animals at 200 mg/kg (Neu, 85+32; C3H, 35+20 IU/L) and increased comparably in both strains at 300 mg/kg (Neu, 8346+2134; C3H, 9197+5116 IU/L). The data indicate that Neu mice are not markedly more susceptible to acetaminophen hepatotoxicity than are the control C3H mice, despite the profound differences in GR activities. Supported by GM44263 from NIH.

1701 CHLORDANE EFFECTS ON HEPATIC GLUTATHIONE LEVELS IN DAMS, PUPS AND VIRGIN RATS.

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Technical chlordane (TC), a complex cyclodiene pesticidal mixture banned in the US in 1988, is still in the food chain, all environmental media & biota and in humans. It poses a risk to humans. Human liver may be a major target organ for chronic oral exposures at environmental levels. Our rat studies identified the liver as a target of chronic exposure since induction of hepatic cytochrome P450 (CYP1A1, 2B1, 2E1) occurred without hepatotoxicity. TC can induce its own metabolism and superoxides, potentially hepatotoxic. Glutathione(GSH) dependent enzyme system can provide major protection against toxic agents produced by xenobiotics. GSH is playing an important function in conjugating xenobiotics with its thiol(-SH) group. Thus, alterations in levels of GSH can also alter the detoxification potential of this enzyme system. We used Sprague-Dawley rats from our TC multigeneration study, to investigate the long-term oral effects of TC on hepatic GSH in dams their preweaned pups and virgin adults. Virgin F3 rats, pregnant F3 dams and their pups were daily exposed to TC at 0.00, 0.125, 0.25 and 1 mg/kg b.w./day. Pups were exposed *in utero* and *via* dam's milk and adults by gavage. Hepatic GSH content was determined using 5, 5'-dithio-bis (2-nitrobenzoic acid) as fluorescent reagent according to the method of Ellman, 1959. TC treatment increased, ($p<0.0001$), hepatic GSH levels in virgin rats at the high dose & in dams at the mid and high doses when compared to controls. In female pups, the GSH level increased ($p<0.0001$) at the low, mid and high dose levels. In male pups, increase ($p< 0.05$) in GSH level occurred only at high TC dose level. Results demonstrate that TC caused sex dependent effects (female>males)and increased GSH level may decrease the potential effects of the toxicant by GSH dependent detoxification.(Supported by MHPF/ATSDR U50/ATU398948)

1702 PROTECTION OF MICE FROM ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY A SULFHYDRYL-PROTECTED PRODRUG OF GLUTATHIONE.

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Acetaminophen (ACP), in high doses, is known to elicit liver toxicity in mice manifested by profound elevation of transferase enzymes in serum with histological evidence of hepatic necrosis. We have previously observed that a number of cysteine prodrugs and double prodrugs can protect against this ACP-induced hepatotoxicity (Crankshaw, et al., J Biochem Mol Toxicol, in press). In developing a ^{14}C-glycine/HPLC method for assessing the incorporation of the cysteinyl moiety (from the prodrug) into GSH in rat lens (AM Holleschau, et al., 1996, Curr Eye Res 15:501-510), we discovered a radioactive peak near GSH which appeared to be produced metabolically. This substance was tentatively identified as the mixed disulfide, L-cysteine-glutathione disulfide (L-CySSG). In the present study, L-CySSG was found to be a highly effective protective agent against ACP-induced hepatotoxicity in mice. Thus, pre- (1.23 mmol/kg) and post- (2.45 mmol/kg) administration of L-CySSG to mice given 2.45 mmol/kg of ACP at 1 hr pre- and 30 min post-ACP (or the reverse protocol, dose-wise) fully protected mice from toxicity, as evidenced by minimal elevation in serum alanine transaminase (ALT) levels. The mixed disulfide prepared from D-cysteine and GSH, *viz.*, D-CySSG, was totally ineffective in protecting mice from ACP-induced hepatotoxicity. This suggests that L-CySSG must be metabolically activated to release GSH, presumably by enzymatic reduction of the disulfide bond. L-CySSG is, therefore, a sulfhydryl-protected prodrug of GSH. GSH monoethyl ester, a known prodrug of GSH, was only moderately hepatoprotective in this system. (Supported by the Department of Veterans Affairs)

1703 TOXICITY OF PHENOLIC COMPOUNDS: DIRECT DETECTION OF GLUTATHIONYL RADICALS PRODUCED BY MYELOPEROXIDASE-CATALYZED METABOLISM IN CELLS.

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Peroxidases (including myeloperoxidase and cyclooxygenase-2) catalyze one-electron oxidation of phenolic compounds to yield potentially cytotoxic phenoxyl radicals which can trigger/enhance oxidative stress. In particular, phenoxyl radicals can react with glutathione, the major regulator of redox-status in cells, to form intermediate glutathionyl radicals ($GS^{\bullet}$). We developed a new sensitive method for the detection of glutathionyl radicals based on a specific interaction of $GS^{\bullet}$ with Ac-Tempo (a conjugate of fluorogenic acridine with paramagnetic nitroxide Tempo). $GS^{\bullet}$ generated by peroxidase-catalyzed reaction through phenoxyl radical recycling or by photolysis of nitrosoglutathione, reacted with Ac-Tempo resulting in the decay of Tempo EPR signal and in the production of acridine fluorescence. Spin trapping of $GS^{\bullet}$ by DMPO and PBN inhibited interaction of $GS^{\bullet}$ with Ac-Tempo. Other radicals resulting from secondary $GS^{\bullet}$ reactions, i.e., the glutathione disulfide and superoxide anion radicals had no significant impact on the fluores-

cence production. The major product of $GS^{\bullet}$ and Ac-Tempo reaction was fluorescent acridine-piperidine as determined by combined HPLC and Mass spectrometry techniques. This method was applied for the fluorescence microscopic imaging of glutathionyl radicals known to be produced by myeloperoxidase-catalyzed metabolism of phenol in HL-60 cells. Indeed, Ac-Tempo fluorescence was observed upon addition of H_2O_2 and phenol, and it was inhibited by pre-incubation of cells with the thiol-blocking reagent, N-ethylmaleimide, or with the inhibitor of heme synthesis, succinyl acetone. Intracellular generation of $GS^{\bullet}$ was also detected in cyclooxygenase-2-transfected PC12 cells upon stimulation with phenol and H_2O_2. Thus, Ac-Tempo can be applied to detect, quantify and to image glutathionyl radical generation in cells. (Supported by NIH/NINDS/WHO #1 F05 NS43922-01)

1704 A ROLE FOR ASCORBATE AND PROTEIN-GLUTATHIONE MIXED DISULFIDES IN CELLULAR PROTECTION AND GSSG/GSH REDOX REGULATION.

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Defects in mitochondrial metabolism and decreases in glutathione are present in idiopathic Parkinson's disease and are thought to contribute to the loss of dopamine (DA) neurons. Malonate, an inhibitor of SDH/complex II, has been used to model the effects of mitochondrial impairment in neurons and results in a greater loss of DA than GABA neurons *in vivo* and *in vitro*. Our past studies have shown that ascorbate, while providing protection to cultured mesencephalic DA and GABA neurons from malonate, significantly increased the formation of protein-glutathione mixed disulfides (PrSSG). To better understand the role of ascorbate and PrSSG in protection, time course studies were conducted with malonate ± ascorbate for 4-8 hr and intra- and extracellular GSH and GSSG, and PrSSG were determined. Ascorbate significantly decreased the efflux of GSH and GSSG, increased PrSSG levels and maintained a more reduced GSSG/GSH ratio compared to control and malonate alone. When glutathione was decreased in cells with buthionine sulfoxamine (BSO), malonate toxicity and reactive oxygen species (ROS) generation were greatly potentiated. Under these conditions ascorbate fully prevented the rise in ROS and provided complete protection. With 12 h exposure to malonate plus ascorbate in BSO treated cultures, the absolute amount of PrSSG formed was lower than in non-BSO treated controls, however, the proportion (‰) of PrSSG to intracellular GSH was significantly elevated. These findings suggest that ascorbate may be protective by acting as a free radical scavenger and by sequestration of GSH in the cell through PrSSG formation. This work was supported by PHS grant NS 36157

1705 ROLE OF GLUTATHIONE DEPLETION IN TOXICANT INDUCED INJURY TO CLARA CELLS.

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The early patterns of toxicity that occur in Clara cells (i.e. non-ciliated airway epithelium) exposed to the metabolically activated pulmonary toxicant naphthalene are closely associated with glutathione (GSH) depletion, and include: swelling, membrane bleb formation, cytoskeletal disruption, mitochondrial injury, and increased membrane permeability, which leads to necrosis. The purpose of this study was to define the cellular events that occur in response to acute GSH depletion alone. Mice were given a single injection of the selective GSH depletor diethylmaleate (DEM; 1g/kg ip). GSH levels in airways were measured directly by HPLC with electrochemical detection, 1-24 hours post-treatment. Changes in cell morphology, ultrastructure, and membrane permeability were examined by light and electron microscopy, and laser-scanning confocal microscopy. Airway GSH levels dropped rapidly following DEM treatment (70% loss within 2 hours) but returned to normal levels within 24 hours. Swelling was apparent in the Clara cells lining the distal (50% volume increase) but not proximal airways. Cell volume increased maximally by 3 hours post-treatment but returned to control levels within 24 hours. Most of the volume increase appeared to be from swollen endoplasmic reticulum. Membrane blebbing was apparent in some distally located Clara cells, where it appeared that the mitochondria were partitioned away from the blebs. Irregularly shaped, membrane bound structures were observed protruding from the apical surfaces of some cells 1 day after treatment, but their nature is uncertain. Airway cells did not appear necrotic nor did their membranes become permeable at any time point. In Clara cells GSH depletion in the absence of reactive metabolite formation, produces some changes associated with bioactivated toxicants (swelling and bleb formation), but does not increase membrane permeability or markedly alter mitochondria, and is not a lethal event.

1706 FORMATION OF A GLUTATHIONE ADDUCT WITH A COCAINE PYROLYSIS PRODUCT, ANHYDROECGONINE METHYL ESTER.

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Introduction: Anhydroecgonine methyl ester (AEME, methylecgonidine) is a pyrolysis product of smoked crack cocaine that appears to play a role in cocaine associated toxicity. AEME and its metabolic product, anhydroecgonine (AE, ecgonidine), have been detected in the urine of crack smokers. Because AEME can undergo a Michael addition reaction with toxicologically relevant nucleophiles, the chemical reactivity of AEME toward glutathione and *N*-acetyl-L-cysteine was studied. A better understanding of the chemical reactivity of AEME with glutathione may assist in the identification of toxic pathways associated with smoked cocaine. **Experimental:** AEME in acetonitrile was combined with reduced glutathione in water and ammonium acetate buffer (pH 7.4) and the mixture was stirred in a sealed tube under N_2. The resulting solution was evaporated to dryness and the residue dissolved in methanol for analysis by electrospray ionization ion trap mass spectrometry. This synthetic method was modified for the reaction of AEME with *N*-acetyl-L-cysteine in water and AE in methanol with glutathione. **Results:** A mass spectral ion at *m/z* 489 was consistent with assignment as a glutathione-AEME adduct. Multiple stage mass spectral analyses (MS^2 and MS^3) of the parent ion confirmed the structure of the product. The reaction of AEME with *N*-acetyl-L-cysteine yielded a base ion at *m/z* 345 for the adduct and daughter ions consistent with an AEME-*N*-acetyl-L-cysteine structure. Similarly, AE formed an adduct with glutathione, shown on mass spectral analysis (*m/z* 475) and further fragmentation by MS-MS. **Conclusions:** Adducts between AEME-glutathione, AEME-*N*-acetyl-L-cysteine and AE-glutathione were detected and their structures elucidated by mass spectrometry. Identification of these adducts *in vitro* suggests that AEME and AE can covalently link with glutathione and possibly with other sulfhydryl-containing cellular constituents, such as proteins.

1707 EXPRESSION AND STABLE TRANSFECTION IN NRK-52E CELLS OF THE MITOCHONDRIAL 2-OXOGLUTARATE CARRIER (OGC), A GLUTATHIONE TRANSPORTER.

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Previous studies of ours showed that the OGC is one of two carrier proteins involved in the transport of glutathione (GSH) from cytoplasm into mitochondrial matrix in rat kidney proximal tubule. The cDNA for rat mitochondrial OGC was amplified from total rat kidney RNA by RT-PCR, expressed as a polyhistidine (His_6)-tagged fusion protein in *E. coli*, purified from inclusion bodies and by nickel-affinity chromatography, and reconstituted into proteoliposomes. Reconstituted rOGC-His_6 transported both 2-oxoglutarate (OG) and GSH, and transport of 1 mM of each of the substrates was inhibited by more than 80% by either 10 mM pyridoxal 5'-phosphate or phenylsuccinate. Further, GSH transport by the reconstituted carrier was inhibited by *S*-alkyl GSH analogues (-methyl to -hexyl), with the extent of inhibition generally increasing with increasing alkyl chain length. OG was transported in proteoliposomes with a K_m of 184 μM and a V_{max} of 475 nmol/min per mg protein. NRK-52E cells, a stable cell line derived from normal rat kidney proximal tubules, was stably transfected with the cDNA for rOGC-His_6. Expression of the carrier in mitochondria from the transfected NRK-52E cells was confirmed by Western blot analysis using an antibody directed to the His_6-tag, and transport activity with OG and GSH as substrates was increased by > 5-fold as compared to that in mitochondria isolated from wild-type NRK-52E cells. These results demonstrate that the expressed and reconstituted OGC exhibits similar properties to the native protein in rat kidney mitochondria. Overexpression in the NRK-52E cells provides a tool to investigate the function of this carrier in regulation of GSH status and function in renal mitochondria. (Supported by NIH Grant DK40725.)

1708 ROTENONE AND/OR CHLORPYRIFOS EXPOSURE OF HUMAN DOPAMINERGIC NEUROBLASTOMA CELLS (SH-SY5Y) AND CORTICAL NEURONS INDUCES ACTIVATION OF c-JUN N-TERMINAL KINASE (JNK) AND p38 MAPK, MITOCHONDRIAL TRANSLOCATION OF BAX, CYTOCHROME C RELEASE, AND APOPTOSIS.

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Recent studies suggest that environmental toxicants including pesticides may contribute to the development of various neurodegenerative disorders including Parkinsons Disease. For example, the insecticide rotenone has been found to reproduce the behavioral and biochemical features of Parkinsons Disease in rats.

Additionally, organophosphate pesticides that target the CNS, like chlorpyrifos, are associated with impaired learning and memory in children. One mechanism implicated in neurodegenerative diseases is neuronal apoptosis. We hypothesize that exposure to pesticides may contribute to cell loss of sensitive neuronal populations through apoptosis. Our studies suggest that both chlorpyrifos and rotenone induce apoptosis in primary cultured cortical neurons and dopaminergic SH-SY5Y cells. Moreover, the combination of rotenone and chlorpyrifos exposure enhances apoptosis compared to either pesticide alone. Exposure to rotenone or chlorpyrifos activates the stress-activated protein kinases, c-Jun NH2-terminal protein kinase (JNK) and the p38 mitogen-activated protein (MAP) kinase. In addition, inhibition of these pathways attenuates the toxicity of these pesticides. We have also found that rotenone treatment results in Bax translocation to mitochondria, release of cytochrome C and caspase activation. Taken together, exposure of neuronal cells to either rotenone or chlorpyrifos alone or in combination triggers differential activation of the JNK and p38 MAPK pathways and induction of mitochondrial-pathway mediated apoptosis. These studies provide novel information concerning the molecular mechanisms of pesticide-induced apoptosis in neuronal cells and new insights concerning the role of environmental toxicants in neurodegeneration.

1709 MITOCHONDRIAL TRANSLOCATION OF PROTEIN KINASE C-DELTA PROMOTES PROTEOLYTIC DEGRADATION OF BCL-2 DURING ENVIRONMENTAL NEUROTOXIC INSULTS IN DOPAMINERGIC CELLS.

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Previously, we demonstrated that exposure to environmental neurotoxins such as 1-methyl-4-phenylpyridinium (MPP+), dieldrin, and methylcyclopentadienyl manganese tricarbonyl (MMT) induces proteolytic activation of protein kinase Cδ (PKCδ) in a caspase-3 dependent manner in dopaminergic cells. Upon further characterization, we found that PKCδ activation not only mediates apoptosis but also regulates the upstream caspase cascade through positive feedback activation. In this study, we investigated the pro-apoptotic role of PKCδ in the upstream caspase cascade following dieldrin or MMT treatment in dopaminergic PC12 cells. Following acute exposure to dieldrin (100 μM) or MMT (200 μM), native PKCδ rapidly translocated to the mitochondria within 1 hr. This was followed by the release of key mitochondrial pro-apoptotic molecules, including cytochrome c and Smac, into the cytosol. The PKCδ specific inhibitor rottlerin blocked the dieldrin- and MMT-induced cytochrome c release but not the translocation of PKCδ. To further understand the role of PKCδ in mitochondrial function, we intracellularly delivered recombinant, catalytically active PKCδ into dopaminergic cells. The catalytically active PKCδ induced depolarization of the mitochondrial membrane potential, cytochrome c release, and caspase-9 activation, further confirming that PKCδ is an important signaling molecule in the caspase cascade. Immunoprecipitation analysis revealed that PKCδ associates with Bcl-2 and caspase-3 during dieldrin or MMT exposure. Furthermore, Bcl-2 was proteolytically cleaved after a 3-hr dieldrin or MMT treatment; both PKCδ and caspase-3 inhibitors blocked the cleavage. Together, our results suggest that the mitochondrial translocation of PKCδ is an initial pro-apoptotic event that subsequently promotes caspase-3 dependent proteolytic inactivation of Bcl-2 during environmental chemical-induced dopaminergic degeneration (supported by NIH ES 10586).

1710 RNAI-MEDIATED KNOCK-DOWN (GENE SILENCING) OF PRO-APOPTOTIC PKCδ *IN VITRO* MODELS OF PARKINSON'S DISEASE.

Y. Yang, V. Anantharam and A. Kanthasamy. *Biomedical Science, Iowa State University, Ames, IA.*

Double strand RNA (dsRNA)-mediated RNA interference (RNAi) is an emerging genetic technique used to ablate specific gene functions in eukaryotes. Recently, this novel approach is extended to mammalian cells to specifically ablate the function of a given gene with small interference RNA (siRNA). The application of siRNA technology in neuronal models to advance the understanding of the degenerative process has not yet been explored. Recently, we demonstrated that caspase-3 dependent proteolytic activation of protein kinase C delta (PKCδ) is critical for the induction of apoptotic cell death in mesencephalic dopaminergic neuronal (N27) cells during exposure to various environmental neurotoxic agents including 1-methyl-4-phenylpyridinium (MPP^+), dieldrin, and methylcyclopentadienyl manganese tricarbonyl (MMT). Herein, we demonstrate that siRNA mediated knockdown of PKCδ effectively protects dopaminergic neurons from environmental neurotoxic insult. Using stringent criteria, we designed four siRNAs for the rat PKCδ gene. *In vitro* transcription was used to synthesize duplex siRNAs. Each duplex siRNA was then transfected into N27 cells and the siRNA cellular localization was confirmed by siRNA-cy3 labeling. Determination of the gene silencing efficiency by both Western blot and immunohistochemical analyses revealed that two of the four siRNAs significantly suppressed PKCδ expression as compared to the control or nonspecific siRNA. PKCε expression was unaltered, demonstrating the isoenzyme specificity of these siRNAs. Furthermore, siRNA-4, the most effective siRNA, almost completely suppressed MPP^+- and dieldrin-induced DNA fragmentation, confirming the pro-apoptotic function of the PKCδ gene. Together, our results indicate that siRNA is a powerful genetic tool for the understanding of the cellular mechanisms underlying dopaminergic degeneration in Parkinson's disease. Perhaps even siRNA-based neuroprotective treatments for various neurodegenerative disorders, including Parkinson's disease, can be developed in the future (supported by NIH grants ES 10586 and NS 38644)

1711 CASPASE INHIBITION SWITCHES THE MODE OF DEATH PRODUCED BY CYANIDE IN CORTICAL NEURONS.

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Execution of apoptosis involves mainly activation of the caspase family of proteases. However recent studies indicate that caspase inhibition occasionally turns the morphology of cell death from apoptotic into necrotic without inhibiting death itself. In the present study the effect of caspase inhibition on cortical cell death induced by cyanide was investigated. In cortical cells exposed to cyanide (400 μM) death was primarily apoptotic as characterized by positive TUNEL staining of apoptotic cells and DNA laddering on gel electrophoresis. Reactive oxygen species generation and subsequent increased caspase activity mediated cyanide-induced cortical apoptosis. Pretreatment of cortical cells with a broad-spectrum caspase inhibitor, Z-VAD-fmk, blocked cyanide-induced apoptosis (TUNEL staining and DNA degradation). Inhibition of caspases resulted in a parallel increase in necrotic-like cell death as measured by increased cellular efflux of LDH and propidium iodide uptake by the cells. The change in the mode of cell death was accompanied by a further increase in ROS generation and reduction in mitochondrial membrane potential. These data suggest that apoptosis and necrosis share common initiation pathways and the level or the extent of caspase activation plays an important role in the cell death pathway decision. (Supported by NIH grant ES 04140)

1712 INVOLVEMENT OF CASPASE-3 PROTEASE IN DOPAMINERGIC DEGENERATION FOLLOWING EXPOSURE TO METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT).

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Exposure to methylcyclopentadienyl manganese tricarbonyl (MMT), (used as an antiknock agent in gasoline) poses a significant health concern. Occupational workers who are exposed MMT manifest neural degeneration resembling Parkinson disease (PD). Identifying molecular mediators and their regulation in dopaminergic neuronal cells would allow us to determine the underlying mechanisms involved in MMT-induced dopaminergic degeneration. Here, we measured molecular responses of dopaminergic cells to different concentrations of MMT (0.1-1.0 mM)-induced cell death. Using substantia nigra cells, we showed that cells exposed to MMT (0.1-1.0 mM) for 24 hrs increased cell death over the control in a concentration dependent manner. This was determined by lactate dehydrogenease (LDH) release and apoptotic TUNEL staining. The increased cell death at various concentrations of MMT was blocked when cells were pretreated with caspase 3 inhibitor, Ac-DEVD suggesting the involvement of caspase 3 activation in MMT-induced dopaminergic cell death. In order to confirm this, we further, measured caspase-3 activity level in cells treated with MMT at the same concentration at different time periods. Like apoptosis and cell death, caspase-3 activity increased peak at 4 hrs after beginning of the exposure, while caspase-3 specific blocker z-VAD significantly reduced the same. There was a significant protection against MMT-induced cell death, when cells were pre-treated with Poly-ADP-Ribose Polymerase (PARP) inhibitor (Benzamide), which reflect the involvement of PARP following caspase-3 activation. These results suggest that the change in the levels of caspase-3 and PARP response to MMT exposure explained the involvement of caspase-3 and associated PARP activation during MMT-induced dopaminergic degeneration.

1713 METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT) INDUCES APOPTOSIS BY PKCδ DEPENDENT ACTIVATION OF NF-κB IN MESENCEPHALIC DOPAMINERGIC NEURONAL CELLS.

A. Kanthasamy, M. Kitazawa, S. Kaul, V. Anantharam and A. G. Kanthasamy. *Biomedical Sciences, Iowa State University, Ames, IA.*

Methylcyclopentadienyl manganese (MMT) is an organic manganese compound that has been legalized for use as a gasoline additive in the United States. Recently, we demonstrated that MMT exposure induces oxidative stress dependent apoptosis *via* capase-3 dependent proteolytic activation of protein kinase-delta (PKC-δ) in dopaminergic cells. The current study was designed to evaluate the effect of MMT on a redox transcription factor, NF-κB and it association with PKCδ in rat mesencephalic dopaminergic neuronal cells (N27 cells). Following a 3 hr exposure to MMT (30, 100, 200 μM) a pronounced increase in NF-κB activation and IκB-a degradation was evidenced. Pretreatment with a PKCδ inhibitor rottlerin or a caspase-3 inhibitor Z-DEVD-fmk, however, produced a near complete blockade of MMT-induced NF-κB activation, indicating the involvement of PKCδ and caspase-3 in the NF-κB activation process. Similarly, MMT-induced NF-kappa B activation was also dramatically reduced following pretreatment of dopaminergic cells with the specific NF-κB inhibitor SN-50. To further determine the importance of NF-κB activation in MMT-induced apoptosis, we tested the efficacy of NF-κB inhibitor and caspase-3 inhibitor on MMT-induced DNA fragmentation. Both SN-50 and Z-DEVD fmk significantly reduced the MMT-induced DNA fragmentation, indicating that the NF-κB pathway is critical for MMT-induced dopaminergic cell death. Together, we conclude that caspase-3 dependent proteolytic activation PKCδ may exert important regulatory effects on NF-κB activation to further facilitate the execution of MMT induced apoptotic cell death in dopaminergic neurons (supported by NIH ES 10586).

1714 LACK OF PHOSPHATIDYLSERINE EXTERNALIZATION IN ETOPOSIDE-INDUCED APOPTOTIC CELLS IS RELATED TO ITS ANTIOXIDANT PROTECTION OF PHOSPHATIDYLSERINE OXIDATION.

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Our previous work showed that clearance and phagocytosis of apoptotic cells is associated with selective oxidation and externalization of phosphatidylserine (PS) in plasma membrane, which could act as preferred ligand for certain macrophage receptors. Etoposide is an effective anticancer agent, which induces apoptosis in different cell lines. We demonstrated that etoposide (10-50 μM), induced apoptosis in HL-60 cells as evidenced by nuclear fragmentation and caspase-3 activation. Under these conditions, J774A.1 macrophages were not able to phagocytize etoposide-stimulated cells. Using Annexin V binding assay, we found that HL-60 cells failed to externalize PS in response to etoposide treatment (50 μM, 2h at 37°C). However a thiol specific reagent, N-ethylmaleimide (10 μM, 10 min at 37°C), inhibited aminophospholipid translocase and triggered externalization of PS in etoposide treated HL-60 cells and stimulated phagocytosis of these cells by macrophages. In addition, etoposide did not cause peroxidation of any phospholipids including PS. Next we utilized an oxidant-induced model of apoptosis, using H_2O_2. We found that H_2O_2-induced apoptosis in HL-60 cells was accompanied by nuclear fragmentation, caspase-3 activation, PS externalization and oxidation of different phospholipids including PS. Etoposide enhanced apoptosis induced by H_2O_2. Etoposide was able to completely block PS H_2O_2-induced oxidation in HL-60 cells and significantly but not completely inhibit PS externalization in HL-60 cells as well as their phagocytosis by macrophages. These results support our hypothesis that oxidation of PS is essential for its exposure on outer leaflet of plasma membrane and hence for recognition and phagocytosis of apoptotic cells. Supported by NIH HL70755 and CA 90787.

1715 FAS-TRIGGERED OXIDATION OF PHOSPHATIDYLSERINE IS ESSENTIAL FOR ITS EXTERNALIZATION DURING APOPTOSIS IN LUNG EPITHELIAL CARCINOMA A-549 CELLS UNDERGOING FAS-MEDIATED APOPTOSIS.

J. Jiang, B. F. Serinkan and V. E. Kagan. *Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.*

We have put forward a hypothesis that oxidation of phosphatidylserine (PS) catalyzed by cytochrome c is essential for PS externalization during apoptosis and for recognition of apoptotic cells. In this report, we used a model of Fas-triggered apoptosis in lung epithelial carcinoma A-549 cells rather than oxidant-induced apoptosis to maximize the apoptosis-associated oxidative stress and eliminate non-specific effects of oxidants on phospholipids. A549 cells were pretreated with interferon gamma (IFN-r, for 24 h) and exposed to anti-Fas antibody (CH-11) underwent apoptosis as evidenced by increased caspase-3 activity and externalized PS. Notably, PS was preferentially oxidized 3 h after anti-Fas triggering while two more abundant phospholipids phosphatidylcholine, phosphatidylethanolamine and the major intracellular antioxidant, glutathione, remained unoxidized. Fas-induced PS oxidation could be prevented by antioxidant enzymes catalase/superoxide dismutase (SOD). PS oxidation was preceded by release of cytochrome c from mitochondria into cytosol (2-h after anti-Fas stimulation. A pan-caspase inhibitor, z-VAD-fmk, blocked Fas-triggered caspase-3 activation, cyt c release, and PS externalization. In contrast, Z-DQMD-fmk, a caspase-3 inhibitor, blocked caspase-3 activity and PS externalization, but did not affect release of cyt c from mitochondria into cytosol. Most importantly, z-VAD-fmk, but not z-DQMD-fmk, inhibited oxidation of PS in Fas-triggered A549 cells. This suggests that cytochrome c-catalyzed PS oxidation participates in execution of apoptotic program and plays an important role in PS-dependent externalization and signaling during non-oxidant induced apoptosis in A549 cells. Supported by NIH grant HL70755-01.

1716 DIRECT INHIBITION OF I KAPPA KINASE ACTIVITY BY BENZENE METABOLITES SENSITIZES BONE MARROW CELLS TO CYTOKINE-INDUCED APOPTOSIS.

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The transcription factor NF-κB plays a critical role in hematopoietic progenitor cell (HPC) function and survival. NF-κB is activated *via* the I kappa kinase complex (IKK) by a variety of cytokines, including tumor necrosis factor-alpha (TNF-α), which also induces apoptosis in cells deficient in NF-κB. We have demonstrated that inhibition of NF-κB by the benzene metabolite hydroquinone (HQ) alters cytokine production in HPC and predisposes them to apoptosis. However, the mechanism by which HQ inhibits NF-κB activation, as well as the possible effects of other benzene metabolites on this important transcription factor, are unknown. We examined the effects of exposure to benzene metabolites on NF-κB in the erythroleukemic cell line TF-1 following activation with TNF-α. The effects of chemical treatment on NF-κB signaling, activation, and function were examined utilizing a combination of kinase assays on isolated IKK complexes and whole cells, electrophoretic mobility shift assays (EMSA), and DNA nick-end labeling to measure apoptosis. HQ, catechol, and p-benzoquinone, which directly inhibited IKK and activation of NF-κB, sensitized the cells to TNF-α induced apoptosis, whereas other benzene metabolites had no effect on IKK, NF-κB, or apoptosis. These findings indicate that benzene metabolites inhibit NF-κB *via* inhibition of IKK and collaborate with TNF-α in inducing apoptosis in HPC, raising the possibility that variations in TNF-α may play a role in mediating individual variation in susceptibility to hematotoxic agents.

1717 CASPASE ACTIVATION AND APOPTOSIS IN SILICA-INDUCED LUNG INJURY.

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Past studies have suggested a pro-inflammatory role for apoptosis and caspase activity in a mouse model of silica exposure (Am J Resp Cell Mol Biol 27: 78-84, 2002). However, caspase activation by silica in lung tissue has not been demonstrated *in vivo*. The goal of the current work is to investigate the contribution of caspase activation in the pathogenesis of silica-induced lung injury both *in vitro* and *in vivo*. To evaluate the *in vivo* activation of lung tissue caspases, C57Bl/5J mice were exposed to silica (α-quartz) particles by intratracheal (IT) instillation. Three days after IT instillation of 2 mg/mouse, a 2-fold increase in the activity of caspase-3, but not caspase-1 was observed. Fourteen days after silica exposure there was an increase in the activity of both caspase-1 and caspase-3. Caspase-3 activity was detected immunohistochemically in both lung macrophages and epithelium at 2 and 14 days post-instillation using an antibody against the large cleaved fragment of activated caspase-3. To further characterize the activation of caspases, the mouse macrophage cell lines RAW 264.7 and MH-S were exposed to α-quartz at 12.5 ug/cm2 or 50 ug/cm2 for either 6 or 24 hours. At both 6 and 24 hours post-exposure to α-quartz, flow cytometric analysis demonstrated pancaspase activation and the externalization of membrane phosphatidylserine, a marker of apoptosis. At 6 hours 18% of macrophages had an apoptotic subdiploid phenotype determined by flow cytometric cell cycle analysis. Apoptosis was attenuated by the pancaspase inhibitor ZVAD-FMK. To differentiate which caspases were activated, α-quartz treated macrophages

were incubated with fluoresceinated substrates to measure the activities of caspase-1, 3, 8, and 9. At 6 hours post-exposure to α-quartz 8-15% of the cell population had increased activity of the pro-apoptotic caspase-3, 8, and 9 whereas the pro-inflammatory caspase-1 had increased activity in 60% of the cells. In summary, our work demonstrates that silica activates lung caspases during both acute and chronic inflammation. (Supported by HL 10360 and ES 09433).

1718 CROSS-TALK BETWEEN DEATH RECEPTOR-MEDIATED AND MITOCHONDRIAL PATHWAYS OF APOPTOSIS INDUCED BY DES IN JURKAT CELLS.

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Exposure to diethylstilbestrol (DES), a synthetic estrogen has been associated with thymic atrophy and previous studies from our lab demonstrated that this occurs through the induction of apoptosis. In the current study, we investigated the mechanism of induction of apoptosis using Jurkat, a human T cell lymphoma line. Culture of Jurkat cells with DES caused a dose-dependent decrease in cellularity and induction of apoptosis. Furthermore, it was observed that DES treatment of FADD-/- and Caspase 8-/- Jurkat, led to decreased apoptosis when compared to wild-type (wt) Jurkat thereby demonstrating the involvement of the death-receptor pathway of induction of apoptosis. The wt, FADD-/-and Caspase8-/- Jurkat following treatment with DES also showed a decrease in mitochondrial membrane potential as determined by DiOC6 staining. Caspase 9-dominant negative (DN) and Bcl-2 over-expressing Jurkat were more resistant to DES induced apoptosis when compared to wt Jurkat cells. These studies therefore demonstrate that both death receptor mediated pathway and the mitochondrial pathway are involved in DES induced apoptosis of Jurkat cells. Further studies confirmed the cleavage of both caspase 8 and caspase 9 following DES treatment by immunoblotting. Caspase 8 may not directly activate the effector caspases but may recruit bid, a member of the bcl-2 family, to activate caspase 9 that could lead to cleavage of downstream effector caspases. It was observed that Bid was cleaved following DES treatment thereby suggesting cross-talk between the death receptor and mitochondrial pathways. In addition, the induction of apoptosis was diminished by treatment with caspase inhibitors Z-VDVAD-FMK, Z-IETD-FMK, Z-LEHD-FMK, Z-AEVD-FMK. Taken together, these studies demonstrate that DES-induced apoptosis in Jurkat is mediated through both death receptor and mitochondrial pathways. (Supported by NIH grants ES09098, DA014885 and HL058641).

1719 DES MEDIATES APOPTOSIS IN HEMATOPOIETIC STEM CELLS AND DEVELOPING THYMOCYTES LEADING TO IMMUNE DYSFUNCTION.

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Exposure to diethylstilbestrol (DES), a synthetic estrogen has been associated with an increased risk for breast cancer in "DES mothers" and a lifetime risk of cervicovaginal cancers and immunological abnormailities in "DES daughters". In the current study we investigated the immunological consequences following prenatal exposure to DES. To this end, pregnant C57BL/6 mice were treated with 10 or 50ug/kg body weight of DES or the vehicle at gestation day 15 and 16. After the end of pregnancy, mothers and pups were analyzed. The data showed severe thymic atrophy as well as increased induction of apoptosis in DES–treated mothers as well as in pups. Moreover, the thymocytes from DES–treated pregnant mice showed increased expression of CD3, αβTCR and CD44 indicative of apoptosis. Furthermore, there was no change in the expression of CD25 and CD69 markers. There was also no change in the percentage of CD4–CD8– (DN), CD4+CD8+ (DP), CD4+ and CD8+ (SP) T cells in these mice. In order to address whether DES affects only developing thymocytes or could also affect pluripotent hematopoietic stem cells, human cord blood CD34+ progenitor cells were examined for their susceptibility to induction of apoptosis. CD34+ cells were cultured in the presence of growth factors such as stem cell factor (SCF) and Flt3L for 2 weeks. Next, the cells were incubated *in vitro* with various concentrations of DES for 24hrs. Such an incubation resulted in a significant reduction in cellularity and induction of apoptosis as detected by using TUNEL and Annexin/PI staining. The DES–exposed cells also showed loss of mitochondrial membrane potential as studied by $DiOC_6$ staining. Together, this study demonstrates that DES induces apoptosis in developing thymocytes as well as in the hematopoietic stem cells. This may account for the immunological abnormalities as seen in DES daughters (Supported by NIH grants ES09098, DA014885 and HL058641).

1720 COMPETING CELL SURVIVAL AND CELL DEATH SIGNALS IN TGHQ TREATED HL-60 CELLS.

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Quinol-thioether metabolites are present in the bone marrow of rats and mice co-treated with hydroquinone and phenol. Several of these metabolites, including 2, 3, 5-*tris*-(glutathion-S-yl)hydroquinone (TGHQ) are hematotoxic in rats and induce DNA damage, growth arrest, and apoptosis in human promyelocytic leukemia (HL-60) cells. The mechanisms by which TGHQ-induces apoptosis in HL-60 cells is unclear and was the focus of the present studies. TGHQ catalyzed the rapid formation of reactive oxygen species (ROS), which reached maximal levels 1 h after treatment. Consistent with previous work, catalase inhibited TGHQ-induced apoptosis, with a concomitant reduction in ROS. Western analysis revealed the presence of cleaved caspase 9, 30 min after exposure of cells to TGHQ. By 2 h, procaspase 9 completely disappeared, with the concomitant appearance of two cleaved forms of caspase 3 (19, 12 kDa). Evidence for caspase 8 cleavage was apparent by 4 h. TGHQ also induced NF-κB-DNA binding activity, which was preceded by the rapid (15 min) phosphorylation and subsequent degradation of IκB-α. IκB-β phosphorylation and degradation occurred only after the initial phosphorylation of IκB-α. Consistent with these findings, the sesquiterpene lactone, parthenolide, which prevents IKKα activation, inhibited NF-κB activation and potentiated TGHQ-induced apoptosis. We investigated the effects of SN50, a cell permeable peptide corresponding to the nuclear localization sequence (VQRKRQKLM) of NF-κB, on TGHQ-induced apoptosis. SN50 potentiated TGHQ-induced apoptosis (PS externalization) consistent with the anti-apoptotic function of NF-κB in most cell types. The data suggest that in HL-60 cells TGHQ treatment activates the caspase-mediated apoptotic signaling pathway, and the NF-κB-mediated cell survival pathway. Factors that determine the functional outcome of these two competing pathways are under investigation. (ES07784, ES09224).

1721 MODULATION OF LIPOCALIN 24P3 EXPRESSION AS AN APOPTOTIC MECHANISM FOR MK886.

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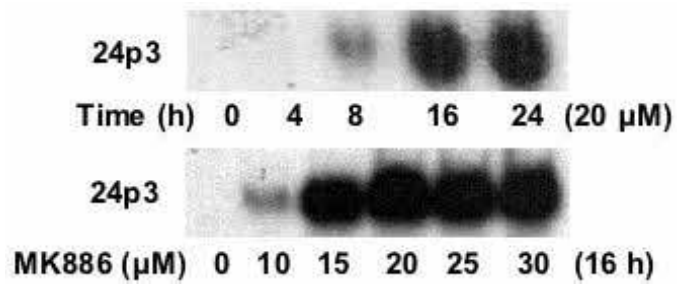
MK886, a strong proapoptotic agent, is an inhibitor of 5-lipoxygenase (LOX) through binding to the 5-LOX-activating protein (FLAP). However, MK886-induced apoptosis is through a FLAP-independent pathway (Biochem. J. 340: 371 [1999]). A possible role of 24p3, a murine lipocalin implicated in apoptosis, in MK886-induced apoptosis was investigated. Exposure of murine prolymphoid progenitor cells (FL5.12) to MK886 dramatically increased 24p3 expression in a dose- and time-dependent manner (Figure). Induction could also be achieved with a structurally distinct FLAP inhibitor, MK591, but not with the structurally analogous compound indomethacin. MK886 is a peroxisome proliferator activated receptor (PPAR) inhibitor (Biochem. J. 356: 899 [2001]).The up-regulated 24p3 expression by MK886 was enhanced a further 2-fold by WY14643, an activator of PPARα, whereas ciglitazone, an activator of PPARγ attenuated expression by 50%. Neither WY14643 nor ciglitazone alone had any effect on the expression of 24p3. The induction of 24p3 by MK886 was inhibited by cycloheximide, suggesting it is dependent on the synthesis of new protein(s). Changes in 24p3 expression closely paralleled the induction of apoptosis. The overexpression of bcl_2 or bcl-x_L in FL5.12 cells prevented apoptosis induced by MK886 as well as the enhancement of apoptosis by WY14643. Thus, 24p3 is an MK886-inducible gene and may play an important role in MK886-induced apoptosis. (Supported by CA83701 and Center Grant ES07784.)



1722 CHANGES IN CELLULAR OXIDANT PRODUCTION IN RESPONSE TO PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR)α AND γ AGONISTS AND THE PPAR ANTAGONIST MK886.

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MK886, an inhibitor of 5-lipoxygenase activating protein (FLAP), induces apoptosis by mechanisms independent of both lipoxygenase and FLAP (Biochem. J. 340: 371 [1999]). MK886 has also been found to inhibit PPARs (Biochem. J. 356: 899 [2001]). PPARs have been implicated in apoptosis, but the mechanistic basis for this observation remains unclear. To assess the role of oxidative stress in MK886-induced apoptosis and its possible relationship with PPARs, we have studied Jurkat cells which express all PPAR isoforms. The PPARα (Wy14643) and PPARγ agonist (ciglitazone) do not cause apoptosis by themselves up to 50 and 25 μM, respectively. However, both agonists substantially increase the production of oxidants as shown by flow cytometry with dichlorofluorescin diacetate. MK886 (20 μM)-induced apoptosis is preceded by only a small increase in oxidants. In combination with 20 μM MK886, Wy14643 further increased the production of oxidants and apoptosis beginning at 4 h. Surprisingly, initial experiments showed that the antioxidants N-acetylcysteine and vitamin E did not prevent the generation of oxidants and appeared to further increase cell death. These data suggest that the production of oxidants is not directly related to the apoptosis induced by MK886 nor the enhancement seen with Wy14643. (This work was supported by CA83701 and Center Grant ES07784.)

1723 GLUTATHIONE AND THIOREDOXIN REDOX STATES ARE INDEPENDENTLY REGULATED DURING DIFFERENTIATION, APOPTOSIS, AND OXIDATIVE STRESS.

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The glutathione (GSH) and thioredoxin (Trx1) systems protect cells from oxidative modification of protein thiols. GSH and its oxidized form (GSSG) constitute the predominant redox buffering system in the cell. The redox state of the GSH/GSSG couple is maintained at −260 mV in proliferating cells, but becomes more oxidizing as cells differentiate, and more positive still in cells undergoing apoptosis. It is unknown whether similar changes occur in Trx1. The purpose of the present study was to compare changes in GSH and Trx1 redox states in cell models of differentiation, apoptosis, and oxidative stress. GSH redox was determined by measuring intracellular GSH and GSSG concentrations by HPLC, and Trx1 redox was determined by the Redox Western blot technique. In spontaneously differentiating Caco2 cells, GSH/GSSG was oxidized and total GSH was decreased, whereas Trx1 was not oxidized and there was an increase in the total amount of Trx1 in these cells. Similarly, in staurosporine-induced apoptosis of HeLa cells, GSH/GSSG redox was dramatically oxidized with a concomitant loss of GSH, yet Trx1 redox and levels were only modestly affected. In contrast, in THP1 cells exposed to the oxidant tert-butylhydroperoxide, both GSH and Trx1 were oxidized to similar extents and over similar time courses. Thus, the redox states of GSH/GSSG and Trx1 are not in equilibrium, suggesting that these two redox-regulating systems have different roles in differentiation and apoptosis.

1724 ELEVATED LEVELS OF PHOSPHORYLATED CA2+/CAMP RESPONSE ELEMENT BINDING PROTEIN (PCREB) IN ISOLATED BRAIN MITOCHONDRIA FOLLOWING FOREBRAIN ISCHEMIA.

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Studies have implicated the Ca2+/cAMP response element binding protein (CREB) in cell survival and brain development as well as in learning and memory. Moreover, CREB has been shown to play a role in neuroprotection against various insults including acute cerebral ischemia. The cellular location of CREB has been identified as residing in the cytosol and the nucleus and recently has been reported to be present in brain mitochondria (J. Neurochem. 72:2272-7, 1999). In the current study, antibodies to CREB and phosphorylated CREB (pCREB) were used to probe for the presence of immunoreactivity in mitochondria isolated from different tissues and cells. Moreover, antibodies to Cytochrome c (Cyt c) and the pro-apoptotic Bax protein were used to probe for immunoreactivity in mitochondria isolated from adult rat brains following a 10 minute forebrain ischemia. At different reperfusion times the vulnerable CA1 subregion of the hippocampus was dissected and fractionated into cytosolic and mitochondrial fractions. Western immunoblots demonstrated the presence of CREB and pCREB in mitochondria isolated from rat and dog brain, rat liver and heart, and from GT1-7 and PC-12 cultured neural cells. Moreover, a transient decrease followed by increased pCREB immunoreactivity was detected in mitochondria isolated from the CA1 region of rat hippocampus during the first hour of reperfusion without any changes in CREB immunoreactivity. Further, decreased pCREB levels occurred concomitantly with Cyt c release from the mitochondria that is probably triggered by an accumulation of Ca2+. These results suggest the ubiquitous presence of CREB in mammalian mitochondria and that the phosphorylation state of mitochondrial CREB following reperfusion is altered. We hypothesize that CREB responds to physiological and pathological signals at the mitochondrial level and that altered posttranslational modifications of CREB may be important for neuronal survival following cerebral ischemia. Supported by: NS34152

1725 GLUCOSE TRANSPORT IN JURKAT CELL: CONCENTRATION-DEPENDENT REGULATION.

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In this study, a mechanism by which glucose level modulates glucose transport in Jurkat cells was investigated. Glucose uptake was more efficient in the cells cultivated in low glucose (2.5 mM) medium than that grown in high glucose (20 mM) medium. Vmax (0.74 nmol·10^6cells^{-1}·min^{-1}) of glucose uptake measured with the cells grown in the low glucose medium was higher than the one (1.06 nmol·10^6cells^{-1}·min^{-1}) in the high glucose medium while Km was almost consistent through the change of glucose levels, indicating the increase of glucose transporter number. However, the RT-PCR showed that Jurkat cells express GLUT1 and 3 but not GLUT2, 4, or 5 and that the levels of the mRNA transcripts did not change with either glucose concentrations or the length of incubation time. This result was consistent with next result that the enhancement of glucose uptake could occur as early as 4 hours after the incubation in low glucose medium. To investigate if the enhance of glucose uptake was mediated by the activation of preexisting transporter, two different kinase inhibitors, SB202190 and wortmannin, were tested. SB202190 was efficient to block the hypoglycemia-induced enhancement of glucose uptake while wartmannin was not successful. This result implies that the signalling pathway from the hypoglycemia to the increase of glucose uptake includes p38MAPK and its upstream kinase, AMPK. The incubation of cells in low glucose medium increases the intracellular AMP and active form of p38MAPK, showing a maximum level at 1 hour. Taken together, these data suggest a signalling pathway for the hypoglycemia; a low glucose condition induces an enhancement in intracellular AMP level with the activation of AMPK, and then this in turn activates p38MAPK and finally glucose transporters.

1726 PHOSPHATIDYLSERINE IS REQUIRED ALONG WITH OXIDIZED PHOSPHOLIPIDS ON CELL SURFACE FOR SYNERGISTIC ENHANCEMENT OF PHAGOCYTOSIS BY MACROPHAGES.

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Externalization of phosphatidylserine (PS) from the inner to the outer leaflet of plasma membrane is a signaling event, which occurs during apoptosis. This signal triggers phagocytosis of apoptotic cells by macrophages. In our previous studies, we have demonstrated that execution of apoptotic program is accompanied by PS oxidation, which precedes externalization of PS and/or oxidized PS (PSox) indicating that PS oxidation stimulates its externalization. In this study, we showed that oxidized phospholipids such as oxidized phosphatidylcholine (PCox) and PSox can synergistically enhance phagocytosis of cells by the macrophage J774.A1 cell line. Liposomes containing PS and phosphatidylcholine (PC) in concentrations ranging from 1 nM to 150 nM were integrated into plasma membranes of HL-60 cells pretreated with N-ethyl maleimide an inhibitor of aminophospholipid translocase. Quantification of amounts of externalized PS/PSox was carried out using a sensitive EPR method based on the specific binding of paramagnetic annexin V-microbeads conjugates with PS on cell surface. We found that phagocytosis of PS-containing cells increased in a dose-dependant manner after a threshold. When PSox was integrated together with PS an almost 2-fold increase in the phagocytosis by macrophages was observed. Incubation of cells with liposomes containing PC or PC+PCox in the absence of PS did not cause any phagocytosis. When PCox was used in combination with PS, an increased phagocytosis was detected. Oxidized phospholipids appeared to decrease the threshold for recognition of target PS-containing cells by macrophages. Overall, these results suggest that PS is crucial for synergistically enhanced recognition of target cells in the presence of oxidized phospholipids. Supported by NIH HL 70755.

1727 P53 PHOSPHORYLATION REGULATES HYPOXIA-MEDIATED APOPTOTIC DEATH IN TUMOR CELLS.

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Tumor hypoxia develops in most solid tumors because of inadequate vasculature and abnormal vascular architecture. Frequently, these hypoxic cancer cells resist radiation and chemotherapy. The current study was undertaken to determine the regulation of p53 in hypoxic tumor cells. p53+/+ and p53-/- mouse embryonic fibroblasts (MEF) transformed with proto-oncogenes Ras and E1A were exposed to hypoxia for 0 to 7 h. Hypoxia increased total p53 protein levels after 2 h of hypoxia compared to normoxia. After hypoxia, 30 to 40% of total p53 protein was phosphorylated at Ser15 as assessed by Western blotting using a phospho-p53 (Ser15) specific antibody. No p53 phosphorylation was detected in normoxic cells. Hypoxia also activated the p53 responsive genes p21 and Bax and increased their protein levels. In addition, hypoxia up-regulated Mdm2 gene expression, a negative regulator of p53 as assessed by gene reporter assay. However, Mdm2 protein levels were only slightly increased during hypoxia compared to normoxia. Inhibition of the MAPK pathway by a specific inhibitor of MEK1, PD98059, increased caspase-3 activation and cell killing but decreased Mdm2 protein levels. This accelerated cell killing is likely due to decreased binding between p53 and Mdm2 proteins resulting in decreased degradation of p53 *via* proteasomes. Collectively, our results indicate that hypoxia induces p53 phosphorylation, increased p53 dependent Bax protein expression, caspase-3 activation, and apoptotic death. Supported by NS39469.

1728 MECHANISM-BASED COMPARISON OF *IN VITRO* AND *IN VIVO* CELL DEATH PATHWAYS IN RETINAL PHOTORECEPTOR CELLS EXPOSED TO THE 2-NITROIMIDAZOLE RADIOSENSITIZER, CI-1010.

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CI-1010 ((R)-a([(2-bromoethyl)-amino]methyl)-2-nitro-1H-imidazole-1-ethanol monohydrobromide), a bifunctional nitroimidazole, produces selective, caspase-3 independent apoptosis in retinal photoreceptors, *in vivo*. The present study attempts to investigate mechanism(s) of CI-1010-induced photoreceptor cell death in the 661W photoreceptor cell line and *in vivo* retina. Cultured 661W cells treated with CI-1010 showed a dose-dependent decrease in viability determined using the MTT assay. Flow cytometry and western blot analysis revealed DNA fragmentation and PARP cleavage characteristic of apoptosis in 661W photoreceptor cells 48 h after exposure to 1 mM CI-1010. Caspase-3 activation, independent of caspase-8 and caspase-9 cleavage, was detected at 18 h, concomitant with decreased Bcl-xL and increased Bax expression. No change in protein expression of Fas and Fas ligand (FasL) was detected at any timepoint. Real-time confocal analysis and immunofluorescence of 661W cells showed retention of mitochondrial membrane potential and mitochondrial staining of cytochrome c up to 24 h following exposure. For comparison, retinas from adult C57BL/6 mice treated with a single intraperitoneal injection (1000 umol/kg) of CI-1010 were removed 6, 12, 18, and 24 h following treatment. Immunoblot analysis of CI-1010-treated retinas revealed activation of caspase-8 at 18 h in the absence of cleaved caspase-3, caspase-9, and PARP. No change in protein expression of Fas, FasL, Bcl-xL, Bax, and Bad, were detected by western blot at any timepoint. The pathway(s) leading to photoreceptor apoptosis *in vivo* after CI-1010 exposure requires further investigation. However, these results confirm activation of a caspase-3-independent pathway(s) of CI-1010-induced photoreceptor apoptosis *in vivo* and suggest that unidentified extrinsic signals may influence the activation of specific cell death pathway(s) in photoreceptors following exposure to CI-1010.

1729 BENZO(a)PYRENE 7, 8-DIHYDRODIOL-INDUCED APOPTOSIS IS AH RECEPTOR DEPENDENT AND REQUIRES MITOGEN ACTIVATED PROTEIN KINASES.

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Benzo[a]pyrene (BP) is a polycyclic aromatic hydrocarbon that is subject to metabolism and formation of BP-7, 8-dihydrodiol (BP-7, 8-diol) and BP-7, 8-dihydrodiol-9, 10-epoxide (BPDE). The formation of BPDE leads to apoptosis and cell death. To study the cellular events underling BP induced cell death, experiments were carried out with the two metabolic intermediates, BP-7, 8-diol and BPDE. BP-7, 8-diol, a non toxic metabolite in mutagenic assays, was found to initiate apoptosis in HepG2 cells, in part through activation of the dioxin or Ah receptor. The treatment of HepG2 cells with BP-7, 8-diol leads to cleavage of the cytosolic Bid protein, an increase in the pro-apoptotic Bak protein and a decrease in the anti-apoptotic Bcl-xl. BP-7, 8-diol initiated changes in the Bcl-2 family of proteins results in the release of cytochrome *c* from mitochondria. This triggers caspases activation and cleavage of caspases specific substrates, such as poly (ADP-ribose) polymerase (PARP-1). These changes in apoptotic markers may occur through CYP1A1 dependent metabolism of BP-7, 8-diol, since BP-7, 8-diol activates *CYP1A1* resulting in the accumulation microsomal CYP1A1. In HepG2 cells, BP-7, 8-diol and BPDE activate mitogen activated protein kinases (MAPKs) p38 and ERK1/2. MAPKs have been implicated in controlling cellular proliferation, apoptosis and death. Specific p38 and ERK1/2 inhibitors block apoptosis in HepG2 cells as monitored by caspases dependent cleavage of PARP-1. To investigate the specific role of MAPKs, mouse embryo fibroblasts deficient in p38 (p38-/-) were treated with BPDE and apoptosis monitored by Annexin V analysis and PARP-1 cleavage. Compared to wild type mouse embryo fibroblasts, p38-/- cells showed a greatly reduced apoptotic index. In addition, markers of apoptosis such as cytochrome *c* release from mitochondria confirmed that p38 plays an important role in BPDE initiated apoptosis. (Supported by ES10337).

1730 RESCUE OF CORTICAL NEURONS FROM CYANIDE-INDUCED APOPTOSIS: DEMANSTRATES BAX TRANSLOCATION IN THE APOPTOTIC PROCESS.

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Cyanide produces an apoptotic death of cortical cells *in vivo*. To determine the time required to initiate apoptotic neuronal death, rat cortical cells were exposed to 300 μM cyanide for varying time (10 min to 8 h) followed by cyanide washout. Cells were then incubated for a total of 24 h and the level of apoptosis estimated by noting oligonucleosomal degradation of DNA on electrophoresis. Ten and 20 min exposures were insufficient to produce apoptosis, but a robust level of apoptosis was detected among the cell population after 30 min or longer. Similarly, translocation of Bax (determined by western blot analysis) from cytoplasm to mitochondria was seen after 30 min but not 10 or 20 min cyanide treatment. Cells exposed for 30 min to cyanide showed cytochrome c release from mitochondria 2 h later and caspase-3 activation after 8 h. Pretreatment of the cells with cyclosporin A blocked cytochrome c release and caspase activation, but not Bax translocation. Cyclohexemide, a protein synthesis inhibitor, blocked cyanide-induced Bax upregulation (cytoplasmic protein levels) but blocked neither Bax translocation to mitochondria nor cyanide-induced apoptosis. PBN, an antioxidant, inhibited Bax translocation, cytochrome c release and caspase-3 activation, as well as cyanide-induced apoptosis. It was concluded that treatment which inhibit Bax translocation (antioxidants) or inhibit the effects of Bax on mitochondria (cyclosporin A) also block apoptosis. Thus translocation of Bax from the cytoplasm to mitochondria occurs during a 30 min exposure of cortical cells cyanide and is a critical regulatory event in initiating subsequent apoptosis. (Supported by NIH grant ES04140).

1731 INHIBITION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION IN THE MECHANISM OF HEAT SHOCK PROTECTION.

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Heat shock proteins have been shown to inhibit apoptotic and necrotic cell death in various cell types after a variety of stresses. However, the specific mechanism underlying the protection by heat shock proteins remains unclear. To test the hypothesis that heat shock proteins inhibit cell death by suppressing the opening of mitochondrial permeability transition (MPT) pores, mitochondria from the livers of control and heat-preconditioned rats were isolated by differential centrifugation. Mitochondrial swelling was measured by absorbance change at 540 nm. Oxygen consumption was determined using a Clark type electrode. Membrane potential, Ca^{2+} uptake and reactive oxygen species formation was monitored using fluorescent dyes, TMRM, Fluo-5N and H_2DCFDA, respectively. Mitochondrial and cytosolic glutathione was measured using Bioxytech GSH-420 kit. Heat shock protein expressions were assessed using Western blot. Heat shock inhibited opening of regulated MPT pores induced by 200 μM $CaCl_2$ and by 50 μM $CaCl_2$ plus 5 μM $HgCl_2$ or 1 μM mastoparan. Half maximal swelling was delayed 15 min or more after heat shock comparison to the control. Heat shock also increased the threshold of unregulated (Ca^{2+}-independent and cyclosporin A-insensitive) MPT pore opening induced by $HgCl_2$ from 20 μM to 40 μM. Heat shock did not alter mitochondrial state 3 and state 4 respiration, respiratory control ratios, membrane potential, Ca^{2+}-uptake, or glutathione levels in mitochondria and the cytosol. However, mitochondrial formation of reactive oxygen species decreased 27% after heat shock. Western blot analysis showed that mitochondrial Hsp25 increased, whereas Hsp10, Hsp60, Grp75 and cyclophilin D did not change after heat shock. These results indicate that heat shock induces resistance to opening of MPT pores, which may con-

tribute to heat shock protection against cellular injury possibly through increased expression of mitochondrial Hsp25 and decreased formation of mitochondrial reactive oxygen species. Supported by NIH 1 P01 DK59340 and 5-R01 AG07218.

1732 EVALUATION OF DR5 MEMBRANE LOCALIZATION IN THE RODENT TESTIS USING FLOW CYTOMETRIC ANALYSIS.

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Previous work by our laboratory has indicated that exposure of rodents to mono-(2-ethylhexyl) phthalate (MEHP) results in the up-regulation of the Fas receptor in testicular germ cells of rats and mice followed by an increase in the apoptotic cell death of these same cells. It has also been demonstrated that the initiation of germ cell apoptosis in MEHP-exposed rodents occurs, in part, due to the activation of the germ cell expressed Fas by Sertoli cell expressed Fas ligand (FasL). More recent research of our laboratory has revealed the presence of additional death receptors, DR4 and DR5, in the testis of C57 mice and in Sprague-Dawley rats. In the present study, we examined the membrane expression of DR5 on germ cells using both western blot analysis and flow cytometry as a first step in characterizing cell specific expression and responsiveness to toxicant exposure. Flow cytometric analyses indicates that multiple subpopulations (4n, 2n and 1n ploidy) of testicular cells stain positive for DR5. Flow cytometry and associated cell sorting along with immunohistochemistry further revealed that 1) approximately 20% of the 1n population (representing the haploid round and elongated spermatids) possessed DR5 on the membrane; 2) between 10-30% of the 4n cell population stained positive with the vast majority of these being spermatocytes; and 3) the 2n population appears to be a mixture of germ and somatic cells. In addition, gld mice, which express a dysfunctional form of FasL, display higher levels of DR5 on germ cell membranes of 4n cells than do C57 mice, as well as greater total receptor levels. Western blot analysis of C57 mice testes revealed an increased DR5 expression on the germ cell membrane after MEHP exposure. In summary, our findings suggest that DR5 is expressed by germ cells, DR5 levels are higher in animals lacking a fully functional Fas system, and that DR5 is responsive to MEHP-induced testicular injury. (This work was supported, in part, by NIH/NIEHS grants ES09145 and ES07784).

1733 THE EXPRESSION OF TNF-RELATED APOPTOSIS INDUCING LIGAND (TRAIL) IN RODENT SERTOLI CELLS.

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TRAIL is a member of the TNF family of ligands. TRAIL binds to the death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). The expression of TRAIL has been observed in many normal tissues. TRAIL has also been found to induce apoptosis in many tumor cell lines. However, only a limited number of non-transformed cell lines have been reported to undergo apoptosis after TRAIL addition. Recent reports have described the presence of TRAIL mRNA in testis. Investigations have also suggested that TRAIL protein is exclusively localized to select subpopulations of germ cells in the rat testis. In the present study we evaluated the expression of TRAIL in primary cultures of enriched rat and mouse Sertoli cells as well as in the mouse-derived TM4 Sertoli cell line. Western blot analyses demonstrate that TRAIL protein is expressed in TM4 cells as well as in enriched cultures of mouse and rat primary Sertoli cells. Immunohistochemical analysis of rat testis reveals that TRAIL protein was localized to both Sertoli cells and germ cells. These results were further confirmed in primary cultures of rat or mouse Sertoli cells and germ cells. In addition, TRAIL mRNA was measured in TM4 cells by RT-PCR. These observations indicate, for the first time, that Sertoli cells of the testis express both TRAIL mRNA as well as TRAIL protein in relatively abundant levels. (Supported, in part, by grants from NIH/NIEHS ES09145 & ES07784).

1734 ORGANIC ANION TRANSPORT INHIBITORS ATTENUATE GLUTATHIONE RELEASE DURING FAS-MEDIATED APOPTOSIS IN HEPG2 CELLS.

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In experimental models of apoptosis, reduced glutathione (GSH) appears to be selectively released from cells leading to cellular GSH depletion. Because GSH has many protective functions in the cell, GSH depletion may contribute to cellular demise. However, neither the mechanism of GSH release during apoptosis, nor its physiological significance has been elucidated. In the present study, the mechanism of GSH release was characterized in HepG2 cells induced to undergo apoptosis by 0.5 μg/mL Fas Ab and 20 μg/mL cyclohexamide (CHX). Approximately 50% of total GSH was released from cells treated with Fas and CHX, whereas untreated cells released 20% GSH over 5 hours. Fas and CHX-treated HepG2 cells showed increased caspase 3-like activity and apoptotic nuclear morphology, but minimal propidium iodide incorporation, suggesting that the cells are apoptotic and that plasma membrane damage is minimal. These results support the hypothesis that GSH is released through a specific transport mechanism. HepG2 cells were also treated with the organic anion transport inhibitors sulfinpyrazone, MK571, probenecid, and dibromosulfophthalein (DBSP) to examine the effects on GSH release and apoptosis. All four compounds inhibited basal GSH release, however only probenecid and DBSP inhibited the Fas and CHX-stimulated GSH release. Inhibiting GSH release with probenecid and DBSP also attenuated lactate dehydrogenase release. These results indicate that GSH release during apoptosis occurs through a different mechanism than basal GSH release. Because sulfinpyrazone and MK571 are known to inhibit the MRP proteins, the GSH release is probably occurring by a mechanism distinct from MRP proteins. In summary, Fas ligand-stimulated GSH efflux is inhibited by the organic anion transport inhibitors probenecid and DBSP, indicating a carrier-mediated GSH export pathway. Inhibition of GSH efflux was protective, suggesting that GSH extrusion may contribute to the cell death process. (Supported by NIH grants ES06484, DK48823, ES01247 and ES07026)

1735 VITAMIN E INDUCED CASPASE-8 MEDIATED APOPTOSIS OCCURS INDEPENDENTLY OF DEATH RECEPTOR ACTIVATION IN NEOPLASTIC MAMMARY EPITHELIAL CELLS.

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Tocotrienols, a subclass of vitamin E compounds, have been shown to suppress mammary tumorigenesis in animal models and inhibit growth of human breast cancer cells in culture. Tocotrienols have also been shown to induce apoptosis in neoplastic mouse mammary epithelial cells, by mechanisms not yet determined. Studies were conducted to determine the intracellular pathways mediating tocotrienol-induced apoptosis in neoplastic +SA mouse mammary epithelial cells. Following 24 hr treatment with γ-tocotrienol, there was a dose-dependent decrease in +SA cell viability resulting from induction of apoptosis. Cell viability studies were performed by MTT assay, and apoptosis induction was detected by TUNEL assay. Additional studies showed that treatment with a cytotoxic dose of 20 μM γ-tocotrienol increased caspase-8 and caspase-3 activity, as measured by colorimetric assay kits. Furthermore, treatment with specific caspase-8 or -3 inhibitor completely blocked the tocotrienol-induced apoptosis in +SA cells. Since activation of death receptors (Fas or TRAIL receptors) is required for the induction of caspase-8 activity and apoptosis, further studies were conducted to determine the exact death receptor(s) and ligand(s) involved in tocotrienol-induced apoptosis. Treatment for 24 hrs with Fas-ligand, Fas-activating antibody, or TRAIL had no effect on +SA cell viability. Moreover, treatment with 20 μM γ-tocotrienol for 0-24 hrs did not alter the total cellular or membrane levels of Fas-receptor or Fas-ligand. Western blot analysis also showed that γ-tocotrienol did not induce translocation of Fas-ligand from the cytosol to the membrane. Finally, 24 hr treatment with Fas-blocking antibody did not reverse the tocotrienol-induced apoptosis in +SA cells, indicating that tocotrienols do not activate these death receptors. In summary, these findings suggest that tocotrienol-induced activation of caspase-8 mediated apoptotic pathways in neoplastic +SA mouse mammary epithelial cells occurs independently of death receptor activation.

1736 1, 2, 3, 4-DIEPOXYBUTANE INDUCES P53 DEPENDENT APOPTOSIS IN HUMAN LYMPHOBLASTS.

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1, 3-Butadiene (BD) is used in the manufacture of styrene-butadiene rubber, and is prevalent in petrochemical industrial areas. This compound is a known mutagen and human carcinogen, and possesses multi-organ systems toxicity that includes bone marrow depletion, spleen and thymus atrophy. Toxic effects of BD are mediated by its epoxy metabolites, 1, 2-epoxybutene (EB), 1, 2, 3, 4-diepoxybutane (DEB), and 1, 2-epoxy-3, 4-butanediol (EBD). The molecular mechanism(s) of BD toxicity however, are not fully understood. In working towards elucidating the cellular and molecular mechanisms of BD toxicity, we investigated the ability of the most potent BD metabolite, DEB, in inducing apoptosis in human TK6 lymphoblasts, as well as the role of the cellular p53 protein in that process. The tumor suppressor p53 protein is known to mediate cellular responses to environmental stress and DNA damage in a variety of systems. Using variety of assays (nuclear fragmentation, DNA ladder and Poly[ADP-ribose]polymerase cleavage), our results demonstrated concentration-dependent and time-dependent apoptosis in DEB exposed human TK6 lymphoblasts. DEB concentrations of 5μM induced apoptosis

in 28% of the cells at 48h post-exposure; under these conditions, control cells exposed to vehicle only did not show any features of apoptosis. The induction of apoptosis by DEB correlated to elevated p53 levels in the same cells in a concentration and time dependent manner. Although DEB-induced apoptosis was observed in the p53 proficient TK6 lymphoblasts, it failed to occur in the p53 deficient NH32 lymphoblasts under the same experimental conditions. Our results demonstrate the occurrence of DEB-induced apoptosis in human lymphoblasts for the first time. In addition, our results reveal that DEB-induced apoptosis is mediated by the tumor suppressor p53 protein. Collectively, this DEB-induced p53 mediated apoptosis may explain butadiene induced bone marrow depletion, as well as spleen and thymus atrophy in exposed animals(supported by grant# ES10018, funded by NIEHS).

1737 THE ROLE OF TNF-α RECEPTOR 2 IN BLEOMYCIN-INDUCED APOPTOSIS IN ALVEOLAR MACROPHAGES.

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Activation of alveolar macrophages (AM) in the production of tumor necrosis factor (TNF)-αby bleomycin (BLM) is linked to AM apoptosis and the development of pulmonary fibrosis. AM may contribute to their own death through expression of TNF receptor (TNFR) 1 and 2. The present study was carried out to characterize the role of the TNFR in the mechanism(s) of BLM-induced apoptosis in AM. Sprague-Dawley rats were instilled intratracheally with saline or BLM at 1 mg/kg body weight. At 1, 3, or 7 days post-exposure, AM were isolated by bronchoalveolar lavage, and apoptosis was determined by ELISA. The activation of caspases 3, 8, and 9, the release of cytochrome c from mitochrondria, the cleavage of nuclear poly(ADP-ribose) polymerase (PARP), and the amount of TNFR1 and TNFR2 in AM were monitored by immunoblotting. The results showed that BLM treatment significantly induced AM apoptosis at all exposure time points, with peak apoptosis occurring at 1 day post BLM exposure. BLM treatment, at 1, 3 or 7 days post exposure, significantly increased active caspase 3 level with enhanced caspase 3 activity and increased PARP cleavage in AM in comparison to the control. The maximum activation of caspase 3 and PARP fragmentation occurred in AM from rats exposed to BLM for 3 days. The amount of cytochrome c released into cytosol was gradually increased with time after BLM treatment and peaked on day 7. This coincides with expression of TNFR2, which was significantly induced in BLM-exposed AM with a peak level at 7-day post exposure. In contrast, BLM treatment did not affect AM expression of TNFR1. BLM exposure also significantly activated caspase 9 but not caspase 8 in AM. These results show that BLM-induced apoptosis is TNFR1/caspase-8 independent, but involves over-expression of TNFR2, the release of cytochrome c from mitochondria, activation of caspases 9 and 3, and the cleavage of the DNA repair protein PARP.

1738 APOPTOTIC ALVEOLAR MACROPHAGES PLAY A ROLE IN THE DEVELOPMENT OF PULMONARY INFLAMMATORY DISEASE IN RATS.

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Increasing evidence suggests that apoptosis plays an important role in pulmonary diseases; however, the molecular mechanism underlying this relationship is unclear. Previous studies from our laboratory have shown that pulmonary instillation of dimethyl sulfoxide (DMSO) induced apoptotic alveolar macrophage (AM) resulted in pulmonary inflammation and fibrosis. In this study, we further investigated the effect of intratracheal instillation of DMSO or UVB induced apoptotic AM and documented the time course of the resulting inflammatory and fibrotic responses in the Brown Norway rat lung. Morphological and immunohistochemical analyses of lung tissues showed an inflammatory response and increased connect tissue in the treatment groups, but not in control groups which were instilled with normal non-apoptotic AM. Instilled apoptotic cells were cleared at 4 weeks post-treatment, but the number of apoptotic lung cells was still higher than those in the control rats. Caspase 8 was elevated in the treatment group at 4 weeks post-treatment, suggesting a continuous induction of apoptosis *in vivo* via the death receptor-mediated pathway. Consistent with this finding, we observed a concomitant increase in TNF-α expression in treated lung tissues. *In vitro* studies also showed an increase in TNF-α production by naive AM incubated with apoptotic AM, but not when exposed to untreated AM. Together, our results indicate a role for AM apoptosis in the induction of inflammatory and fibrotic lung disorders, which may be mediated though cytokines, such as TNF-α.

1739 C2-CERAMIDE *VS.* TNF-α INDUCED CYTOTOXICITY AND APOPTOSIS IN A RAT HEPATOMA (H4IIE) CELL LINE.

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Understanding the various mechanisms of cellular toxicity and cell death can aid in drug design. Previously, we noted that TNF-α and C2-ceramide produced different toxicity and apoptosis profiles in rat hepatoma (H4IIE) cells. Tumor necrosis factor alpha (TNF-α) is a cytokine involved in certain apoptotic pathways and can stimulate the hydrolysis of sphingomyelin to ceramides. Ceramides are putative lipid second messengers implicated in apoptotic responses. In this study, the effects of TNF-α and ceramide on toxicity and apoptosis were investigated in H4IIE cells using multiple biochemical markers. H4IIE cells were exposed to ceramide (0-300 μM) or TNF-α (0-150 ng/mL, specific activity 1000 University/ng) for 24 hours. Changes in general cell health were determined by monitoring mitochondrial function, membrane leakage, and cell proliferation. Oxidative stress was assessed by measuring changes in reduced glutathione (GSH) and membrane lipid peroxidation. Apoptosis was evaluated by caspase (3, 8, and 9) activity, DNA fragmentation, and using a Cellomics™ ArrayScan®: mitochondrial mass potential, f-actin levels, and nuclear condensation/fragmentation. Cellular calcium changes were measured for up to 2 hours after treatment using Fluo3-AM dye and fluorescence microscopy or a Molecular Devices FLIPR system. As expected, TNF-α caused a marked increase in caspase 3, 8, and 9 activities as well as DNA fragmentation and f-actin content. TNF-α was cytotoxic above 5ng/mL, however, no significant change was observed in GSH or in membrane lipid peroxidation prior to toxicity. In contrast, ceramide caused a significant increase in membrane lipid peroxidation and DNA fragmentation but had no effect on caspase 3, 8, and 9 activities. Ceramide increased intracellular calcium levels while TNF-α did not produce significant calcium influx. These data suggest that apoptosis induced by TNF-α occurs through caspase dependent pathways whereas exogenous C2-ceramide induced cell death involves calcium influx, oxidative stress, or other caspase independent pathways.

1740 MECHANISMS OF OCHRATOXIN A-INDUCED INHIBITION OF HUMAN LYMPHOCYTE PROLIFERATION.

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Ochratoxin A (OTA) is a fungal secondary metabolite produced in agricultural commodities by Aspergillus and Penicillium fungi species. Human exposure to OTA is both due to consumption of directly contaminated food or of food products derived from animals consuming contaminated feedstuffs. OTA was implicated in the pathogenesis of Balkan endemic nephropathy due to its nephrotoxicity ; in addition, it is hepatotoxic, genotoxic, carcinogenic and immunosuppressive. This latter toxicity has been widely studied in experimental animals such as mice and chickens. In this study, we investigated the effects of OTA on human peripheral blood mononuclear cells (PBMC). PBMC stimulated with phytohaemagglutinin (PHA) and incubated with OTA at different concentrations for 48 h showed an inhibition of proliferation at $5x10^{-6}$ M and 10^{-5} M of OTA as measured by ^{3}H-thymidine uptake. However, IL-2 secretion was not affected for all concentrations tested as determined by ELISA. In addition, exogenous IL-2 added to cell culture at 1 and 10 ng/ml did not overcome the inhibition of lymphocyte proliferation. A slight decrease in CD25 (IL-2R alpha) surface expression at $5x10^{-6}$ M of OTA was observed compared to control cells (60 vs 73 % and 47 vs 72 % at 24 h and 48 h respectively). Moreover, stimulated and unstimulated PBMC incubated with OTA entered apoptosis in a time and concentration-dependent manner. The mechanism is likely to be mediated by caspases since the pan-caspase inhibitor VAD reduced apoptosis induced by OTA. Our results suggest that downregulation of CD25 and induction of apoptosis are involved in OTA inhibition of lymphocyte proliferation. The role of cell cycle proteins such as cdk2 and cyclin E, as well as Bcl-2 family proteins, are currently under investigation.

1741 CHANGES IN LYMPHOCYTE SUBSETS AND APOPTOSIS IN LYMPHOID TISSUES OF NIVALENOL-TREATED MICE.

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Nivalenol (NIV) is the major group B trichothecene mycotoxin and NIV widely contaminates agricultural commodities. In this study, ICR mice were orally administered with 15 mg/kg of NIV to elucidate the development of apoptosis and

changes in lymphocyte subsets in lymphoid tissues. The TUNEL-positive cells showing ultrastructural characteristics of apoptosis, were observed in lymphoid tissues, and the ladder pattern was detected by agarose gel electrophoresis on DNA extracted from thymus. Moreover, FACS analysis revealed that NIV attacked Peyer's patches first and thymus most severely, and the selective damage in CD4+CD8+ cells in thymus while CD4+ and CD8+ cells were unexceptionally reduced in other lymphoid tissues. As to B cell subsets, all B cell subsets, especially IgA+ cells showed significant increase in Peyer's patches while B cell subsets generally decreased in spleen and mesenteric lymph nodes. These results indicate that NIV induces apoptosis and changes in lymphocyte subsets in lymphoid tissues of mice.

1742 EFFECTS OF T-2 TOXIN ON KERATINOCYTE PRIMARY CULTURES.

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T-2 Toxin (12-13 epoxytrichothecene mycotoxin) induces apoptosis in basal keratinocytes when topically applied on the dorsal skin of Wistar-derived WBN/ILA-*Ht*rats. In the present study, effects of T-2 toxin on keratinocyte primary cultures were examined. Cells which were obtained from newborn Wistar rats and cultured by the method of Reynwald and Green were used after the third passage. Keratinocyte medium containing 0.25μg/ml of T-2 Toxin dissolved in DMSO or solvent alone was added to four-days-cultures and incubated at 37°C. At 0.5, 1, 3, 5, 7, and 9 hours after treatment (HAT), feeder layer was separated from T-2 toxin-treated and control flasks, and cells were trypsinized. Cell viability was estimated by trypan blue exclusion method. In addition, RNA was obtained and RT-PCR was perfomed. Samples obtained from slides cultures at 3, 6, 9 and 12HAT were fixed in 4% paraformaldehide or 2.5% glutaraldehide for morphological studies. After T-2 toxin application, cell viability decreased to 40% at 12HAT. Pyknotic and karyorrectic nuclei with shrinkage of cell body were found in small keratinocytes at 6HAT, and there number increased until 12HAT. At the same points, large squamous keratinocytes showed degeneration characterized by intracytoplasmic edema. The number of PCNA-positive cells dramatically decreased from 3 to 12 HAT. Apoptosis related genes expression (*c-fos* and *c-jun* oncogenes) and cytokines (TNF-α) mRNA expression markedly increased at 0.5 HAT and drecreased thereafter. These findings were also observed in our previous *in vivo* studies suggesting that *c-fos* and *c-jun* oncogenes and TNF-α have some relation to the mechanisms of wich T-2 toxin-induced apoptosis in basal keratinocytes.

1743 SUBCYTOTOXIC INORGANIC ARSENIC AFFECTS MITOCHONDRIA IN HK-2 HUMAN PROXIMAL TUBULAR CELLS: POTENTIAL FOR APOPTOTIC CELL DEATH.

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The kidney is a known target organ for arsenic (As) and is critical for both As biotransformation and elimination. Previous studies have demonstrated that at high doses (ppm) inorganic As is toxic to mitochondria primarily by affecting cellular respiration. However the effect of As on mitochondria after low level exposures is not known, particularly in the kidney. Thus the mitochondrial toxicity of low level inorganic As was investigated in a human proximal tubular cell line, HK-2. Subcytotoxic concentrations of arsenite (1-10 μM) and arsenate (<25 μM) were found to affect MTT processing by mitochondria in the HK-2 cells. Therefore, even at low concentrations, the mitochondria are a primary target for inorganic As toxicity. Mitochondrial injury was further assessed by examining the alteration of the mitochondrial membrane potential using Mito Tracker Red, a mitochondrion selective dye, with detection of staining by confocal microscopy. Mitochondrial staining was punctate in untreated cells, indicative of selective uptake by actively respiring mitochondria. Following 24 h exposure to As concentrations as low as 100 nM, changes in the membrane potential were evident by alterations in staining patterns. Fluorescence became more intense and eventually more diffuse throughout the cytoplasm with concentrations in the μM range. In general, arsenite effects were seen at a lower concentration than for arsenate, which confirms data from the MTT studies. Disruption of mitochondrial membrane potential by low-level inorganic As exposure could initiate cell death *via* apoptosis. To determine apoptosis, the presence of plasma membrane phosphatidyl serine was measured by Annexin-V-FLUOS labeling with detection by fluorescence microscopy. Apoptotic cells were observed with 100 nM arsenite exposures and a concentration-response increase was seen up to 10 μM. Therefore, the induction of apoptosis *via* the action of low-level As on mitochondria could contribute to the pathogenesis of renal tubular injury. (NIH Superfund ES 04940)

1744 ARSENITE STIMULATES APOPTOSIS IN MYELOID LEUKEMIA CELLS DURING G2/M PHASE.

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Arsenic, notorious for its toxic and carcinogenic properties, may also be effective in the treatment of a variety of cancers. Arsenite (As(III)) is thought to be the most bioactive form of arsenic. As(III) is cytostatic and induces apoptosis in the myeloid leukemia cell line U937. We used analysis of 5-bromo-2′-deoxyuridine (BrdU) incorporation together with propidium iodide (PI) staining to test the hypothesis that As(III) induces apoptosis in G2/M phase cells. U937 cells were exposed to $NaAsO_2$ (0-10 μM) for 24-48 h and pulsed with BrdU for 15 min. Anti-BrdU labeling and PI staining were measured *via* flow cytometry. As(III) induced apoptotic DNA degradation in cells with G2/M DNA content as shown by the dose-dependent appearance of a population of BrdU-negative cells with sub-G2 DNA content. This population represents cells triggered by As(III) to undergo apoptosis after entering G2/M phase. These cells do not stain with BrdU, so although they have an S phase DNA content, they are not active S phase cells. Therefore, it may not be possible to define the cell cycle status of cells with S phase DNA content by PI staining alone. To clarify the source of the sub-G2 cell population, we used a BrdU pulse-chase technique. Following a 15 min pulse with BrdU, cells were treated with $NaAsO_2$ (0-10 μM) for 0-24 h. Cells were assayed as described above. A distinct population of BrdU-positive cells with G1 DNA content developed in untreated samples after 8 h, indicating that these cells have moved from S phase through G2/M phase and back to G1. The hypothesis that As(III)-treated cells undergo apoptosis during G2/M phase predicts that fewer cells will transit from S phase through G2/M to G1. Indeed, the population of BrdU-positive cells with G1 DNA content decreases in a dose-dependent manner with As(III) treatment. Also, As(III)-treated BrdU-positive cells undergo DNA degradation only after they attain G2/M DNA content. We conclude that As(III) targets U937 cells for apoptosis during G2/M phase. Supported by NIH grants P30 ES01247 and T32 ES07026.

1745 COPPER-INDUCED CASPASE-3 ACTIVATION AND APOPTOSIS IN RAT HEPATOMA CELL LINE (H4IIE).

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Increased hepatic copper (Cu) accumulation in Wilson's disease and Indian Childhood Cirrhosis cause severe liver damage. Although these changes can result in cirrhosis and hepatocellular carcinoma, the mechanisms involved in the pathogenesis of Cu-induced damage are not well understood. In order to study the initial changes on Cu-induced DNA damage and apoptosis, the present study was undertaken in rat hepatoma cell line (H4IIE) after exposure to Cu salts (250 to 1000 μM). The cell viability was not affected at 8 hrs of Cu exposure but was markedly reduced at 12 hrs with Cu concentration >750 μM. More apoptotic cells were observed by TUNEL staining at 8 hrs of exposure to Cu (> 500 μM) than control cells. This was confirmed by changes in DNA ladder formation. TNF alpha was not detected in the cells. There was an increase in Caspase-3 activity at 4 hrs of incubation with more than 750 μM of Cu exposure. The results suggest that Caspase-3 activation is an early event in Cu-induced injury *in vitro*. Therefore, Caspase-3 activation plays an important role in the Copper induced apoptosis. (Supported by a Grant from CIHR).

1746 EFFECTS OF DIETHYDITHIOCARBAMATE (DDC) ON RAT HIPPOCAMPAL ASTROCYTES ON CASPASE-1, C-MYC, BCL-2 AND P53 WITH AND WITHOUT GLUTATHIONE (GSH).

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Diethyldithiocarbamate (DDC), a potent metal chelator, is a carbamate derivative. It has been documented that DDC binds and redistributes copper in a lipophilic complex to the central nervous system. Apoptosis is a morphologically defined process in which cells die in a controlled manner. Previously, we have demonstrated by TUNEL and DNA laddering techniques that 35 μg/ml and 350 μg/ml DDC induced apoptosis or programmed cell death in rat hippocampal astrocytes treated for 1hr. Caspase-3 was also shown to be induced. This study investigates the activation of 4 apoptotic genes in astrocytes, caspase-1, c-myc, bcl-2 and p53, following the recovery from DDC treatment with and without glutathione (GSH). Caspase-1 belongs to the cysteine protease family and plays a major role in apoptosis. It mediates inflammatory response through cleavage activation of IL-1β and IFN-γ promolecules. c-myc is a proto-oncogene documented to play a role in the induction of apoptosis. Bcl-2 blocks cell death following a variety of stimuli. Induction of p53 protein expression is usually considered as one of the primary events involved in the

apoptotic reaction cascade following DNA damage. Rat hippocampal astrocytes grown in Dulbecco's modified Eagle's essential media were treated with 35 and 350 μg/ml DDC at subconfluency for 1 hr. Astrocytes were then left to recover with or without 10mM glutathione in DMEM for 24 hr at which time cells were collected for RT-PCR. Caspase-1 was significantly activated in astrocytes treated with 35 and 350 μg/ml DDC in a dose dependent manner. Glutathione post-treatment prevents the activation of caspase-1 in both treatment groups. c-myc was significantly induced but not in a dose dependent manner. Bcl-2 and p53 did not show any significant induction or inhibition of apoptosis.

1747 EFFECT OF ACUTE MERCURY VAPOR EXPOSURE TO MURINE THYMUS.

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Elemental mercury is a toxic metal which causes various health effects. Acute poisoning causes pulmonary damage that is characterized by edema and hyaline membrane formation. However, toxic effects of mercury vapor *in vivo* on immune system are not well understood. Therefore, this study was performed to examine the *in vivo* toxic effect of mercury vapor exposure to thymus. Sixteen C57BL/6J Jcl mice were exposed to mercury vapor at 2.71 to 3.74 mg/m^3 for 1 hour. Mice were killed under diethylether anesthesia at 24 hours after exposure. The liver, spleen, kidney, heart, lung, brain and thymus were taken from each mouse. Histopathological examinations including autometallography for mercury distribution were performed. Mercury concentrations in the lung, thymus, kidney, brain and liver were measured by cold vapor atomic absorption method. The highest mercury concentrations were detected in the kidney, followed by the lung. Mercury concentration in the thymus was not significantly different from that in the liver. Mercury granules were demonstrated in the histological sections of the all organs examined. In the thymus, mercury granules were seen in the phagocytic cells and vascular endothelial cells. Histopathological changes were restricted to the lung and thymus. In the lung, mild inflammation and edema were observed. In the thymus, there was an increase of the number of phagocytic cells containing apoptotic lymphocytes compared with control mice. Agarose gel electrophoresis of the thymuses showed a ladder pattern that indicating the presence of fragmented DNA. These results suggest that apoptosis of lymphocytes may be caused by the mercury vapor in the thymus. Mechanisms of apoptosis induced by mercury vapor are to be elucidated.

1748 POSSIBLE INVOLVEMENT OF CALPAIN/P35/CDK5 CASCADE IN METHYLMERCURY-INDUCED DEATH OF CEREBELLAR NEURONS.

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It has been shown that methylmercury-induced death of cerebellar granule neurons is apoptotic both *in vitro* and *in vivo* (Kunimoto (1994) Biochem Biophys Res Comm. 204, 310-317: Nagashima et al (1996) Acta Neuropathol 91, 72-77). To clarify the molecular mechanism for the induction of apoptosis in cerebellar neurons by methylmercury, several lines of experiments have been performed using rat cerebellar neurons in primary culture. Cerebellar neurons prepared from neonatal rats were exposed to methylmercury at very low concentrations (up to 30nM) *in vitro* and possible involvement of calpain/p35/cdk5 cascade in the death process was examined, since the cascade is shown to be activated during the neuronal death in Alzheimer?s disease. Calpain inhibitor and cdk5 inhibitor (butyrolactone I) were both effective in preventing or delaying the methylmercury-induced death of cerebellar neurons. In addition, 440 kD ankyrinB, an axonal protein and a substrate for calpain, was degraded in the cerebellar neurons treated with methylmercury. These results suggest that methylmercury-induced activation of calpain plays important role in the death process of cerebellar neurons.

1749 TRAIL AND DEATH RECEPTOR RESPONSE IN RODENT TESTIS AFTER CISPLATIN EXPOSURE.

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Testicular germ cell tumors are extremely vulnerable to elimination by cisplatin-based chemotherapeutics. Non-cancerous germ cells are also sensitive to cisplatin and undergo apoptotic death following chemotherapy. However, the mechanism(s) responsible for initiating apoptosis in germ cells is still unclear. Here, we evaluate the response of death receptors and their cognate ligands in the testis following cisplatin treatment and analyze the correlations between death receptor changes and our previously reported massive apoptotic die-off of germ cells. Adult (11wk) C57/BL/6 mice were exposed to a single acute dose of cisplatin (5 mg/kg I.P.). Western blots from testis homogenates revealed a marked increase in membrane levels of DR4, DR5 and TRAIL by 3 h after cisplatin exposure. Exposure the TM4 Sertoli cell line to 150 micromolar cisplatin demonstrated increased levels of TRAIL by western blot analysis similar to those seen *in vivo*. Alterations in TRAIL protein expression were not observed in the F9 germ cell line. Pro-caspase-8 processing was also observed *in vivo* and in F9 cells after cisplatin treatment. Immunohistochemical analysis of frozen rat testis cross-sections suggests membrane localization of TRAIL in both Sertoli cells and germ cells and localization of the death receptors DR4 and DR5 in germ cells alone. Finally, an increase in NF-κB DNA binding activity was seen in both TM4 and F9 cells following cisplatin treatment. Up-regulation of DR5 occurs in germ cell concomitantly with TRAIL up-regulation in Sertoli cells. These data suggest that TRAIL-dependent signaling in germ cells is regulated by Sertoli cells in response to cisplatin treatment. Whether this pathway is an active participant in germ cell apoptosis or elicits another response in germ cells remains to be tested. (Supported, in part by grants from NIH/NIEHS ES09145, ES07784 and from the Lance Armstrong Foundation).

1750 6(5*H*)-PHENANTHRIDINONE ATTENUATES THE HEPATOTOXICITY OF CARBON TETRACHLORIDE.

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Carbon tetrachloride (CCl_4) is routinely used as a model for eliciting centrilobular (zone 3) hepatotoxicity. CCl_4 is bioactivated to the trichloromethyl radical, which causes extensive lipid peroxidation, and ultimately cell death by necrosis. Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme involved in the identification of DNA strand termini. Extensive cellular damage can overactivate PARP-1, which rapidly depletes the cellular stores of NAD^+ and ATP, resulting in necrotic cell death. The purpose of the present study was to determine whether 6(5*H*)-phenanthridinone (10 mg/kg, i.p.), a potent inhibitor of PARP-1, could attenuate the toxicity of CCl_4 (572 mg/kg, i.p.; LD_{50}) in male ICR mice. Animals treated with CCl_4 exhibited severe necrotic centrilobular lesions and significantly elevated serum glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), and lactate dehydrogenase (LDH) levels. In contrast, the histopathology and serum biochemistry (GPT, GOT, LDH) of animals treated concomitantly with CCl_4 and 6(5*H*)-phenanthridinone were not significantly different versus controls. The results of this study demonstrate that the hepatoxicity of CCl_4 can be blocked independently of its metabolism, and further indicate the role of PARP-1 in chemical-induced toxicity. (Supported in part by the Department of Defense)

1751 QUANTIFICATION OF HEPATOCYTE PROLIFERATION AND APOPTOSIS IN RATS TREATED FOR FIVE DAYS WITH VARIOUS HEPATOCARCINOGENS.

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Increased hepatocyte proliferation and apoptosis are common responses to various hepatic toxicants, especially nongenotoxic carcinogens. Cell proliferation and apoptosis are traditionally evaluated by histologic examination, but can also be quickly quantified using immunohistochemistry in combination with computer-assisted image analysis. The purpose of this study was to evaluate and validate a method for the quantification of hepatocyte proliferation and apoptosis following a 5-day daily treatment with various genotoxic or non-genotoxic carcinogens. Male CD IGS rats were treated orally for 5 days with vehicle, methapyrilene at 35, 70 or 140 mg/kg/day, phenobarbital at 60, 100 or 200 mg/kg/day, clofibrate at 200, 400 or 800 mg/kg/day, 2-AAF at 8, 50 or 100 mg/kg/day, 4-AAF at 8, 50 or 100 mg/kg/day, or tamoxifen at 25, 100 or 200 mg/kg/day. Livers were collected for histology and immunohistochemistry. Hepatocyte proliferation and apoptosis were quantified using computer-assisted image analysis following immunohistochemical staining for PCNA and activated caspase 3. As previously reported, treatment with the three non-genotoxic carcinogens (methapyrilene, phenobarbital and clofibrate) caused a dose-dependent increase in hepatocellular proliferation at all dose levels, but no changes in the apoptosis labeling index. In contrast, 4-AAF, a non-genotoxic and non-carcinogenic compound, only caused a minimal increase in hepatocyte proliferation at the high dose only. 2-AAF, a potent genotoxic carcinogen, induced apoptosis at the low- and mid-dose, but decreased cell proliferation at all doses. Tamoxifen, a carcinogenic agent potentially by hormonal mechanisms, did not affect apoptosis, but slightly decreased hepatocyte proliferation. Collectively, these results correlate with previously reported data and indicate that our method to quantify hepatocyte proliferation and apoptosis is suitable for the quantification of changes in cellular kinetics after a short-term treatment with hepatotoxicants.

1752 ACUTE ETHANOL (ETOH) EXPOSURE *IN VIVO* POTENTIATES ACETAMINOPHEN (AAP)-INDUCED HEPATOCELLULAR APOPTOSIS BY MODULATING OXIDATIVE STRESS AND EXPRESSION OF BCL-XL AND P53 GENES IN THE LIVER.

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As modes of cell death induced by AAP have been undergoing intense debate in recent years, the effect of EtOH on AAP-induced liver injury has been the focus of investigation for many decades. Although EtOH-potentiated AAP hepatotoxicity has been primarily attributed to bioactivation by CYP450-IIE1, this study, for the first time, examined whether majority of the hepatocellular biochemical events are surrendered to apoptotic pathways, and if so, whether potentiation is due to accelerated induction of apoptotic death. Since, oxidative stress, genomic DNA fragmentation, and select expression of bcl-xl and p53 are considered instrumental to apoptotic process, these parameters were analyzed in the mouse liver. Male ICR mice (3 months old) were orally administered two consecutive doses of ethanol on Day-1 and Day-2 (7.6 ml/kg; 10% solution), followed by AAP on day-4 (300 and 400 mg/kg; ip). Animals were sacrificed after 18-hrs of AAP exposure. Serum ALT, and liver histopathology were considered key markers for the potentiation reaction. Lipid peroxidation, DNA fragmentaion (quantitative and qualitative) and expression of bcl-XL and P53 genes in the livers were compared in various groups to assess the extent of apoptosis. Data show that EtOH significantly potentiated AAP-induced liver injury coupled with massive genomic DNA fragmentation and oxidative stress (malondialdehyde formation). Expression of bcl-XL was considerably reduced (compared to AAP alone), and the p53 level was relatively higher in EtOH+AAP group (compared to AAP alone). No major changes were noted in the control and EtOH-alone groups. Histopathological evaluation of PAS-stained liver sections revealed, EtOH significantly potentiated AAP-induced apoptotic death in addition to necrotic death of hepatocytes. However, it is unclear whether both apoptogens acted synergistically, or orchestrated the suicidal process independently. [supported by Division of Pharmacology/Tox]

1753 DIFFERENCES IN ACUTE TOXICITY SYNDROMES OF TCDD AND HEXACHLORODIBENZO-*p*-DIOXIN IN RATS.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent congener of polychlorinated dibenzo-*p*-dioxins. The potency of 1, 2, 3, 4, 7, 8-hexachlorodibenzo-*p*-dioxin (HxCDD) is only 10 % of that of TCDD for typical Ah receptor (AhR) -mediated effects. Han/Wistar (Kuopio), (H/W) rats are exceptionally resistant to TCDD and the resistance is related to the mutated AhR. However, in terms of acute lethality, HxCDD is more potent than TCDD in H/W rats. Acute lethality and macroscopical effects of HxCDD and TCDD were compared in male rats of strain H/W, line A, and line B. H/W and line A rats are very resistant to TCDD due to an altered AhR, while line B rats are moderately resistant. The rats received 10000 and 2000 μg/kg of either TCDD or HxCDD i.g. Additionally line B rats received 1000, 600 or 200 μg/kg of TCDD or HxCDD. Body weight loss and mortality were monitored for 42 or 46 days. In H/W and line A rats the estimated LD50 values were 1500-12000 μg/kg and >10000 μg/kg for HxCDD and TCDD, respectively; for line B rats they were 1200-2000 μg/kg and 200-600 μg/kg, respectively. Necropsies revealed a severe liver toxicity syndrome. All rats that died from HxCDD had an exceptionally pale liver. TCDD caused a similar pale liver syndrome only in line B rats and only at the highest dose. In addition, HxCDD reduced body weight more rapidly than TCDD and caused diarrhea. The mean time to death was only 14 days for HxCDD compared with 33 days for TCDD. Interestingly, 10000 μg/kg HxCDD caused this syndrome in TCDD-resistant H/W and in line A rats, while the same dose of TCDD did not. The differences in toxic effects between HxCDD and TCDD suggest that the molecular mechanisms by which HxCDD causes lethality are different from those of TCDD. (Supported by the Academy of Finland, Project 42551, and the European Commission, Contract QLK4-CT-1999-01446.)

1754 DIFFERENTIAL REVERSIBILITY OF CYP1A1 INDUCTION IN TCDD VERSUS HxCDD TREATED RATS.

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2, 3, 7, 8–tetrachlorodibenzo–*p*–dioxin (TCDD) and 1, 2, 3, 4, 7, 8–hexachlorodibenzo–*p*–dioxin (HxCDD) were chosen for this study because of their differential half lives (20 and 60 days, respectively). An aim of this study was to elucidate the contribution of pharmacokinetics vs. pharmacodynamics in the recovery of CYP1A1 in TCDD vs. HxCDD treated animals. Female Sprague-Dawley rats were administered a loading dose of either TCDD or HxCDD in corn oil *via* oral gavage (n=5); controls were dosed with only vehicle. For the next 64 days, animals were dosed with maintenance doses of TCDD or HxCDD every third or ninth day, respectively, to maintain pharmacokinetic steady state. Iso–effective doses of TCDD/HxCDD (0.0125/0.3125 and 0.05/1.25 μg/kg) were selected based on their toxic equivalence. Following the 64 day dosing period, rats were allowed to recover. Rats were terminated on recovery days 2, 4, 8, 16, 32, 64, 128, and 256 after the final day of dosing (day 64). Livers were removed, weighed and snap frozen. The relative liver weight for each treatment remained constant throughout the recovery period. Hepatic EROD activity, measured fluorometrically, decreased in a dose– and time–dependent manner during the recovery period. The EROD activity of the TCDD treated rats declined more than that of the HxCDD treated rats during the recovery period. Liver samples were also analyzed for CYP1A1 and CYP1A2 mRNA and CYP1A1 protein expression by the branched DNA (bDNA) signal amplification assay and Western blotting, respectively. Semiquantitation of Western blots were obtained using GelPro image analysis. Both CYP1A1 mRNA and protein decreased in a dose– and time–dependent manner. In conclusion, these data demonstrate a differential reversibility of EROD activity, CYP1A1 mRNA and protein in TCDD vs. HxCDD treated rats. Moreover, there is a lack of complete reversibility of EROD induction in TCDD treated rats even after the elapse of almost 7 half–lives.

1755 THE EFFECT OF TCDD AND LEPTIN ON mRNA EXPRESSION OF NEUROPEPTIDES REGULATING FOOD INTAKE IN HYPOTHALAMUS.

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A complex and only partially known CNS network controls food intake and body weight. This control is based on integration of sensory input from external environment and from internal energetic state using leptin as the major peripheral adiposity signal. The main integrative centre of appetite control is hypothalamus with a large array of neurotransmitters involved. Wasting syndrome precedes lethality in 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD)-treated laboratory animals and seems to be caused by a derangement of a fundamental mechanism controlling body weight. Here, we examined the effects of TCDD and/or leptin on mRNA expression levels of hypothalamic neuropeptides and their receptors by real-time quantitative RT-PCR in two rat strains. The dose of TCDD employed (50 μg/kg ig.) is lethal to one of the strains but nonlethal to the other. The rats were killed by decapitation 6, 24 or 96h after TCDD exposure and were further treated with leptin (1.3 mg/kg ip.) or saline 2h before termination. Total RNA was isolated from the hypothalami of the rats. Every neuropeptide mRNA fragment measured (over 20) was cloned, sequenced and used as an external standard in real-time RT-PCR (LightCycler). The concentrations were normalized to beta-actin and the results evaluated by two-way ANOVAs. TCDD induced a drastic increase in hypothalamic expression of the positive control mRNA, CYP1A1, but the changes in neuropeptides and their receptors were smaller and dependent on time and strain. At the first time-point (6h), TCDD potentiated the effects of leptin on CART, insulin receptor and CRF receptor in the TCDD-sensitive rat strain alone. At later time points, TCDD elicited a different pattern of responses in the two strains. These findings suggest involvement of hypothalamic control centres in the wasting syndrome.

1756 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)-INDUCED ACCUMULATION OF BILIVERDIN AND HEPATIC PELIOSIS IN RATS.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread, persistent and highly toxic environmental pollutant. The most TCDD-sensitive and the most TCDD-resistant rat strains [Long-Evans (*Turku/AB*) and Han/Wistar (*Kuopio*), respectively] were cross-bred to separate the alleles of two genes (*Ahr* and an unidentified gene "*B*") mediating resistance against TCDD toxicity. During cross-breeding, a new type of toxicity in livers of both sexes was detected characterized macroscopically by intense dark green to black color and swelling that appeared most frequently after a large dose (300 μg/kg or more as a single i.g. dose) and a follow-up period of more than three weeks. Therefore, studies were undertaken to identify the causative pigment chemically and to examine the hepatotoxicity histologically. The pigment fractions were separated by thin layer chromatography and

then analyzed by HPLC and electrospray mass spectrometry. The pigment was found out to consist of biliverdin and several biliverdin-related compounds. In liver histopathology carried out on male rats, progressive sinusoidal distension and hepatic peliosis with membrane-bound cysts were seen. The clinical manifestations of pigment accumulation were recorded most often in intermediately resistant rat lines such as line B (homozygous for the gene *B*), but never occurred in rats expressing only the Han/Wistar (*Kuopio*)-type Ah receptor with an altered transactivation domain structure. (Supported by the Academy of Finland, the Finnish Research Program on Environmental Health, Project 42551 and the European Commission, Contracts ENV4-CT96-0336 and QLK4-1999-01446).

1757 CHARACTERIZATION OF BRONCHIAL METAPLASIA IN RATS EXPOSED TO 3, 3, 4, 4, 5-PENTACHLOROBIPHENYL (PCB 126).

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The National Toxicology Program has conducted a series of two-year rat bioassays on dioxin-like compounds. One of the effects observed after exposure of Harlan Sprague-Dawley female rats with PCB 126, by gavage, was a dose-related increase in bronchiolar metaplasia. This lesion was found at the junction of terminal bronchioles and alveolar ducts throughout the lung. Alveolar epithelium in this location was replaced by cuboidal to columnar ciliated cells. Scattered among these were cells that lacked cilia and had a smooth apical surface that protruded into the alveolar space. To help elucidate the character of this lesion, staining was done with Periodic Acid Schiff (PAS), Alcian blue (AB) and immunohistochemistry [glutathione S-transferase Pi (GSTPi)]. Both PAS and AB staining were more positive in areas of bronchiolar metaplasia than alveolar hyperplasia (+1.7 of 4 vs. +0.8 for PAS, +2 of 4 vs. +1 for AB), indicating the presence of more mucus with bronchiolar metaplasia. Lung slides from 17 control and 17 treated rats were stained with GSTPi. Normal lung tissue exhibited intense positivity in the bronchiolar epithelium, with Clara cells staining the most intensely (+2.6 of 4). Diffuse, light staining (+1.1) occurred in alveolar epithelial type I cells. Foci of chronic inflammation with alveolar epithelial type II hyperplasia were positive in 5 of the 8 animals with this lesion, with an average score of +1.3. Areas of bronchiolar metaplasia stained with greater intensity than foci of inflammation and alveolar epithelial type II hyperplasia (average score +1.8). These areas also contained cells consistent with Clara cells that stained more intensely than the remainder of the cells. These results provide evidence that this lesion is actually metaplasia (considered an adaptive response), from typical alveolar to bronchiolar epithelium and should not be confused with alveolar epithelial hyperplasia.

1758 EXPOSURE TO TCDD RENDERS VIRUS-SPECIFIC CD8+ T CELLS UNRESPONSIVE DURING PRIMARY INFECTION AND DELAYS THEIR EXPANSION FOLLOWING REINFECTION.

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Numerous studies implicate a role for impaired T cell function in the immune suppression resulting from exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). To elucidate mechanisms by which TCDD drives T cell dysfunction, we use a murine model of infection with human influenza A virus. Cell-mediated immunity to influenza virus depends on the activation, differentiation and proliferation of CD8+ T cells into cytotoxic T lymphocytes (CTL). Exposure to TCDD suppresses the proliferation of CD8+ T cells, reduces their production of IFNg, and impairs the generation of virus-specific CTL. We atribute this to impaired activation of CD8+ T cells. To examine this further, we tested the hypothesis that treatment with TCDD induces anergy, characterized by the inability of antigen-specific T cells to respond under optimal conditions. Mice were gavaged with TCDD (10 ug/kg) or vehicle control one day prior to intranasal infection with a sublethal dose of influenza virus. Following infection, mediastinal lymph node (MLN) cells were restimulated *in vitro* with an immunodominant influenza virus peptide and IL-2. Stimulation of CD8+ cells from vehicle-treated mice induced proliferation and IFNg production. However, optimal stimulation conditions did not fully restore these responses in CD8+ cells from TCDD-treated mice, suggesting they are anergic. To study this unresponsive state *in vivo*, we tested the ability of virus-specific CD8+ T cells to respond to a second encounter with antigen. Eight weeks after primary infection the number of residual virus-specific memory cells was diminished 50% in the TCDD-treated mice. In response to re-infection, the expansion of virus-specific CD8+ T cells was delayed in mice treated with TCDD, such that 70% fewer cells were detected in the MLN three days after secondary challenge. These findings indicate that a single dose of TCDD induces anergy in CD8+ T cells during a primary response, diminishes the size of the memory T cell pool and delays the recall response.

1759 THYMOCYTE ALTERATIONS IN CD2-DRIVEN CONSTITUTIVELY ACTIVE ARYLHYDROCARBON RECEPTOR (AHR) TRANSGENIC MICE.

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Arylhydrocarbon receptor (AhR) is the transcription factor that mediates the toxic effects of dioxin. Upon binding dioxin, AhR in the cytoplasm is activated and translocated to the nucleus, where it heterodimerizes with another transcription factor, arylhydrocarbon receptor nuclear translocator (ARNT). The AhR/ARNT complex binds to the specific DNA sequence, the xenobiotic responsive element (XRE), and alters the expressions of various genes. These AhR-dependent alterations in gene expressions are thought to be involved in the majority of adverse effects, including the immunotoxicity of dioxin. In order to clarify the cellular targets and mechanisms of dioxin-induced immunotoxicity, we generated transgenic (Tg) mice expressing constitutively active (CA) mutants of AhR in T cells using a CD2 promoter. We used a CA-AhR mutant having a deletion in the PAS B domain, which constitutively activated AhR/ARNT-dependent transcription, independently of ligands. Expression of CA-AhR mRNA was detected in the thymus and spleen of Tg mice. Furthermore, expression of CYP1A1, one of the genes activated by the AhR/ARNT heterodimer, was detected in the thymus and spleen without exposure to ligands. In these Tg mice, the weight of and total cell number in the thymus were decreased. In addition, the ratio of CD8 single positive/CD4 single positive cells in the thymus was elevated when compared with wild-type mice, just as observed in dioxin-treated wild-type mice. These results demonstrate that the changes in thymocyte population induced by dioxin are solely dependent on activation of AhR in T cells

1760 INDUCTION OF OXIDATIVE STRESS IN THE REPRODUCTIVE SYSTEM OF RATS AFTER SUBCHRONIC EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

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2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant. TCDD is formed as a by-product in the manufacture of chlorinated hydrocarbons and in the emissions from steel foundries and motor vehicles. TCDD is classified as a classic reproductive toxicant. It causes oxidative stress in a variety of animal tissues. The ability of TCDD to induce reproductive toxicity in both males and females after exposure during pregnancy, lactation or before puberty has been reported. However, its effect on mature animals is not clear. This study was conducted to assess the health risks associated with exposure to TCDD on testicular function of mature male rats and to assess *in vivo* its effect on the oxidative enzymes in mitochondrial and microsomal fractions of testis. Mature male Sprague-Dawley rats were orally administered 50, 100 or 200 ng/kg/day TCDD for 60 days. TCDD has no effect on the body weight and food intake during the course of the study. TCDD significantly reduced the relative weight of testis, epididymis, seminal vesicles and prostate. The number of the epididymal sperm count and the percentage of sperm motility were decreased. There was a significant increase in both percentages of sperm mortality and abnormality associated with testicular histopathological alterations. TCDD induced oxidative stress in both mitochondrial and microsomal fractions of testis. There was a significant decrease in the activities of superoxide dismutase (SOD), glutathione perioxidase (GPX) and catalase (CAT) and a significant increase in the level of thiobarbituric acid reactive substances (TBARS) as a measurement of lipid peroxidation. These changes were more prominent in microsomal than mitochondrial fraction. This study may suggest that TCDD caused exhaustion of antioxidant defense system in rat testis inducing an oxidative stress, which could explain the disruption of the testicular functions.

1761 BONE STATUS AND ALL-TRANS-RETINOIC ACID (ATRA) HOMEOSTASIS IN MICE LACKING CELLULAR RETINOL-BINDING PROTEIN I (CRBPI-KO) BEFORE AND AFTER CHEMICAL INSULT BY TCDD.

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Dioxins are known to affect retinoid homeostasis, but the toxicological consequences are unknown. To clarify the link between vitamin A homeostasis and dioxin toxicity, we studied bone status (pQCT and three-point-bending tests of

tibia and femur), hepatic retinoid levels (HPLC of total retinyl ester, all-trans-retinol, and atRA levels), and CYP1A1/2, 1A2, and 2B1/2 activities (E/P/MROD assay) in adult male CRBPI-KO mice before and after a single dose of TCDD. Wildtype (WT) mice of the same strain background were used as controls. We saw no differences in bodyweight or bone length between untreated CRBPI-KO and WT mice. However, bone mineral density (BMD) and strength were lower in CRBPI-KO mice. After TCDD exposure, CRBPI-KO bones were unaffected, whereas WT bones displayed reduced BMD and strength. Retinyl esters in CRBPI-KO livers were significantly lower than WT at all times and doses. After TCDD exposure CRBPI-KO mice lost nearly all their retinyl ester stores, whereas WT mice were almost unaffected. TCDD-treated CRBPI-KO livers displayed reduced atRA levels, whereas atRA levels in WT were unchanged. Baseline CYP1A activities were lower in CRBPI-KO than in WT but after TCDD exposure, CYP1A was twice as much induced in the CRBPI-KO than in WT. This study clearly shows that a binding protein specific for the vitamin A system plays a role not only for proper retinoid storage as earlier shown but also for bone status and for the homeostasis of the hormone RA. The effects of TCDD on bone could however not be associated with an altered RA homeostasis. Nonetheless, the absence of CRBP I was indeed protective against the bone-disrupting effects of TCDD and the role of this protein in bone homeostasis must therefore be further explored.

1762 REGULATION OF CYCLIN D1 GENE EXPRESSION IN THE MOUSE UTERUS BY ESTROGEN AND 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN.

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Induction of uterine cyclin D1 mRNA levels was investigated in 25 day-old B6C3F1 mice treated with 200 ng of 17β-estradiol (E2) for 1, 3, 6, and 12 h followed by *in situ* hybridization of uterine sections. Increased cyclin D1 mRNA staining was observed in the luminal epithelium 6 and 12 h after treatment, whereas background cyclin D1 mRNA staining in stromal cells was observed in solvent (corn oil)-treated animals and was not increased after 6 and 12 h treatment with E2. Comparable hormone-responsiveness was observed in wild-type estrogen receptor α (ERα) knockout (αERKO)+/+ and aryl hydrocarbon receptor knockout (AhRKO) mice, whereas responsiveness was not observed in homozygous αERKO-/- mice, confirming the role of ERα in E2-induced cyclin D1 expression in the uterus. Moreover, since previous studies indicate that induction of uterine cyclin D1 mRNA is not affected by cycloheximide, our results suggest that induction of this gene in luminal epithelial cells is a direct effect of E2 and not related to induced stromal factors. Inhibition of E2-responsiveness in the uterus by the AhR agonist 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is well established. TCDD alone does not affect stromal or epithelial expression of cyclin D1; however, in B6C3F1 mice cotreated with E2+TCDD, there was a decrease in E2-induced uterine cyclin D1 mRNA expression in the luminal epithelial cells. This inhibitory TCDD-ERα crosstalk on cyclin D1 mRNA was not observed in AhRKO mice confirming the role of the AhR in mediating this response which was similar to inhibitory AhR-ER crosstalk observed in breast cancer cells and rodent mammary tumors. (Supported by NIH ES09106 and ES04176)

1763 HEPATIC RETINOID LEVELS IN A TCDD-SENSITIVE (LONG-EVANS) AND TCDD-RESISTANT (HAN/WISTAR) RAT STRAIN FOLLOWING LONG-TERM LOW-DOSE TCDD EXPOSURE.

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental contaminant that alters retinoid homeostasis by mechanisms believed to be mediated by the aryl hydrocarbon receptor (AhR). In this study we investigated whether the noted structural differences between the AhRs of Long-Evans (L-E) and Han/Wistar (H/W) rat strains influenced polar and apolar retinoid homeostasis following long-term TCDD treatment. Female L-E and H/W rats were given TCDD by s.c. injection once per week at calculated daily doses of 0, 1, 10, 100, or 1000 (H/W only) ng/kg bw/day for 20 weeks. Total hepatic retinoid levels were dose-dependently decreased in both strains. However, effects were seen at 1 ng/kg bw/day in the L-E strain whereas significant effects were observed at 10 ng/kg bw/day in the H/W strain. BMD05 levels, defined as a 5% change from mean control values, were approximately 140 and 1400 pg/kg bw/day for L-E and H/W rats respectively. Preliminary analyses of hepatic polar retinoid levels showed no change in retinoic acid levels of either strain. On the other hand, a recently discovered retinoic acid metabolite, 9-cis-4-oxo-13, 14-dihydro-retinoic acid was all but eliminated in the liver of both strains at the low-dose. Thus the data suggest that long-term low-dose TCDD exposure markedly increases hepatic retinoid turnover without significant effects on hepatic retinoic acid levels. Moreover, the 10-fold difference between the L-E and H/W strain provide further support for a role of the AhR in TCDD altered retinoid homeostasis.

1764 USE OF CYP1A2 (-/-) KNOCKOUT AND CYP1A2 (+/+) C57BL/6N PARENTAL STRAINS OF MICE TO COMPARE METABOLISM OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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The most toxic dioxin congener, TCDD, induces hepatic cytochrome CYP1A2 to which it subsequently binds, resulting in whole body half lives of 5-11 years in humans and 30 days in rats. Metabolism of TCDD is very limited in both species. Whether TCDD is a poor substrate for metabolizing enzymes or is unavailable for metabolism due to its strong affinity to CYP1A2 has not been firmly established. Thus, we tested the hypothesis that sequestration of TCDD by CYP1A2 makes TCDD unavailable for metabolism that would readily occur in the absence of CYP1A2. Male C57BL/6N mice which possess or lack the CYP1A2 gene were given a single oral dose of 156 ug[14C]TCDD/kg. After 4 days, the mice (housed in metabolism cages with separate collection of urine and feces every 24h) were killed and tissues collected. Tissue deposition and overall metabolism in urine and feces were quantitated. Similar to previously reported studies, liver:fat ratio of the two groups was vastly different, i.e. 4.09 (C57BL/6N) vs. 0.57 (knockout, KO). Slightly higher levels of 14C-derived TCDD were excreted in urine and feces of the parental strain at each time point when compared to KO mice. The overall level of metabolism of TCDD was determined as sum of 14C in 0-96h urine, non-extractable feces, and metabolites in extractable feces. The parental strain of mice had greater overall metabolism than the KO mice, i.e. 11.1% vs. 6.5% of the dose, respectively. The lower overall metabolism in the KO than the parental strain of mice is probably due to low hepatic retention and rapid redistribution of TCDD into lipophilic tissues for storage, which made the TCDD unavailable to hepatic metabolizing enzymes. In conclusion, the data presented in this study contradicts the original hypothesis and confirms that TCDD has an inherently slow metabolism in mammals, perhaps *via* the inducible CYP1A1, 1A2, and 1B1 isozymes and/or non-P450 dependant mechanisms. (This abstract does not reflect USEPA and USDA policies.)

1765 A COMPARISON OF THE METABOLISM OF METHOXYRESORUFIN, ACETANILIDE AND CAFFIENE IN RAT AND HUMAN CYP1A2 SUPERSOMES AND THEIR INHIBITION BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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CYP1A2 is highly expressed in both rats and humans. TCDD and related chemicals induce and bind to these proteins. The binding of TCDD and related chemicals to CYP1A2 leads to their sequestration in hepatic tissue. The present study compares the metabolism of prototype CYP1A2 substrates in rat and human CYP1A2 + P450reductase SUPERSOMES (GenTest Corporation, Woburn, MA) and the ability of TCDD to inhibit these reactions. For the O-demethylation of methoxyresorufin, the K_M was similar between human and rat CYP1A2, 0.10 and 0.09 nM respectively. Rat supersomes had a slightly higher V_{max} than human supersomes, 3.5 and 2.4 pmol/min/mg CYP1A2. TCDD inhibited methoxyresorufin metabolism with $K_I$values of 0.3 and 0.06 μM in the human and rat supersomes respectively. Caffeine was metabolized by both human and rat CYP1A2 supersomes. The estimated V_{max} was higher in the human compared to the rat supersomes, 5.0 and 2.2 nmol/min/mg CYP1A2, respectively. The Km was also higher in the human (6.5 mM) compared to the rat (0.9 mM) supersomes. The metabolism of acetanilide to 4-hydroxy acetanilide was similar in the human and rat supersomes. The Vmax was 2.8 and 5.9 nmol/min/mg protein, in human and rat supersomes, respectively. The K_M was 19.1 mM in human supersomes and 74.5 mM in rat supersomes. Initial studies indicate that TCDD inhibits both caffeine and acetanilide metabolism in rat and human supersomes. These data demonstrate that the *in vitro* metabolism of prototype substrates is similar between the rat and human CYP1A2 supersome preparations and that TCDD inhibits the metabolism of these substrates by both rat and human CYP1A2. Because of the potential for inhibition of CYP1A2 activity by TCDD, studies examining CYP1A2 induction in TCDD exposed populations using these substrates should be viewed cautiously. (This abstract does not reflect EPA policy)

1766 INFLUENCE OF DIABETES, OBESITY AND 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)EXPOSURE ON THE EXPRESSION OF HEPATIC CYP1A2 IN A MURINE MODEL OF TYPE II DIABETES.

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Recent epidemiology studies indicate an association between TCDD exposure and an increased incidence of type II diabetes. One criticism of these results is that diabetes and/or body fat composition may influence the disposition and/or elimination of TCDD. In the present study the influence of body fat composition and type II diabetes was examined on the expression of CYP1A2, a major determinant of the disposition of TCDD. In this study a high fat, high simple carbohydrate (HFHSC) diet was used to develop murine models of type II diabetes and obesity. After exposure to the HFHSC diet for 13 weeks, C57BL/6J mice develop obesity, hyperinsulinemia and hyperglucosenemia, consistent with type II diabetes. Following 13 weeks on the HFHSC diet AJ mice develop greater body fat mass without developing hyperinsulinemia or hyperglucosenemia. These models have allowed us to compare the influence of diabetes, body fat composition and TCDD exposure on the expression of CYP 1A2. Following 13 weeks on either a normal or HFHSC diet animals were exposed to 0, 0.1 or 5 ug/kg of 3H-TCDD by oral gavage and killed 1, 30 and 40 days later. Hepatic CYP1A2 was determined using western blot analysis. Initial results indicate that on day 1, animals on the normal diet have a greater fold induction of CYP1A2 than those on the HFHSC diet. This effect is more pronounced at the higher dose. For example in C57 mice on the normal diet, CYP1A2 is induced 3 and 43 fold by 0.1 and 5 ug/kg TCDD while in C57 mice on the HFHSC diet CYP1A2 is induced 2 and 13 fold at these same doses. On days 30 and 40, fold induction of CYP1A2 by 5 ug/kg TCDD is approximately 7-10 fold greater in the animals on the HFHSC diet. These data suggest that diet can influence the response to TCDD. This influence may be related to alterations in body fat composition. (This abstract does not represent USEPA policy. SJG supported by NIEHS training grant T32 ES07126)

1767 INFLUENCE OF TYPE II DIABETES AND OBESITY ON THE DISPOSTION AND ELIMINATION OF TCDD IN MICE.

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One possible explanation for the relationship between TCDD exposure and diabetes in some epidemiological studies may be that diabetics have slower elimination of TCDD compared to non-diabetics. The present study examines the effects of type II diabetes and body fat mass on the elimination of TCDD in mice. A high fat, high simple carbohydrate (HFHSC) diet was used to develop murine models of type II diabetes and obesity. After exposure to the HFHSC diet for 13 weeks, C57BL/6J mice develop obesity with signs of type II diabetes, while AJ mice develop greater body fat mass without alterations in serum glucose or insulin. Thus we can compare the effects of diabetes and body fat mass on the elimination of TCDD. After 13 weeks on either a normal or HFHSC diet male mice were exposed (po) to 5 ug 3H-TCDD/kg. Mice were killed 1, 3, 10, 20, 30, 40 and 60 days later and concentrations of TCDD were determined in blood, liver and fat. The elimination half-life of TCDD from the blood was similar in the C57 and AJ mice on the normal diet and the AJ mice on the HFHSC diet, 15.9, 17.9 and 14.9 days, respectively. C57 mice on the HFHSC diet had a blood half-life of 36.5 days. The HFHSC diet increased the liver half-life of TCDD from 9.8 to 23.8 days in the C57 mice, but did not significantly affect the hepatic elimination in the AJ mice. The half-life of TCDD from adipose tissue was slightly elevated by the HFHSC diet in C57 mice, 16.8 vs. 23.8 days, in the normal vs. the HFHSC diet. The HFHSC diet had no effect on the half-life of TCDD from adipose tissue in the AJ mice. These data suggest that type II diabetes may influence the half-life to TCDD and that this is independent of body fat composition. This work was funded in part by an Interagency Agreement with the USAir Force # FQ7624-00-YA085) and a cooperative agreement (CR 828790) with NRC. (This abstract does not represent USEPA policy).

1768 COMPARING ENVIRONMENTALLY RELEVANT PCBs TO TCDD.

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Environmental exposures to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) always occur as part of a complex mixture. In order to assess the potential risk associated with these exposures, the Toxic Equivalency Factor (TEF) method was developed, and uses toxic equivalents (TEQ) to relate a chemical to TCDD. While this method adequately accounts for the effects of mixtures of TCDD or dioxin-like (DL) chemicals, there are almost always non-dioxin-like (NDL) chemicals present, in particular, the NDL polychlorinated biphenyls (PCBs). To examine the interactions of TCDD with NDL PCBs, the present study compared the effects on multiple responses of different laboratory-defined mixtures, based on mass ratios found in food, of TCDD and NDL PCBs in both wildtype C57BL/6J (WT) and CYP1A2 null (KO) male and female mice. These chemical groups are: TCDD alone; DL Mix A; NDL Mix B; and Mix C - a combination of Mixes A and B. No effects were seen in body weight or other tissues, in male or female WT or KO mice. Although we expected an increase in liver weights in Mix B and Mix C, liver weight was increased only in Mix C in both male and female, WT and KO. No effects were observed in levels of glutathione or uric acid, markers of oxidative stress. Both male WT and KO showed greater decreases in levels of total triiodothyronine (TT3) while Mix A and Mix C resulted in a 50% reduction in levels of total thyroxine in all groups. With exposure to TCDD and DL mixes, but not to NDL PCBs, there is an increase in ascorbate levels. Based on these findings, there does not appear to be a strain difference in response to chemical exposure, indicating CYP1A2 is not necessary for these responses. There does appear to be a gender difference, reflecting a possible endogenous estrogenic component to maintenance of levels of TT3 and ascorbic acid cycling. Based on these findings, it appears that these mixtures have a synergistic effect greater than the sum of the individual congeners, or TCDD alone. (This abstract does not reflect EPA policy. DB supported by EPA CT902908 and NIEHS T32-ES07126)

1769 EVALUATION OF PCB EXPOSURE ROUTES IN ANNISTON, ALABAMA: ASSESSMENTS OF FISH CONSUMPTION USING PBPK MODELING OF PCB CONGENERS IN LOCALLY-CAUGHT FISH.

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The forty-year history of polychlorinated biphenyls (PCB) production in Anniston, Alabama was associated with releases of PCBs to the local watershed. Although PCB contamination in the Anniston area is well documented, the PCB exposure pathway(s) are not well characterized. This investigation examined a likely PCB exposure pathway: the consumption of PCB contaminated fish taken from a local waterway. To evaluate PCB exposures, physiologically based pharmacokinetic (PBPK) models were used in conjunction with knowledge of fish consumption behavior and serum PCB levels found in Anniston residents. The model parameters included physiological and biochemical factors, parameters affecting exposure duration, the distribution of PCB congeners in specific tissues, the PCB congener patterns from local fish, and serum PCB congener patterns from area residents who participated in an exposure investigation. PCB congener analysis of locally-caught fish showed the Anniston-area fish contain appreciable levels of higher chlorinated PCB congeners. Simulations from the model provided estimates of levels of PCBs in blood under exposure scenarios based on fish consumption rates that vary between demographic groups. The modeled estimates indicate that, for demographic groups that consume large amounts of local fish, the fish-eating pathway could contribute significant amounts of higher chlorinated PCBs to an individual's body burden.

1770 ACUTE EFFECTS OF *ortho*-PCB CONGENERS ON THE HYPOTHALAMO-PITUITARY-THYROID AXIS.

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ortho-Substituted polychlorinated biphenyl (PCB) congeners represent a large proportion of total PCBs in the environment and produce, resulting in episodes of exposure. Information is scanty regarding the effects of these PCBs on the hypothalamo-pituitary-thyroid (HPT) axis in developing animals. Acute effects of ortho-PCB congeners, 95 (2, 3, 6-2', 5'), 101 (2, 4, 5, -2', 5'), 110 (2, 3, 6-3', 4'), 149 (2, 3, 6-2', 4', 5'), and PCB153 (2, 4, 5-2', 4', 5') were investigated on the HPT-axis. Weanling female Sprague-Dawley rats were given a single ip dose of chosen PCB congeners at 16 or 32 mg/kg for 2 days. Selected endpoints were examined 48 hr after the last dose. Serum thyroxine (T4) levels decreased in PCB 95, 101, or 149 treated rats as compared to the respective controls. No changes were seen in serum triiodothyronine (T3), thyroid stimulating hormone (TSH), and prolactin (PRL) levels. PCB 110 exposure did not reduce serum T4, but increased serum T3 levels. PCB 153 exposure elevated serum T4 levels, but serum T3, TSH, or PRL levels did not change. Hypothalamic dopamine (DA) levels decreased following PCB 95, 101, 149, or 153 treatment, but did not change after PCB 110 exposure. The thyrotropin releasing hormone (TRH) test was administered to investigate the functionality of HPT-axis following exposure to PCB 95 or 101 at 32 mg/kg (as described above). Synthetic TRH was administered to weanling rats ip 30 min or 2 hr before decapitation. Serum T4 levels decreased following PCB 95

treatment at both times, but only at 2 hr after PCB 101 exposure as compared to control. Serum TSH levels were lower than control in PCB 95 treated rats, but did not change after PCB 101 exposure. No changes were seen in serum PRL and hypothalamic DA levels after exposure to TRH, or PCBs 95 or 101. These results indicate that acute exposure to ortho-PCB congeners cause changes in the HPT-axis, probably through multiple mechanisms. A less than maximal response of the pituitary and thyroid to TRH suggests an inhibition of T4, TSH, and PRL release.

1771 LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN TESTES OF RATS TREATED WITH 2, 2', 4, 4', 5, 5'-HEXACHLOROBIPHENYL (PCB-153) AND EFFECTS OF CORN OIL, MEDIUM-CHAIN TRIGLYCERIDE (MCT) OIL AND OLIVE OIL.

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The testes are vulnerable to injury by various chemicals, including polychlorinated biphenyls (PCBs). This damage may be mediated in part through oxidative stress. PCBs cause oxidative stress in liver and other organs. To study the effect of PCB-153 on oxidative stress and antioxidant enzymes in testes, and to account for the influence of different vehicles on these parameters, we measured thiobarbituric acid-reactive substances [TBARS] and the activities of antioxidant enzymes in rat testes after one i.p. injection of PCB-153 (300 umol/kg) dissolved in 3 different vehicles [corn oil, olive oil, and MCT oil; 1 ml/kg rat] or vehicle alone. Control animals received no injection. All animals were euthanized 2 days after treatment and testes subcellular fractions were prepared by differential centrifugation. Our preliminary results show that PCB-153 treatment in any vehicle produced an increase in TBARS level in testes cytosols and homogenates. The oils alone had no effect on TBARS compared to control. All PCB-treatment groups showed reduced catalase activity, but a reduction in activity was also seen in rats that received corn oil or olive oil alone. Similarly total glutathione peroxidase activity was lower in animals treated with corn oil, olive oil, or PCB-153 in olive oil. However, treatment with MCT oil, or PCB-153 in MCT or in corn oil had no effect. Glutathione reductase activity was at the control level in all vehicle treatment groups, but was increased in rats receiving PCB-153 in MCT oil and decreased in rats injected with PCB-153 in corn oil or olive oil. Glutathione S-transferase activity was not affected by any treatment. This study shows that PCB-153 may induce oxidative stress in testes, possibly in part through a reduction in antioxidant enzyme levels. It also shows, however, that the choice of vehicle may have an influence on enzyme activities in testes. (Supported by ES07380 and DAMD17-02-1-0241)

1772 COMPARATIVE STUDY OF PCB, PBB, AND PBDE MIXTURES ON SERUM PARAMETERS IN THE RAT.

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Mixtures of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) were or are currently used by industry for multiple applications. Unfortunately these mixtures are released into the environment and enter the food chain. They are found in breast milk samples and in the serum and tissues of humans and animals where they may cause health problems. PCBs have been studied extensively, but very little is known about the replacement compounds, the PBDEs. To compare the toxic potentials of these compounds, we treated male and female Sprague-Dawley rats (5 weeks old, about 150g) with weekly injections of equimolar amounts (0.765 mmol/kg rat/injection) of the PCB mixture Aroclor 1254, the PBB mixture Firemaster BP-6, the PBDE mixture DE-71, or vehicle alone (corn oil, 1 ml/100 g rat). One week after the second injection serum was collected. Results from the serum analysis show that male and female rats may have different sensitivities. For example, triglycerides were reduced by all three mixtures in male rats, whereas uric acid was reduced by all three mixtures in female rats. Differences were also found in the order of potency of the three mixtures. All three mixtures increased cholesterol and HDL levels in both geners with the order of potency being PBB > PCB >> PBDE, whereas the PCB mixture had the strongest effect on the levels of total triiodothyronine, total thyroxine and free thyroxine. In addition there was a difference in sensitivity to these mixtures between the sexes. In males the order of potency was PCB > PBB > PBDE, whereas in females the PBDE mixture caused a stronger reduction of all three thyroid hormone levels than the PBB mixture. These first results show that PCBs, PBBs, and PBDEs may cause adverse health effects as shown here for lipids and thyroid hormone levels, but that the degree in sensitivity towards the different mixtures varies from end point to end point and between the two genders. (Supported by ES07380 and grant # GOK20395 from the EPA)

1773 CONTAMINATION OF DIOXINS AND CO-PLANAR PCBS IN WHALE MEAT PRODUCTS FROM JAPANESE MARKET.

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Whale meat products in Japanese market were surveyed for contamination of dioxins and co-planar PCBs for human consumption. The TEQ levels (pg/g wet wt) in fresh meats from Antarctic and Northern Pacific minke whales ranged from 0.02 to 0.25 (mean, 0.07, n = 6) and from 0.16 to 0.34 (mean, 0.26, n = 6), respectively. Meat products of small cetaceans (pilot whales, Risso's and bottle-nosed dolphins) from Japanese coastal water were highly contaminated with dioxin-like PCBs at the range of 1.0 - 51 pg-TEQ/g wet wt (mean, 12.3, n = 13). The concentrations on lipid weight basis corresponded to 82 - 240 pg-TEQ/g, which were comparable to the levels in small cetacean blubbers. Relative contribution of dioxins and co-planar PCBs to the total toxic potencies accounted for 1-12% and 78-99%, respectively. The major contributors were 2, 3, 4, 7, 8-pentaCDF for dioxins, and 3, 4, 5, 3', 4'-pentaCB (#126), 2, 4, 5, 3', 4'-pentaCB (#118) and 2, 3, 4, 5, 3', 4'-hexaCB (#156) for co-planar PCBs. The TEQ levels and their composition in meats were species-dependent and variable according to the lipid contents. Human consumption of lipid-rich products from small cetaceans in the market would exceed the TDI (4 pg-TEQ/kg bw/day) set by Japanese authorities and lead to increase in body burden of the contaminants. (Supported by IFAW)

1774 INCREASED LEVEL AND ASYMMETRICAL LOCALIZATION OF SECRETED FRIZZLED-RELATED PROTEIN 2 (SFRP2) MRNA IN THE MURINE FETAL BRAIN PERINATALLY EXPOSED TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN.

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Exposure of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) to rodents is known to exert various biochemical alterations in central and peripheral nervous systems. To investigate possible changes in TCDD-dependent gene expression in the brain, we have first identified up- and down-regulated genes by using differential mRNA display techniques. Pregnant C57BL/6N mice were given an oral dose of 5 μg TCDD/kg bw on gestational day 12.5 (GD12.5) or an equivalent volume of corn oil as vehicle control. Fetal brains were collected on GD18.5, postnatal day 5 (PND5), and PND21. Two genes were found to be up-regulated by perinatal exposure of mice to TCDD, one was secreted frizzled-related protein 2 (sfrp2) that encode a Wnt modulator and the other was c-myc, a Wnt target gene. Next we utilized *in situ* hybridization and analyzed the expression profile of sfrp2 gene in the total brains collected on GD18.5. We found that sfrp2 mRNA was localized asymmetrically around the ventricular zone of the third ventricle in the brain from TCDD-exposed fetuses in contrast to the symmetrical localization in the brain from the vehicle-control fetuses. We suggest that perinatal exposure to TCDD may cause defects in development and function of the mouse brain *via* the Wnt signal transduction pathway.

1775 DIOXIN ALTERS DEVELOPMENT OF THE COMMON CARDINAL VEIN IN THE ZEBRAFISH EMBRYO.

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A role for the aryl hydrocarbon receptor (AHR) pathway in vascular development has been implicated by studies in AHR null mice. Certain fetal vascular structures that normally regress during development fail to regress in AHR null mice. Therefore, we hypothesized that dioxin alters vascular remodeling and maturation. We chose to use the developing common cardinal vein (CCV) in the zebrafish embryo as a model to investigate this hypothesis. The CCV emerges from the junction of the anterior and posterior cardinal veins, grows across the yolk eventually connecting to the heart, and is extensively remodeled during its development. Fli1-eGFP transgenic zebrafish*, in which the vascular endothelium expresses GFP, were used for this study. The development of the CCV was followed from 27-74 hours post fertilization (hpf) in control or dioxin (10 ng/ml) exposed embryos. A significant reduction in the area of the CCV was detected as early as 44 hpf. The CCV area was reduced to 63% of control at 62 hpf. The dioxin dose-response curve for this reduction in area was similar to those for pericardial edema and reduction in

body length (determined in the same fish). There was no significant effect of dioxin on the time at which maximal CCV area occurred. Thus dioxin reduces the total area of the CCV but does not alter the time at which the maximal size is reached. The dependence of this effect on AHR2 was investigated using a zebrafish AhR2 specific morpholino to knockdown expression of AhR2. No significant effect of the AHR2 morpholino was found on the dioxin induced reduction in area of the CCV. This suggests that this is not an AHR2 dependent process, because both pericardial and yolk sac edema were completely blocked by the AHR2 morpholino in the same fish. This effect of dioxin on the CCV occurs well before overt signs of dioxin developmental toxicity are manifested, suggesting that alteration of vascular development may play a role in dioxin developmental toxicity in zebrafish. (UW SeaGrant, NRSA) Provided by B. M. Weinstein and N. D. Lawson, NIH.

1776 MORPHOLINO KNOCKDOWN OF AHR2 IN THE ZEBRAFISH EMBRYO PROTECTS AGAINST TCDD DEVELOPMENTAL TOXICITY.

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TCDD (2, 3, 7, 8 tetrachlorodibenzo-p-dioxin) is an environmental contaminant known to cause developmental toxicity in fish, mammals, and birds. In mammals it has been demonstrated that TCDD causes its toxicity by activating the AHR signaling pathway. Zebrafish have been found to express two forms of the AHR, zfAHR1 and zfAHR2, with zfAHR1 being most closely related to the mammalian AHR. Interestingly, *in vitro* molecular evidence has suggested that zfAHR2 is the form involved in mediating TCDD toxicity in zebrafish, however this has not been demonstrated *in vivo*. We have used an antisense morpholino to knockdown expression of the zfAHR2 protein in order to test the hypothesis that zfAHR2 is in fact responsible for mediating the developmental toxicity of TCDD. Embryos were microinjected with a morpholino targeted against zfAHR2 or a control morpholino immediately after fertilization and then exposed to TCDD within 4 hours. Embryos were then observed for endpoints of TCDD developmental toxicity to determine which were dependent on zfAHR2 signaling. The presence of the zfAhR2 morpholino produced a significant decrease in the ability of TCDD to induce transcription of cyp1A mRNA through at least 96 hpf. The morpholino was also able to protect the embryos from TCDD induced pericardial and yolk sac edema, craniofacial malformations, and anemia. The presence of the morpholino was initially able to protect against TCDD induced reduction in peripheral blood flow. However, the embryos still develop reduced blood flow at later times likely due to the persistence of TCDD and the decreasing effectiveness of the morpholino. Similarly, the morpholino provided minimal protection against TCDD's block on swim bladder inflation which also occurs at a later time. No effect of the zfAHR2 morpholino could be seen on normal development. These results support previous *in vitro* studies and demonstrate that zfAHR2 is the receptor involved in mediating several endpoints of TCDD developmental toxicity in the zebrafish embryo. Supported by UW Sea Grant.

1777 INVOLVEMENT OF HEDGEHOG SIGNALING IN IMPAIRED JAW DEVELOPMENT BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN IN DEVELOPING ZEBRAFISH.

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Previously, we reported that 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) retarded lower jaw development with only a minor inhibitory effect on total body length (Teraoka et al., 2002). Jaw growth retardation was significant long before circulation failure in lower jaw primordia emerged, suggesting direct effects of TCDD on jaw primordia. In the present study, the mechanism of impaired jaw development was investigated with special reference to the involvement of hedgehog signaling. Lower jaw primordia expressed sonic hedgehog (shh), tiggy-winkle hedgehog (twhh) and their receptors (patched 1, 2), as well as their transcription factors (gli1, 2). Shh function defective mutant embryos (Sonic you) with relatively normal circulation around jaw primordia showed marked retardation of jaw growth. When cyclopamine, an inhibitor for hedgehog signaling, was applied from 48hpf, lower jaw growth was significantly inhibited. On the other hand, embryos treated with TCDD showed marked reduction of shh and twhh expression in lower jaw primordia at 48 and 54hpf, while both patched expressions were not affected at all. Furthermore, zebrafish aryl hydrocarbon receptor 2 (zfAHR2) mRNA was strongly expressed in lower jaw primordia at 48 hpf. Injection of morpholino antisense oligo against zfAHR2 (zfAHR2-MO) but not its negative homologue with 4 different nucleotides (4-Mis) in two cells stage embryos inhibited reduction of hedgehog expressions and these toxic effects induced by TCDD. These results suggest the involvement of hedgehog signaling in lower jaw growth retardation by TCDD in zebrafish embryos.

1778 MICROARRAY ANALYSIS FOR MOUSE FETUS GENES ALTERED BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) EXPOSURE ON GESTATIONAL DAY 13.

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A relatively low dose of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure during pregnancy has been reported to result in developmental defects in reproductive organs on male offspring, e.g., delay of prostate development. In the mouse, the magnitude of the effect is more severe on gestational day 13 (GD13)-exposure than on the later stage, suggesting that this stage fetus is more sensitive to TCDD (Lin, T.M., *et al.*, 2002). In the present study, we exposed pregnant C57BL/6J mice to TCDD (10 μg/kg, p.o.) or vehicle (oil) on GD13 or GD17 and collected fetuses 24-hours post-administration. Total RNAs from fetal whole body were extracted and 5 male fetuses from each group were combined and analyzed by Atlas Mouse Glass 3.8I microarray (BD Biosciences Clontech, CA, USA). Among 3, 756 genes in the array, 822 genes were counted as detectable spots either of the 4 treatment groups. Among them, 146 genes and 239 genes were detected as genes up (ratio > 1.5)- and down (ratio < 0.67)-regulated by GD 13-TCDD treatment, respectively. Interestingly only 14 and 38 genes were also detected as altered genes by GD17-TCDD treatment. Semiquantitative RT-PCR analysis clearly showed that CYP1A1, 1A2, and 1B1 mRNA levels were increased in all fetuses exposed TCDD on both GD13- and GD17, however, CYP1A2 was classified as non-detectable level even in TCDD-exposed groups in the microarray. Above results suggested that GD13 mouse fetuses have a different set of genes from GD-17 fetuses in terms of specific response to TCDD.

1779 TEMPORAL EFFECTS OF AH RECEPTOR LIGANDS ON CH12LX MURINE B-CELL LYMPHOMA CELL GENE EXPRESSION: CDNA MICROARRAY ANALYSIS, REAL-TIME PCR VERIFICATION AND BIOINFORMATIC ASSESSMENT.

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The B-cell, a major component of humoral immunity, is a sensitive target for the immunotoxic effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) possibly by rendering B-cells less responsive to antigen or mitogen stimulation. The potential mechanisms of TCDD action on B-cells were examined in murine B-cell lymphoma cells (CH12LX) treated with 3 nM TCDD or DMSO vehicle for 0, 2, 4, 6, 8, 12 and 24 hrs using a sequence verified cDNA microarray representing 3068 genes/ESTs. Quantitative real-time PCR was used to verify the microarray results and statistical significance was determined using the t-test ($p<0.05$). Cyp1a1 and Cyp1a2 displayed characteristic induction profiles with maximum induction of 300- and 6-fold, respectively, at 4 hrs. Semaphorin 3b (Sema3b), phosphitidyl-inositol-3-kinase delta (pik3cd), signal transducer and activator of transcription 1 (Stat1), and an EST similar to T-cell surface glycoprotein CD1.2 precursor exhibited similar induction profiles and were maximally induced 6-, 2.3-, 1.8- and 10-fold, respectively. A 5000 bp region upstream of the transcriptional start site was extracted from the UCSC Genome Browser database. Putative DREs were identified within the promoter regions that had position weight matrix scores comparable to 15 bona fide functional DREs suggesting that Sema3B, pik3cd and Stat1 may be regulated by the AHR. Induction by structurally diverse AHR ligands further implicated regulation by the AHR. These genes are involved in mitogen-stimulated signaling pathways and therefore, may play a role in the compromised responsiveness of B-cells following treatment with TCDD and related ligands.

1780 TRANSCRIPTIONAL REGULATION OF THE HS4 DOMAIN THROUGH A DRE AND κB MOTIF.

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The B-cell is a sensitive cellular target of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) with the inhibition of immunoglobulin (Ig) secretion being a sensitive endpoint of TCDD toxicity. Our previous results describing the CH12.LX (AhR-

expressing) and BCL-1 (AhR-deficient) B-cell lines have supported an AhR/dioxin-responsive enhancer (DRE)-mediated mechanism for TCDD-induced inhibition of μ heavy chain expression and thus of IgM secretion. Transcriptional regulation of the Ig heavy chain genes involves several regulatory elements including the 3'α enhancer, which is composed of four regulatory domains. One of these domains, hs4, contains a DRE overlapping a κB motif. We have previously demonstrated binding of the AhR nuclear complex and NF-κB proteins to these motifs as well as TCDD and LPS-induced transcriptional activity through the hs4 domain. To determine if the DRE and κB motifs contribute to this enhancer activity, a luciferase reporter gene containing a variable heavy chain (V_H) promoter and a 42 bp fragment of hs4, which contains the overlapping DRE and κB motifs or mutations of these motifs, was transiently transfected into the CH12.LX or BCL-1 cells. In the CH12.LX cells, TCDD and/or LPS modestly affected promoter activity. Addition of the hs4 fragment resulted in a significant increase in activity following TCDD or LPS treatment with the cotreatment inducing the greatest activity. Mutation of either or both of the DRE and κB motifs diminished the effect of TCDD and LPS, resembling the effect of these treatments on the promoter alone. In the BCL-1 cells, only LPS activated the V_H promoter and addition of the hs4 fragment with or without mutations of the DRE and κB motifs did not alter this profile of activity. These results suggest that binding to both the DRE and κB motifs is necessary for enhancement of promoter activity by the hs4 fragment and perhaps activity of the entire hs4 domain. (Supported by NIH ES02520 and NIEHS ES05914)

1781 THE VASCULAR ENDOTHELIUM OF THE BLOOD-BRAIN BARRIER AS A TARGET FOR DIOXIN TOXICITY.

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Numerous functions regulated by the central nervous system are targeted by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), demonstrating its potential neuroactivity. Due to its highly lipophilic structure, TCDD should transverse the vascular blood-brain barrier (BBB), enter the brain parenchyma and elicit damage. However, *in vivo*, TCDD distribution and accumulation into the brain is unexpectedly low. As a result, it is hypothesized the barrier endothelial cells play a role in mediating dioxin penetration, and by doing so are an initial cellular target of TCDD neurotoxicity. The goal of these studies was to characterize TCDD toxicity in the cells types comprising the blood brain barrier, namely endothelial cells and supporting astrocytes. Primary cortical culture models of these cell types, characterized by cell specific markers, have been established for these studies. Western blot analysis has localized the expression of the aryl hydrocarbon receptor (AhR), the predominant modulator of TCDD toxicity, in both endothelial cells and astrocytes. In addition, the prototypical CYP1A1 and CYP1B1protein induction in response to TCDD treatment has been characterized. While both CYP1A1 and CYP1B1 are induced in endothelial cells after four and eight hours respectively, CYP1B1 is the predominant CYP450 isoform induced in astrocytes at twenty-four hours. The CYP450 protein induction in both cell types is sustained for greater than 72 hours and is mediated by the AhR as indicated by co-treatment of cells with the receptor specific antagonist, 3-methoxy-4-nitroflavone. These data indicate endothelial cells and astrocytes are responsive to TCDD through the AhR mediated pathway. It is further hypothesized that CYP450 induction and consequential enzymatic activity is a mechanism for TCDD toxicity in these cells and may lead to vascular breakdown and potentiate neurotoxicity. (Supported by NIH grant ES09430, Training Grant ES07026, and Center Grant ES01247)

1782 ARYL HYDROCARBON RECEPTOR GENE SILENCING WITH SMALL INHIBITORY RNA DIFFERENTIALLY MODULATES Ah-RESPONSIVENESS IN MCF-7 AND HEPG2 CANCER CELLS.

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Sequence-specific small interfering RNA (siRNA) duplexes can be used for gene silencing in mammalian cells and as mechanistic probes for determining gene function. Transfection of siRNAs for the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) mRNAs in MCF-7 breast cancer cells resulted in a 60-80% decrease in levels of AhR and ARNT proteins in whole cell extracts and decreased binding of nuclear extracts to ^{32}P-labeled dioxin responsive element (DRE). siRNA for the AhR also decreased 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced CYP1A1 protein, CYP1A1-dependent activity, and luciferase activity in cells transfected with an Ah-responsive construct containing three tandem DREs linked to a bacterial luciferase gene. 17β-Estradiol (E2) induces proliferation of MCF-7 cells through enhanced G_0/G_1 to S phase progression, and this response is inhibited in cells cotreated with E2 plus TCDD. The effects of TCDD on E2-induced cell cycle progress were partially blocked in MCF-7 cells transfected with siRNA for AhR. The results also indicated that siRNA-dependent decreases in AhR protein in MCF-7 cells were accompanied by increased G_0/G_1 to S phase progression, suggesting a growth inhibitory role for the "endogenous" AhR. Surprisingly, TCDD alone induced G_0/G_1 to S phase progression and exhibited estrogenic activity in MCF-7 cells transfected with siRNA for the AhR. In contrast, degradation of the AhR in HepG2 liver cancer cells resulted in decreased G_0/G_1 to S phase progression, suggesting that in the absence of ligand, the AhR enhances growth of this cell line.

1783 A CONSTITUTIVELY ACTIVE ARYL HYDROCARBON RECEPTOR INDUCES GROWTH INHIBITION BY CELL CYCLE ARREST AND APOPTOSIS IN JURKAT T CELLS.

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to suppress T cell-dependent immune reactions, such as antibody production, in an aryl hydrocarbon receptor (AhR)-dependent manner. We previously reported that TCDD exposure inhibits T cell expansion prior to suppression of T cell-dependent antibody production in mice. However, the mechanism by which TCDD affects the growth of T cells remains to be clarified. In this study, we investigated the effects of AhR activation on the growth of Jurkat T cells by transiently transfecting them with an expression vector for green fluorescence protein (GFP) alone or GFP-constitutively active AhR (CA-AhR) fusion protein. We used a CA-AhR mutant lacking the minimal PAS B motif, which constitutively existed in the nucleus and activated AhR-dependent transcriptions independently of the ligand. GFP-positive cells were sorted out 2 days after transfection and cultured in order to measure cell growth. The growth of Jurkat T cells was completely inhibited by expression of CA-AhR whereas it was not affected by expression of GFP alone. In order to elucidate the mechanism of inhibition of T cell growth by CA-AhR, cell cycle stage and apoptosis in the transfected cells were investigated. Expression of CA-AhR increased the percentage of cells in G1 phase and decreased the percentage of cells in S phase, indicating that CA-AhR induced G1 arrest. In addition, expression of CA-AhR increased the population of Annexin V-positive apoptotic cells when compared to expression of GFP alone. These results suggest that activated AhR modulates T cell growth *via* cell cycle arrest and apoptosis, and thus prevents T cell expansion.

1784 THE ARYL HYDROCARBON RECEPTOR MEDIATES DEGRADATION OF THE ESTROGEN RECEPTOR α THROUGH ACTIVATION OF PROTEASOMES.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and other aryl hydrocarbon (AhR) ligands suppress 17β-estradiol (E2)-induced responses in the rodent uterus and mammary tumors and in human breast cancer cells. Treatment of ZR-75, T47D and MCF-7 human breast cancer cells with TCDD induces proteasome-dependent degradation of endogenous estrogen receptor α (ERα). The proteasome inhibitors MG132, PSI and PSII ablate the proteasome-dependent effects induced by TCDD, whereas the protease inhibitors EST, calpain inhibitor II and chloroquine do not affect this response. ERα levels in the mouse uterus and breast cancer cells were significantly lower after cotreatment with E2+TCDD than after treatment with E2 or TCDD alone. Our results indicate that AhR-mediated inhibition of E2-induced transactivation is due to limiting levels of ERα in cells cotreated with E2+TCDD as measured by both transient transfection and Western blot analysis. Gene silencing by RNA interference assays using small inhibitory RNA molecules demonstrate that the AhR is required for TCDD-induced degradation of ERα. TCDD alone or in combination with E2 increases formation of ubiquitinated forms of ERα, and both coimmunoprecipitation and mammalian two hybrid assays demonstrate that TCDD induces interaction of the AhR with ERα in the presence or absence of E2. In contrast, E2 does not induce AhR-ERα interactions. Thus, inhibitory AhR-ERα crosstalk is linked to a novel pathway for degradation of ERα in which TCDD initially induces formation of a nuclear AhR complex which coordinately recruits ERα and the proteasome complex resulting in degradation of both receptors. (Supported by NIH ES09106 and ES04176)

1785 ARYL HYDROCARBON RECEPTOR AGONISTS INHIBIT HORMONE-INDUCED TRANSACTIVATION IN PROSTATE CANCER CELLS.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and 6-methyl-1, 3, 8-trichlorodibenzofuran (6-MCDF) are ligands for the aryl hydrocarbon receptor (AhR), where 6-MCDF is a weak AhR agonist for several TCDD-like toxic responses. This study investigated the interactive effects of AhR ligands and steroid hormones on receptor proteins, hormone-induced transcriptional activation, and proliferative responses in human prostate (LNCaP and 22RV1) cancer cells. Both LNCaP and 22RV1 cells express the androgen receptor (AR), and after treatment with 10 nM dihydrotestosterone (DHT), significant 2- to 4-fold upregulation of immunoreactive AR protein is observed within 3 h, and remains elevated for up to 24 h. DHT-induced upregulation of AR is inhibited in cells treated with TCDD or 6-MCDF. In prostate cancer cells transfected with a construct (PB-luc) containing an androgen-responsive probasin gene promoter insert, both TCDD and 6-MCDF significantly inhibited DHT-induced reporter activity, whereas this activity was not inhibited in cells transfected with a construct containing only the strongly androgen responsive region (ARR3TK-luc). Ten nM 17β-estradiol (E2) also upregulated AR levels in LNCaP and 22RV1 prostate cancer cells and E2 significantly induced reporter gene (luciferase) activity in cells transfected with PB-luc or ARR3TK-luc. TCDD and 6-MCDF inhibited E2-induced activity only in cells transfected with PB-luc. E2-induced activity in cells transfected with PB-luc was inhibited by the estrogen receptor β (ERβ) antagonist 2, 2-bis(*p*-hydroxyphenyl)-1, 1, 1-trichloroethane (HPTE) and the pure antiestrogen ICI 182, 780, whereas the latter compound did not inhibit DHT-mediated transactivation. These studies indicate that AhR-AR crosstalk is dependent on promoter context (i.e. PB vs. ARR3TK). Moreover, our results also suggest that E2-responsiveness of probasin may be ERβ-dependent and the inhibitory effects of TCDD and 6-MCDF indicate that the AhR may inhibit ERβ-induced genes in prostate cancer cells. (Supported by DAMD17-02-1-0147 and NIH ES09106)

1786 CROSS TALK BETWEEN DIOXIN AND HYPOXIA SENSING PATHWAYS: A GENE ARRAY STUDY.

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Although the cellular responses to 2, 3, 7, 8-tetrachlorodebenzo-p-dioxin (TCDD) and the environmental stress of hypoxia are well studied investigations of potential cross talk are limited. The endothelial response to these combined stresses is unknown. Aryl hydrocarbon receptor nuclear translocator is a common dimerization partner for both aryl hydrocarbon receptor and hypoxia inducible factor. Possible cross talk between these two pathways was studied using gene array technology. Human lung microvascular endothelial cells were exposed to 10nM TCDD, hypoxia (0%O2) or both TCDD and hypoxia for 24 hrs and resulting RNA was analyzed using Affymetrix gene arrays. Statistical analysis of triplicate studies was performed using Affymetrix microarray suite and Affymetrix data mining tool. Ten genes were upregulated during exposure to TCDD alone and 194 genes were upregulated during exposure to hypoxia alone. A total of 147 genes were upregulated during exposure to both TCDD and hypoxia combined. The 6 genes that were upregulated only during TCDD exposure (i.e. hypoxia prevented their upregulation) encoded proteins involved in oxidant stress response, certain growth factors and cellular receptors. The 76 genes that were upregulated only during hypoxia (i.e. TCDD prevented their upregulation) encoded proteins involved in cell adhesion, extracellular matrix formation, histone binding and certain cellular receptors. The common genes upregulated under TCDD alone and in combination with hypoxia represented members of the cytochrome p450 family. The common genes upregulated under hypoxia alone and in combination with TCDD represented a wide variety of genes involved in the endothelial hypoxic response. These results demonstrate that there is cross talk between the TCDD and hypoxia sensing pathways.

1787 ARYL HYDROCARBON RECEPTOR-MEDIATED INHIBITION OF ESTROGEN RECEPTOR-NEGATIVE BREAST CANCER CELL GROWTH.

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The mechanisms of inhibitory aryl hydrocarbon receptor (AhR)-estrogen receptor α (ERα) crosstalk in breast cancer cells have been extensively investigated, and selective AhR modulators (SAhRMs) have been developed for inhibition of ER-positive breast cancer cell/tumor growth. MDA-MB-453 and BT-474 human breast cancer cells are ER-negative and their growth is due, in part, to overexpression of the oncogene, epidermal growth factor receptor 2 (EGFR2/ErbB2/neu). In these cell lines, there is constitutive activation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3-K) signaling pathways. Treatment of these cells with 1 or 10 nM 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces CYP1A1- or dioxin response element (DRE)-dependent activities confirming that both cell lines are Ah-responsive. Treatment of these cells with 25 μM LY294002 or 10 μM U1026 which inhibit PI3-K or MAPK signaling also inhibits growth of both cell lines. Growth inhibition of these cells is also observed after treatment with 10 nM TCDD, 1 - 5 μM 6-methyl-1, 3, 8-trichlorodibenzofuran (6-MCDF) and other SAhRMs. These AhR-mediated growth inhibitory responses are also accompanied by alterations of cell cycle progression. The effects of TCDD and SAhRMs on MAPK and PI3-K signaling pathways were investigated by determining phosphorylation of MAPK and Akt. After short term (3 hr) treatment with LY294002 or U1026, there was significant inhibition of both MAPK- and PI3-K-dependent phosphorylation, whereas inhibitory effects were not observed for SAhRMs. In contrast, after treatment for up to 72 hr, SAhRMs significantly decreased activity of both kinases pathways, and the mechanisms of this response are currently being investigated. (Supported by NIH ES09106 and ES04176)

1788 TCDD INDUCES A SUPPRESSION OF PPARγ EXPRESSION THAT INHIBITS ADIPOCYTE DIFFERENTIATION.

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C3H10T1/2 cells (10T1/2) are a multipotential mouse embryo fibroblast cell line that can differentiate into adipocytes when administered insulin, dexamethasone, and isobutylmethylxanthine at 100% confluency; the PPARγ ligand BRL46593 increases this differentiation. We have previously shown that PPARγ expression is inhibited and subsequent lipid droplet accumulation is greatly diminished if TCDD is administered 48 hours prior to hormonal stimulation. Here we demonstrate that changes in TCDD-induced AhR activation positively correlate with TCDD-mediated inhibition of PPARγ. The down regulation of AhR induction of Cyp1B1, suppression of PPARγ expression, and suppression of the transformation into adipocytes each show a similar dependence on TCDD concentration (EC_{50} < ~ 2 — 5pM). TCDD suppressed PPARγ in an equally effective manner whether it was added 48 hours prior to or up to 12 hours after hormonal stimulation; however, if TCDD is administered 24 hours after the hormonal stimulation, suppression of PPARγ does not occur. We have also shown that the PPARγ suppression, whether TCDD treatment was initiated 48 hours prior to or 12 hours after hormonal stimulation, depended on MEK/ERK activation within the same time period of 6 – 16 hours post hormonal administration. The AhR antagonist 3'-methoxy-4'-nitroflavone (3-MNF) similarly antagonizes TCDD activation of AhR, as measured by inhibition of Cyp1B1 protein expression and suppression of PPARγ expression. In order for 3-MNF to antagonize the TCDD effect on PPARγ, 3-MNF must be present during the period of TCDD/MEK activity prior to the onset of PPARγ stimulation. We conclude that TCDD induces a protein that, in conjunction with MEK activity, suppresses PPARγ expression.

1789 TCDD INDUCES INCREASED EXPRESSION OF RETINOIC ACID METABOLIZING GENES: POSSIBLE ROLE IN ALTERING PROLIFERATION AND DIFFERENTIATION IN HUMAN KERATINOCYTES.

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The aryl hydrocarbon receptor (AhR), is thought to mediate most of the carcinogenic and toxic effects of TCDD and related chemicals through the formation of a heterodimer with its DNA binding partner, ARNT and transcriptional regulation of AHR/ARNT target genes. We have previously shown that administration of TCDD to primary, human keratinocytes results in an immediate increase in proliferation, and a decrease in replicative senescence and a subsequent increase in late differentiation. In an effort to identify the gene pathways that regulate proliferation and differentiation and may be modified by TCDD and the AHR/ARNT heterodimer, we have performed similar experiments and analyzed the TCDD-induced changes in gene expression using microarray and western blot analysis. We have found that TCDD induces the mRNA expression levels of a number of genes involved in retinoic acid metabolism (i.e., retinol and retinoic acid dehydrogenases) in a time dependent manner. In addition, significant changes in retinoic acid receptor (RAR) isoforms were observed in the TCDD-treated cells. Given that retinoic acid is a powerful modulator of proliferation and differentiation, we suggest that the ability of TCDD to alter proliferation and differentatiation in these cells may involve the alteration of retinoic acid homeostasis.

1790 EFFECT OF ARYL HYDROCARBON RECEPTOR (AhR) AGONISTS ON THE ACTIVATION OF TELOMERASE IN HUMAN MAMMARY EPITHELIAL CELLS.

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Telomerase, a reverse transcriptase inactive in normal somatic tissue, permits indefinite cell growth *via* the elongation and maintenance of telomeres, the repetitive sequence capping chromosome ends. Epidermal Growth Factor markedly stimulates telomerase activity in keratinocytes and 2, 3, 7, 8-tetrachlorodibenzodioxin (TCDD) prevents this effect. Telomerase is increased in the vast majority of human mammary tumors. Thus, the effects of TCDD and other AhR agonists on telomerase activity in human mammary epithelial cells were evaluated in order to assess the role of AhR agonists in breast carcinogenesis. Telomerase was measured using a quantitative PCR-based method (telomerase repeat amplification protocol). MCF-7 cells were exposed to 4 nM TCDD or the vehicle alone for a period up to 25 days. Compared to controls a larger proportion of cells exposed to TCDD detached from the Petri dish after 6 to 8 days in culture, but no reduction in cell proliferation was observed throughout the study. A marked reduction of telomerase activity was measured in all viable floating cells in comparison to adherent cells. TCDD did not change telomerase activity in the latter. In another series of experiments, a mixture of 16 AhR agonists (3 coplanar PCBs, 7 PCDFs and 6 PCDDs) was tested for up to 25 days in the non tumorigenic human mammary cell line, MCF-10A. The AhR-mixture caused a progressive reduction in telomerase activity from day 0 to day 3, whereas after day 6 no more activity was detected. A very small fraction of cells became floating and this was not influenced by TCDD treatment. A gradual growth arrest was observed from day 9 to day 25. Thus, a reduction of telomerase activity preceded the marked reduction in cell proliferation demonstrating a strong link between telomerase and proliferation. The effect of AhR agonists on telomerase activity in human cell lines does not support a role for these compounds in breast tumor development. (Supported by TSRI and PREECAN)

1791 THE ROLE OF DNA OXIDATION IN TCDD-INDUCED HOMOLOGOUS RECOMBINATION.

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The environmental toxicant 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) elicits a spectrum of deleterious biological responses. One of the toxic effects of TCDD is carcinogenicity, but the molecular mechanism by which TCDD exerts its tumorgenic effects is unclear, since it is not directly genotoxic. Therefore, the molecular mechanism leading to the development of cancer as a result of TCDD exposure needs to be elucidated. DNA damage, including DNA oxidation, can induce DNA double-strand breaks (DSB), which can be repaired through homologous recombination (HR). Excessive DSBs may promote aberrant DNA recombination, which can mediate detrimental genetic changes. We hypothesize that these genetic changes lead to increased genomic instability and ultimately TCDD-induced carcinogenesis. To investigate this hypothesis we used a Chinese hamster ovary (CHO) cell line containing a neo direct repeat recombination substrate (CHO3-6) to determine whether TCDD-induced HR is initiated by DNA oxidation. CHO 3-6 cells were exposed to TCDD (50, 500 or 1000 pM) for 6 or 24 hr and 2 weeks later HR frequencies were determined by counting the number of functional neo expressing, G418-resistant colonies per live cells plated. TCDD-initiated DNA oxidation was determined by measuring the formation of 8-hydroxy-2'-deoxyguanosine *via* HLPC and electrochemical detection. While TCDD did not increase HR after the 6 hr exposure period, exposure to 500 pM TCDD for 24 hr significantly increased HR frequency. DNA oxidation was not increased in cells exposure to TCDD for either 6 or 24 hr. Therefore, our results suggest that TCDD-initiated HR in CHO 3-6 cells is not mediated by oxidative DNA damage. (Supported by the Canadian Institute of Health Research and Queen's University ARC)

1792 METABOLIC DEGRADATION OF A SUGGESTED ENDOGENOUS ARYLHYDROCARBON RECEPTOR LIGAND, THE TRYPTOPHAN PHOTOPRODUCT 6-FORMYLINDOLO[3, 2-B]CARBAZOLE.

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Purified extracts of irradiated tryptophan exhibit 4- to 7-fold higher binding affinity to the arylhydrocarbon receptor (AhR) compared to TCDD. In different cells, *in vitro*, the synthesized pure photoproduct 6-formylindolo[3, 2-b]carbazole (FICZ) as well as extracts of UV-irradiated tryptophan induce a rapid and transient expression of AhR-regulated genes. The transient induction suggests that the biotransformation gene battery induced *via* AhR activation takes part in a metabolic degradation of the ligand. The *in vitro* metabolism of the tryptophan photoproduct FICZ has been characterized by LC-MS and NMR spectroscopy. Five metabolites have been identified and their structures elucidated. In the present study, using rat hepatic S9 incubation and specific enzyme inhibitors, the involvement of different phase I and phase II metabolizing enzymes were investigated. The P4501A1enzyme is the most important enzyme for the first step in the metabolism of FICZ. Monohydroxylated metabolites seem to be formed by both CYP1A1 and CYP1A2, although with slower kinetics by CYP1A2. Dihydroxylated secondary metabolites were also identified. By use of specific inhibitors, the enzymes CYP1A1, CYP1A2, and CYP1B1 were indicated to participate in the formation of three different dihydroxylated compounds. The conjugating enzymes SULT and UGT take part in the further metabolism as indicated by decreased levels of the dihydroxylated metabolites after addition of the necessary conjugating cofactors PAPS and UDPGA, respectively. Our experimental data on the metabolism of the tryptophan derived AhR ligand FICZ by the battery of AhR-regulated enzymes explain the important role of the functional CYP1A1 enzyme in the autoregulatory feedback loop that has been proposed to exist for controlling transcription of AhR-regulated genes.

1793 2, 3, 7, 8 TETRACHLORODIBENZO-P-DIOXIN (TCDD) INCREASES MITOMYCIN C (MMC) TOXICITY AND ALTERS METABOLISING ENZYMES THROUGH ARYL HYDROCARBON RECEPTOR (AHR) INTERACTION.

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Selective manipulation of metabolic enzymes and intracellular events to increase drug efficacy is currently an area of much interest. Commonly the compounds used, such as broussochalcone, are AhR agonists. It is the relative induction of activation and detoxification enzymes which determines the efficacy of the agonist-AhR interaction to modulate drug toxicity. We have investigated the toxicity of the enzyme-activated prodrug MMC when TCDD, a prototypical AhR agonist, is co-administered. Human mammary carcinoma cells (MCF7) were maintained in MEM with non essential amino acids, 10% FBS, 1 mM sodium pyruvate and 10 μg/mL bovine insulin and were co-treated with TCDD (10 nM) and MMC (0, 1, 10 or 100 μM) for 2h or pre-treated with TCDD then MMC for 2h each in 95% air/5% CO2. Cells were subsequently assessed for viability or enzyme activity with S9 protein prepared by differential centrifugation. In clonogenic assays significantly more cell death was observed with TCDD and MMC co-treatment at 1 and 10 μM compared to cells treated with MMC alone although no significant differences in cell death were observed with TCDD pre-treatment. Enzyme activities increased by TCDD when compared to vehicle were DT-diaphorase (NQO1), xanthine dehydrogenase (XDH) and cytochrome P450 reductase (CYPR) in both pre- and co-treated cells. With TCDD pre-treatment, XDH activity when compared to both vehicle and 1 μM MMC increased as opposed to MMC alone, while CYPR activity increased at all doses when compared to MMC alone. The activities of xanthine oxidase and glutathione-S-transferase were not altered. Dose-dependent decreases in NQO1 and XDH activity were observed as MMC concentration increased. Co-administration of MMC and AhR agonists may result in greater toxicity through increased activity of activation enzymes, particularly XDH and CYPR without a concomitant increase in detoxification enzymes.

1794 AGONISTIC AND ANTAGONISTIC EFFECTS OF POLYBROMINATED DIPHENYL ETHERS (PBDE) IN MCF7 CELLS.

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Polybrominated diphenylethers (PBDEs) are used as flame-retardants in consumer products to reduce the chances of ignition and burning. Levels of some PBDE congeners have been increasing in fish, wildlife, and in human tissues. Certain PBDEs have been found to be highly lipophilic and persistent, resulting in bioaccumulation in the environment. The structural similarity of PBDEs to other polyhalogenated aromatic hydrocarbons has raised concerns that PBDEs might act as agonists for the aryl hydrocarbon (Ah) receptor. To study the possible dioxin-like effects of the environmentally relevant PBDEs (BDE47, 77, 99, 100, 153, 154, 183, 209), the Ah receptor-mediated induction of the cytochrome P450 enzyme CYP1A1 was studied in human breast carcinoma cells (MCF7). 7-Ethoxyresorufin-O-deethylase (EROD) was used as a marker for CYP1A1 activity. MCF7 cells (ATCC) were exposed to several concentrations (0.01-25 μM) of these PBDEs (Å Bergman, Sweden). Positive controls were 2, 3, 7, 8-TCDD (0.001-2.5 nM) and PCB126 (1-100 μM). EROD activity was measured after 72 hours. None of these

PBDEs was capable of inducing EROD activity. However, when the cells were exposed to BDE153 (2, 2', 4, 4', 5, 5'-hexabromodiphenyl ether) in combination with TCDD, there was a strong concentration dependent antagonistic effect. The positive control TCDD caused maximal EROD induction at 1 nM (100% efficacy). In the presence of BDE153 at concentrations of 2.5 and 10 μM, the maximum efficacy of TCDD was reduced to 38% and 23%, respectively. Similarly, 10, 25 and 50 μM PCB153 reduced the efficacy to 53%, 38% and 2%, respectively. At the present, these PBDEs are tested in either human and mammalian *in vitro* systems for possible Ah receptor agonistic and antagonistic properties. Whether or not this antagonism is due to direct interaction with the Ah receptor or some other unknown mechanism of downregulation will be further assessed.

1795 SPECTRAL INTERACTIONS OF POLYCHLORINATED BIPHENYLS (PCBS) WITH RAT HEPATIC MICROSOMAL CYTOCHROME P450 ENZYMES.

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Rat hepatic microsomes contain multiple cytochrome P450 (CYP) enzymes that interact with xenobiotics to produce a type I spectral change indicative of a CYP-substrate complex. The purpose of this study was to determine whether the tetrachlorobiphenyls (PCB 47, 52, 54, and 77) elicit type I spectral changes with microsomal CYP and if so, which CYP forms are involved. Rats were treated with phenobarbital (PB) (75 mg/kg/day), dexamethasone (DEX) (100 mg/kg/day), or 3-methylcholanthrene (MC) (25 mg/kg/day) given i.p. once daily for 4 days. Hepatic microsomes were prepared for subsequent CYP determination, spectral binding, antibody inhibition studies, gel electrophoresis, and CYP immunoquantitation. All 4 PCBs elicited a type I difference spectrum with microsomal CYP showing an absorbance maximum and minimum at approximately 390 and 420 nm, respectively. The apparent spectral binding constant (Ks) and maximum absorbance change (ΔAmax) were derived from double reciprocal plots. Apparent Ks values of 67, 53, 11, and 5 μM were obtained for PCB 47, 52, 54, and 77, respectively, with control microsomes; 11, 18, 2, and 7 μM with PB microsomes; 78, 52, 9, and 12 μM with DEX microsomes; and 270, 469, 47, and 7 μM with MC microsomes. PCB 47, 52 and 54 bound avidly to PB microsomes with a binding efficiency (ΔAmax/Ks) that was on average 137 and 7 times greater, respectively, than that obtained with MC and DEX microsomes. The absorbance change elicited by PCB 54 in PB microsomes was decreased by 37, 29 and 3%, respectively, in the presence of antibodies against CYP2B, CYP3A and CYP 2C enzymes, and in control microsomes by 52 and 38% in the presence of antibodies against CYP3A and CYP2C enzymes. In conclusion results of antibody inhibition and CYP immunoquantitation experiments suggest that CYP2B and to a lesser extent CYP3A enzymes bind the non-coplanar PCBs (47, 52 and 54) in PB and DEX microsomes whereas CYP1A enzymes bind the coplanar PCB 77 in MC microsomes. CYP2C enzymes also bind PCB 47, 52 and 54 but only in control microsomes.

1796 CYP1B1 INDUCTION BY TCDD OR CELL SUSPENSION REQUIRES SMALL PROPORTION OF NUCLEAR AHR IN MEF 10T1/2 CELLS.

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Cytosolic AhR translocates to the nucleus after binding ligand, such as TCDD. A portion of nuclear AhR acquires transcriptional capability by forming a heterodimeric complex with the ARNT and, thereby, induces target genes. We hypothesize that only a small portion of AhR, translocated to the nucleus following treatment with 1 to 10 nM TCDD is utilized for target gene induction. Therefore, the concentration dependence for AhR translocation, turnover, and Cyp1B1 induction was compared. Gene induction was still maximum at 10 pM TCDD even though nuclear translocation and turnover of AhR were substantially decreased. This result suggests, first, that the quantity of nuclear translocation at 1 nM TCDD far exceeds the level required for target gene induction. Second, that the nuclear AhR that is unbound to DNA is immediately targeted for nuclear export and degradation. This nuclear export is, therefore, increased in proportion to the capacity of TCDD to generate this nuclear excess of AhR as seen in ER (Alarid, Mol Endocr 1999). AhR-mediated transcription is activated not only by TCDD, but also by cell suspension. Cytosolic AhR is complexed with Hsp90. When AhR is activated by TCDD or by suspension, the translocation of AhR to the nucleus is accompanied by translocation of Hsp90. Translocations of AhR and Hsp90 were comparable for TCDD and suspension activations. Geldanamycin, an inhibitor of Hsp90 ATPase, caused a large loss of AhR and prevented translocation of Hsp90 to the nucleus in each process. AhR turnover was also similar and equally inhibited by MG132, a proteosome inhibitor. A major difference between these two, otherwise very similar processes of AhR turnover, was that suspension induction of Cyp1B1 was insensitive to αNF, a competitive inhibitor of TCDD activation. This suggests that the suspension activation does not involve an endogenous AhR ligand. In conclusion, AhR activation by either TCDD or by cell suspension is equally effective and both require a low proportion of nuclear AhR for target gene induction where its down-regulation is slow.

1797 THE EFFECTS OF GREEN TEA CATECHINS ON ARYL HYDROCARBON RECEPTOR-MEDIATED GENE REGULATION.

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Green tea (GT) has been implicated in affording protection against cancer in numerous animal models of chemical carcinogenesis. The active constituents and mechanisms responsible for these observations are not fully understood. Among the proposed mechanisms is one involving inhibition of carcinogen activation by modulating bioactivating enzymes, such as CYP1A1, through an interaction with the AhR pathway. Previous investigations in our laboratory have shown that treatment of mouse hepatoma cells with GT extracts results in inhibition of 2, 3, 7, 8-tetrachloro-p-dioxin (TCDD) induced activity of a DRE-dependent luciferase reporter gene. Further fractionation of GT led to the identification of epigallocatechin gallate (EGCG) and epigallocatechin (EGC) as the catechins responsible for this inhibition. From this data, it was hypothesized that EGCG and EGC function as AhR antagonists. However, due to the high (uM) concentrations of catechins used as well as their strong antioxidant activity, it is possible that this decrease in luciferase activity resulted from some indirect, non-AhR effect. Further studies demonstrated that neither cytotoxicity nor antioxidant activity contribute to this decrease in TCDD induced luciferase activity. Furthermore, western blotting of mouse hepatoma cells treated with TCDD together with either EGCG or EGC showed a concentration dependent decrease in both luciferase and CYP1A1 protein levels, strongly supporting a role for the AhR in this inhibition. Interestingly, although both catechins inhibited TCDD induced luciferase with equal potency, EGCG was a much more potent inhibitor of CYP1A1. Further insight into the mechanism of action revealed that although EGCG inhibits TCDD induced DRE binding in cell free systems EGC does not. Identical results were observed in whole cell DRE binding assays indicating the parent compounds are responsible for the antagonist activity. Together, these data suggest that EGCG and EGC function as AhR antagonists through different mechanisms resulting in differential inhibition of AhR mediated gene induction.

1798 CHARACTERIZATION OF REGULATORY ELEMENTS IN THE HUMAN AH RECEPTOR PROMOTER.

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The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor belonging to the PAS protein family of transcription factors. It mediates biological and toxic responses of e. g. halogenated aromatic hydrocarbons and certain indole derivatives. In the absence of a ligand, the AhR is located in the cytoplasm in a complex with a dimer of heat-shock protein 90 and XAP2 (X-associated protein 2). After ligand binding, AhR translocates to the nucleus where it heterodimerizes with the PAS protein Arnt (Ah receptor nuclear translocator). The AhR-Arnt heterodimer is able to activate specific xenobiotic regulatory elements (XREs) in enhancers of target genes. In many vertebrate as well as in invertebrate species the AhR or homologues have been identified. In humans, it has been found in various tissues such as lung, liver and placenta. Although much is known about the biochemical and molecular mechanisms of AhR action, little is known about the control of the expression of the AhR gene itself. In the present study, we aimed at the identification and characterisation of regions important for constitutive AhR gene expression. We generated a luciferase reporter gene construct including 2.7 kb of the 5'-flanking region of the AhR. Reporter gene assays demonstrated that this part of the AhR promoter strongly activates the reporter gene compared with the empty reporter vector. Reporter gene assays with deletion mutants led to the identification of a region containing putative binding sites for Sp1, c-myc and CREB. Interestingly, reporter gene activities of two constructs with a deletion in this region differed significantly. The functional relevance of putative regulatory sequences was investigated by gel mobility shift analysis and site-directed mutagenesis. Summarizing, our results indicate that a Sp1-site contributes to AhR regulation. Further experiments including super shift assays and investigation of the influence of Sp1 expression plasmids are necessary to reveal the regulatory relevance of the region identified in the present study.

1799 ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β (PPARβ) IN MOUSE KERATINOCYTE PROLIFERATION AND DIFFERENTIATION.

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Previous work has shown an enhanced epidermal hyperplastic response induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in PPARβ-null mice, suggesting a critical role of PPARβ in attenuating epidermal cell proliferation. In this study, the functional role of PPARβ in keratinoctye proliferation and differentiation was investigated using primary keratinocytes from wild-type and PPARβ-null mice. Differentiation induced by the classical method of increasing culture medium Ca^{++} level resulted in induction of atypical mRNA markers of differentiation and morphological changes including the formation of cornified cells in both genotypes, independent of PPARβ. Differentiation induced by TPA also resulted in induction of standard mRNA markers of differentiation in both genotypes, and the hallmark morphological features of dendritic cell-like appearance in keratinocytes from both genotypes. Despite induction of a known TNFα-responsive gene (mtCLIC) in both genotypes, induction of known mRNA markers of differentiation and atypical morphological changes associated with differentiation were not found in TNFα-treated cells from either genotypes. PPARβ-null keratinocytes showed high expression of cyclin B1 and PCNA mRNA compared to wild-type keratinocytes in low calcium medium. In addition, expression of both mRNAs initially decreased, following by a significant increase, after 1hr pulse treatment of TPA in PPARβ-null keratinocytes, whereas their expression was gradually decreased in wild-type keratinocytes during the same time frame. These results show that PPARβ does not regulate keratinocyte differentiation induced *in vitro*, consistent with previous *in vivo* results. These studies also provide strong evidence that PPARβ attenuates cell proliferation consistent with previous *in vivo* data. (Supported by NIH, CA89607)

1800 THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA IN CELL CYCLE REGULATION.

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. PPARs can be activated by a large group of chemicals called peroxisome proliferators (PPs) that include industrial pollutants and hypolipidemic drugs. It has been shown in murine models that administration of PPs results in hepatomegaly, mitogenesis, and eventually carcinogenesis. PPs are thought to cause cancer by altering gene expression of the target cell and, ultimately, affect cell cycle regulation. To study the role of PPAR alpha-dependent regulation of the cell cycle, hepatoma cell lines from the PPARα wild type and knockout mice were developed. The immortalized hepatoma cells reflect the PPARα-dependent mitogenic response to PPs. The activity, phosphorylation status, and expression of key cell cycle regulatory proteins were examined in these immortalized hepatocytes treated with Wy 14, 643. A significant difference in p53 and pRb activity between phenotypes, but not upon Wy 14, 643 treatment was observed. Similarly, the phosphorylation status of pRb, CDK4, CDK6, p21, and GSK3α/β was affected by PPARα status, but not by Wy 14, 643 treatment. Through quantitative RT-PCR, we are currently examining the role of PPARα in regulating the expression of cyclin dependent kinase inhibitors such as p15, p16, p18, p19, and p21. Although we have shown that PPARα affects the constitutive activity of certain cell cycle regulatory proteins, the key genes involved in the PP-dependent mitogenic response need to be determined.

1801 THE PROTECTIVE EFFECT OF GW4064X ON ALPHA-NAPHTHYLISOTHIOCYANATE (ANIT)-INDUCED HEPATOTOXICITY IN MICE: ROLE OF ACTIVATED FXR.

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ANIT is a well-known hepatotoxicant that induces obstructive cholestasis in rodents. GW4064X, a farnesoid X-activated receptor (FXR; NR1H4) agonist, has been shown to protect against bile-duct ligation induced hepatotoxicity in rats. This study was designed to determine whether GW4064X is also effective in reducing ANIT-induced hepatotoxicity in mice. Adult CD-1 mice were given vehicle (Veh) (10 ml/kg, ip) or GW4064X (50 mg/kg, ip) daily for 4 days. Two hours after the second dose of Veh or GW4064X, mice were orally dosed with ANIT (150 mg/kg). Hepatotoxicity was assessed 48 hours after ANIT administration. GW4064X treatment significantly decreased ANIT-induced hepatotoxicity, as determined by serum activities of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase. Serum levels of total bilirubin and bile acid were also significantly decreased by GW4064X treatment. Histopathological analysis of hepatocellular necrosis indicated GW4064X-treated mice had fewer and smaller necrotic areas than Veh-treated mice. To examine the mechanism of the protection, total RNA from livers was extracted and subjected to real time quantitative RT-PCR analysis. Compared to Veh, GW4064X treatment resulted in an enhanced expression of small heterodimer partner (SHP) and bile salt export pump (BSEP). ANIT treatment decreased cytochrome P450 7a (CYP7A) and cytochrome P450 8b (CYP8B) expression by 95% and 70%, respectively. GW4064X treatment had no further suppression in both CYP7A and CYP8B. In summary, GW4064X protected against ANIT-induced liver injury in mice. This provides additional evidence that FXR agonists may be useful in the prevention of hepatic damage during conditions of cholestasis.

1802 INDUCTION OF THE TRUNCATED ERYTHROPOIETIN RECEPTOR BY CHLORAMPHENICOL IN MOUSE BONE MARROW.

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Interaction between erythropoietin (Epo) and its surface receptor EpoR plays a fundamental role in erythropoiesis. Erythropoiesis is a multi-stage process requiring proliferation and differentiation of erythroblasts to form mature erythrocytes, which repopulate the circulating erythroid pool. There are two forms of EpoR: a full length (EpoR-F) expressed predominantly in mature erythroids and a truncated receptor (EpoR-T) expressed predominantly in immature erythroids. Expression of EpoR-T is thought to have a dominant-negative effect on EpoR-F thus regulating erythropoiesis depending on circulating Epo levels. Chloramphenicol is a broad-spectrum antibiotic used in the treatment of serious infections including typhoid fever and meningitis. However, chloramphenicol is hematotoxic in humans and animals causing reversible anemia, but in extremely rare cases a more serious irreversible aplastic anemia may occur. The aim of our work was to study the mechanism by which chloramphenicol is hematotoxic. We hypothesised that chloramphenicol partially mediates its bone marrow toxicity by modulating the expression of EpoR in erythroblasts. In our studies, treatment of mice with 3000 mg/kg/day chloramphenicol resulted in a 50% or more decline in blood reticulocytes, which was preceded by a depletion of erythroid precursors in bone marrow as determined histochemically in sternums. Immunoblot analysis of bone marrow showed a time-dependent elevation of EpoR-T that corresponded temporally to reticulocyte decline. The overall increase in EpoR levels after treatment was also confirmed by immunofluorescence in bone marrow smears. Although the presence of EpoR-T is physiologically important to maintain tight regulation of erythropoiesis, increased expression of EpoR-T by chloramphenicol may deregulate erythropoiesis and perhaps contribute to the erythroblast-specific toxicity observed with chloramphenicol.

1803 SNURF AND MMS19 AS COACTIVATORS OF ESTROGEN RECEPTOR α-MEDIATED GENE EXPRESSION IN BREAST CANCER CELLS.

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At least two mechanisms of 17β-estradiol (E2)-induced transactivation have been identified in human breast cancer cell lines. These include estrogen receptor α (ERα) interactions with estrogen response elements (EREs) and ERα/Sp1 interaction with GC-rich motifs in promoters of E2-responsive genes. ERα-dependent transcriptional activation of genes through interaction with EREs is dependent upon activation function 2 (AF2) in the ligand binding domain of ERα which specifically interacts with steroid receptor coactivators and other coactivators containing LXXLL motifs. Preliminary studies demonstrated that AF2-interacting coactivators do not enhance ERα/Sp1-mediated transactivation. This study reports the effects of SNURF, a DNA binding domain (DBD) interacting protein, and MMS19, an activation function 1 (AF1)-interacting protein on coactivation of ERα in ZR-75 human breast cancer cells. SNURF cooperatively coactivates ERα through recruitment and interaction with TATA-binding protein (TBP). SNURF coactivates E2-mediated induction by 4- to 5-fold in ZR-75 cells transfected with an ERE promoter construct ($pERE_3$) and ERα. Our results also indicate that interaction of SNURF with DNA is not sufficient, and that SNURF must recruit TBP for efficient coactivation by SNURF. SNURF-mediated coactivation of ERα/Sp1 in cells transfected with a GC-rich promoter ($pSp1_3$) was approximately 2-fold;

however, the mechanism for this coactivation has not been determined. Peptide competition studies using an AF1 peptide resulted in complete inhibition of E2-induced reporter activity in ZR-75 cells transfected with $pSp1_3$. Only minimal decreases were observed using a GRIP NR-box peptide that contains an LXXLL motif. These results demonstrate that ERα/Sp1-mediated transactivation is primarily AF1-dependent. AF1-interacting coactivator MMS19 coactivates ERα 2- to 3-fold in ZR-75 cells cotransfected with $pERE_3$. In contrast, MMS19 does not significantly coactivate ERα/Sp1 in ZR-75 breast cancer cells. (Supported by NIH ES09106 and CA76636)

1804 VITAMIN D RECEPTOR INTERACTING PROTEIN 150 (DRIP 150) AS A COACTIVATOR OF ESTROGEN RECEPTOR α

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Estrogen-induced gene expression is dependent on hormone interactions with the estrogen receptor α (ERα) and recruitment of nuclear cofactors that facilitate transactivation. Vitamin D receptor interacting protein 150 (DRIP 150) is a component of the mediator complex of nuclear cofactors, and in ZR-75 human breast cancer cells, DRIP 150 enhances 17β-estradiol (E2)-induced transactivation in cells transfected with a construct ($pERE_3$) containing 3 tandem estrogen response elements (EREs). In experiments using wild-type and variant ERα mutants, it was shown that DRIP 150 coactivation was dependent on the C-terminal activation function 2 (AF2) of ERα. DRIP 150 did not coactivate a variant ERα construct that contained mutations in helix 12 (D538A, E542A and D545A) that are required for interactions with coactivators that contain LXXLL motifs. Moreover, DRIP 150-mediated coactivation of ERα was blocked in cells cotransfected with an NR-box peptide containing LXXLL box motifs. DRIP 150 contains N- and C-terminal LXXLL motifs at aa 69-73 and 1182-1186, respectively; however, C-terminal deletions of DRIP 150 which did not contain the latter motif (aa 1-1144, 1-976, 1-885, 1-869, and 1-864) enhanced ERα-mediated coactivation. Amino acids between 864 and 789 were essential for coactivation of ERα by DRIP 150, and the specific sequences responsible for coactivation are currently being characterized. (Supported by NIH ES09106 and CA76636).

1805 PREGNANE X RECEPTOR (PXR) INTERACTS WITH THE NUCLEAR COREPRESSOR SMRT TO REPRESS BASAL CP3A4 GENE EXPRESSION.

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The Pregnane X Receptor (PXR; NR1I2) is a member of the nuclear receptor superfamily that heterodimerizes with the Retinoid X Receptor (RXR) on numerous nuclear receptor response element DNA motifs, resulting in the transactivation of target genes. PXR is found primarily in liver and intestine, and is primarily responsible for the tissue-specific up-regulation of Cytochrome P450 3A4 (CYP3A4) in humans by numerous inducer chemicals. Recent PXR knockout mouse studies suggest that PXR may repress basal expression of target genes. We therefore examined whether PXR repressed basal gene expression, and whether the nuclear receptor corepressor SMRT plays a role in such repression. PXR binds to SMRT *in vitro* at the ID2 region. The interaction between PXR and SMRT also occurs *in vivo* in a ligand-dependent manner. SMRT interacts with the PXR ligand-binding domain (LBD), and this interaction is specifically regulated by the AF2-helix. Gal4-PXR-mediated repression of reporter activation appears to be due to an interaction with endogenous SMRT. Furthermore, SMRT represses PXR-mediated activation of the 13-kb CYP3A4 promoter, yet the addition of ligand abrogates this effect. These data demonstrate that PXR interacts with SMRT in the absence of ligand to repress basal expression of target genes.

1806 RESISTANCE TO SILICA-INDUCED LUNG FIBROSIS IN SENESCENT RATS: ROLE OF TNF-ALPHA AND FAS-L.

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Aging is associated with a progressive decline in the ability of alveolar macrophages to produce TNF-a in response to LPS, which reflects a defective protein kinase C (PKC) translocation due to decreased expression of its anchoring protein RACK-1 (1). The purpose of the present study was to investigate the immunotoxicological consequences of this defective activation in an experimental model of silicosis. Young and old rats were intratracheally instilled with silica (30 mg) or saline as control. In young animals, as expected, silica induced a significant increase in bronchoalveolar lavage fluid (BAL) of TNF-alpha, lactate dehydrogenase and cell numbers, which correlated with increased collagen deposition and silicotic nodule formations. In old rats no changes in BAL fluid or lung parameters were observed, indicating that senescent rats are resistant to silica-induced lung toxicity. These *in vivo* results were confirmed *in vitro*, where silica failed to induce TNF-alpha release in alveolar macrophages obtained from old animals. This could be explained with a defective PKC translocation in aged macrophages. Use of RACK-1 antisense oligonucleotide reduced the response of young macrophages to silica, demonstrating its role in silica-induced TNF-alpha production. Furthermore, a decrease in FAS-L expression and silica-induced apoptosis in old macrophages were observed, supporting the idea that age-associated alterations in signal transduction pathways contribute to decreased sensitivity to silica-induced lung fibrosis in old animals. References 1. J. Immunol. 163: 3468-3473, 1999.

1807 IMMUNOGLOBULIN RESPONSE TO SILICA EXPOSURE IN LUPUS PRONE NEW ZEALAND MIXED MICE.

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Crystalline silica exposure in New Zealand mixed (NZM) mice leads to an exacerbated autoimmune disease measured by increases in autoantibodies, immune complexes, proteinuria and pulmonary fibrosis. To determine the effect of long-term silica exposure on immunoglobulin responses in autoimmune prone mice, NZM mice were intranasally instilled with saline or saline suspensions of 1 mg silica. Immunoglobulin levels were measured by ELISA 14 weeks following instillation of silica. IgM levels were slightly elevated in silica exposed NZM mice compared to saline exposed mice (979 μg/ml ± 213 vs 792 μg/ml ± 104). IgG levels were significantly decreased in silica exposed mice compared to saline exposed mice (1639 μg/ml ± 193 vs 3024 μg/ml ± 501 $p<0.05$). IgG2a levels were similar between silica and saline treated mice (864 μg/ml ± 133 vs 920 μg/ml ± 203). IgG2b and IgG3 levels were also similar between silica and saline exposed mice (246 μg/ml ± 29 vs 227 μg/ml ± 52 and 160 μg/ml ± 37 vs 203 μg/ml ± 42). IgG1 levels were significantly lower in silica exposed mice compared to saline exposed mice (650 μg/ml ± 204 vs 1104 μg/ml ± 224 $p<0.05$). Due to these decreases in immunoglobulin, B cell numbers were examined by flow cytometry in NZM mice following silica exposure. B cell numbers in the spleen and the superficial cervical lymph nodes were decreased 58% and 10% respectively in silica exposed NZM mice 14 weeks following exposure. The immunoglobulin isotype pattern suggests silica leads to a Th1 type immune response with a decrease in total IgG due to decreased B cell numbers. This work was supported by NIH grant ES-04804.

1808 IMMUNOREGULATORY ADJUVANT EFFECT OF NO IN DRUG-INDUCED IMMUNOSENSITIZATION.

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Many drugs elicit autoimmune-like features in susceptible patients. The chemodrug streptozotocin (STZ) is commonly used as a diabetogenic compound in animals. STZ-induced diabetes resembles immune-dependent diabetes as it involves macrophages, CD8+ cells and high production of IFNγ. STZ contains a nitrosamine-group and releases nitric oxide (NO) *in vivo*. Previous studies suggested that NO is responsible for inducing type-1 immunological features and activity of macrophages by STZ. We further investigated this hypothesis using the reporter antigen popliteal lymph node assay (RA-PLNA) by testing the effects of modulation of NO-production and macrophage depletion on STZ effects and also on the effects the type-2 drug (D-penicillamine, D-pen). This assay is particularly suitable to study the adjuvant and immune deviating activity of compounds, using the same antigen (TNP-OVA) and mouse strain (BALB/c). Mice were s.c. injected with the compounds and 7 days later the PLN was excised and we measured antibodies, cytokines and performed flowcytometry. We found that NO-free STZ was ineffective in eliciting a response. Additionally, methylnitrosurea (MNU, i.e. STZ without sugar moiety), but not NO-free MNU, also induced a type-1 response. However, a non-related NO-donor S-nitroso-N-acetylpenicillamine (SNAP) induced a clear type-2 response to TNP-OVA and SNAP shifted the response induced by STZ towards a type-2 phenotype. The D-pen-induced response was also elevated after SNAP-treatment. As STZ causes a marked influx of macrophages, iNOS-derived NO may also affect the T and B cell responses. The iNOS inhibitor aminoguanidine inhibited both type-1 and type-2 drug-induced responses, whereas *in vivo* depletion of macrophages resulted in a decrease in type-1 features (IgG2a) but an increase in type-2 (IgG1) in STZ-induced responses and no change at all in D-Pen responses. Together, both endogenous and exogenous NO adjuvate drug-induced type-2 responses, whereas it shifts STZ-induced type-1 responses to type-2. The type-1 deviating capacity of STZ may thus not be due to NO per se but may rather result from STZ-dependent activation of macrophages.

1809 COSTIMULATORY CD80, CD86 AND CTLA-4 SIGNALING ARE REQUIRED IN TYPE-2 BUT INDISPENSIBLE IN TYPE-1 IMMUNE RESPONSES ELICITED BY AUTOIMMUNOGENIC DRUGS.

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Some pharmaceutical drugs provoke autoimmune-like derangements in susceptible subjects. Sensitization responses by these chemicals can broadly be subdivided in two types. Type-1 responses, elicited by the diabetogen streptozotocin (STZ), are characterized by macrophages, CD8+ cells, IFN-γ and IgG2a whereas the anti-rheumatic drug D-Penicillamine (D-Pen) induces B cells, IL-4, IL-5, IgG1 and IgE; features of type-2 responses. Besides the specific recognition of antigens, costimulatory signals (CD80, CD86, CTLA-4) between immune cells are required to regulate the response and they contribute to deviation of responses in type-1 or type-2. In this study we used STZ and D-Pen in the reporter antigen popliteal lymph node assay (RA-PLNA) to create typical type-1 or type-2 features in responses to TNP-OVA in BALB/c mice. The PLN was excised 7 days after s.c. exposure. We observed an increase in CD86+ B cells in the type-2 response and less CD86+ but far more CD80+ non-B cells (macrophages, dendritic cells) and CTLA-4 expression in the type-1 response. When treating D-Pen-exposed mice with anti-CD80, anti-CD86 or both, IL-4, IgG1, IgE, IgM and ICAM-1 expression were decreased. Anti-CLTA-4 increased all these type-2 parameters. On the contrary, STZ-induced type-1 features were not influenced or even slightly increased after anti-CD80 and/or anti-CD86 or anti-CTLA-4 treatments. In this STZ-induced response CD80 and CD86 might preferentially bind to CTLA-4, which is significantly upregulated, and may therefore provide a downregulating signal. Additionally, we showed the lack of a type-2 response in CD80/CD86 KO mice whereas the STZ-induced type-1 phenomena were still operational in these mice. In summary, we conclude that interactions between CD28/CTLA-4 and CD80/CD86 are highly balanced and crucial in eliciting a D-Pen-induced type-2 response. The STZ-induced type-1 features, however, are indisputably operational when blocking these molecules or in CD80/CD86 KO mice and thus not dependent on this costimulatory interaction.

1810 PENICILLAMINE (PA)-INDUCED AUTOIMMUNITY: NEW INSIGHT INTO THE MECHANISM OF COVALENT BINDING *IN VIVO*.

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BACKGROUND: Protein covalent binding of drugs/reactive metabolites has been associated with idiosyncratic drug reactions (IDRs). To study the mechanism of IDRs, and the role of covalent binding *in vivo*, we use an animal model in which Brown Norway (BN) rats develop an autoimmune syndrome after exposure to PA. We have shown covalent binding in BN rats that developed signs of autoimmunity. Moreover, immunohistochemistry (IHC) revealed a pattern of binding to collagen-like fibers. **OBJECTIVES**: (1)perform a time course for binding in BN rats before they develop signs of the syndrome. (2)test for covalent binding in Lewis rats (LW), a strain that is not susceptible to PA-induced autoimmunity. (3)determine if the binding involves a disulphide or thiazolidine link. **METHODS**: Covalent binding studies were done with anti-PA rabbit immune serum by Western Blotting (WB) and IHC. Male rats were treated with PA and tested for covalent binding on days 3, 7, and 14 (BN) or 3 weeks (LW). For *in vitro* studies, liver, skin and spleen from control were incubated with PA at 37°C for 24 hours. WB under reducing versus non-reducing conditions was compared to our results from *in vivo* covalent binding studies. **RESULTS and CONCLUSIONS**: Binding was present by 3 days but was greater in sick animals than in animals that did not get sick. Binding was as great in LW rats as in BN rats. *In vitro* binding was absent (skin/liver) or low (spleen) suggesting that the equilibrium with protein disulfides favors the native disulfide; yet, binding was eliminated under reducing conditions which suggests that most of the binding detected involves a disulfide bond. In contrast, *in vivo*, binding in spleen was not eliminated under reducing conditions suggesting thiazolidine linkage is involved. Since BN (day 3) and LW rats displayed the same pattern of covalent binding, we suspect that binding is required but not sufficient to cause an IDR. We cannot determine with these experiments whether increased binding in sick animals contributes to sickness or is a result of unmasking of antigen during inflammation.

1811 EFFECT OF TWO PEDIATRIC VACCINES ON INSULIN-DEPENDENT DIABETES MELLITUS (IDDM) IN FEMALE NOD MICE.

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The induction or exacerbation of autoimmune diseases is a potential adverse effect of immunostimulating drugs. Vaccines have been suspected to increase the incidence of, or reveal autoimmune diseases, even though epidemiological studies have so far failed to demonstrate any causal relationship between vaccination and autoimmune diseases. In this study, groups of 15 female NOD mice received three intraperitoneal injections of the multivalent diphtheria, tetanus, pertussis, poliomyelitis, haemophilus vaccines DTaP-IVP or DTaP-IVP/Hib (1/100 the human dose) at 2-week intervals from 10 weeks of age. The immunostimulant tilorone hydrochloride (2 mg/mouse) was used as a positive control. Both vaccines and tilorone had a similar effect on IDDM as evidenced by a significant reduction in blood and urinary glucose levels. At the end of the study, 90 % of control mice had elevated blood glucose levels in contrast to 60 % of mice treated with either vaccine. Positive urinary glucose levels (according to a standard dipstick) were noted in 15 %, 40 %, and 33 % of mice treated with DTaP-IVP, DTaP-IVP/Hib and tilorone respectively, and in 53 % of control mice. A slight prolongation in survival was noted in mice treated with either vaccine. This study is the first report of a possible protecting effect of pediatric vaccines in IDDM-prone mice.

1812 HCB-INDUCED IMMUNOPATHOLOGY IS PARTLY MEDIATED BY T CELLS.

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Hexachlorobenzene (HCB) is a persistent environmental pollutant with (auto)immune effects in man and rat. The Brown Norway (BN) rat is susceptible to HCB and oral exposure causes inflammatory skin and lung lesions, splenomegaly, lymph node (LN) enlargement and increased serum levels of IgE and autoantibodies. Whether this is caused by HCB or by its metabolites and if T cells are involved is unknown. By using thymectomized BN rats it was shown that induction of lung lesions was thymus-independent, but the induction of skin lesions was delayed and seemed partly T cell dependent (TAP, 1999, 161(2):180). Since there were still functional T cells present in these rats, the role of T cells needed further investigation. To elucidate the role of T cells we used Cyclosporin A (CyA) to inhibit T cell function. BN rats were exposed to either a control diet or a diet supplemented with 450 mg/kg diet HCB for 21 days. CyA treatment started 2 days prior to HCB exposure. Rats were injected daily with 20 mg/kg bwt CyA. Treatment with CyA diminished the HCB-induced immunopathology significantly. Development of skin lesions was delayed and the severity was also strongly decreased. Also, CyA declined the HCB-induced increase in liver, spleen and LN weights. T cell dependent processes like increases in serum IgE and autoantibodies were reduced to background levels. Lungs were still infiltrated by macrophages, but the perivascular eosinophilic infiltrate was greatly reduced. Together, our results indicate that T cells seem to play a prominent role in HCB-induced skin lesions, the enlargement of spleen and LN, the increase in serum IgE and autoantibody levels and the attraction of eosinophils into the lungs. However, macrophage infiltration into the lungs is T cell independent. We hypothesize that HCB initially attracts phagocytes which adjuvates an adaptive immune response. The interplay of both macrophages and T cells in the HCB-induced immunopathology needs further investigation.

1813 ACCELERATION OF THE DEVELOPMENT OF LUPUS IN NZBXNZWF1 MICE BY CHLORDECONE.

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Systemic lupus erythematosus (SLE) is a debilitating disease affecting women nearly nine times as often as men. Previously, we reported the ability of three organochlorine pesticides with estrogenic effects (methoxychlor, 50 mcg/day; chlordecone, 30 mcg/day; and o, p-DDT, 15 mcg/day) to accelerate the onset of disease in lupus-prone (NZBxNZW)F1 mice. Ovariectomized female mice treated continuously with any of these pesticides experienced earlier mortality from immune glomerulonephritis, the most severe manifestation of SLE in this mouse strain, compared with placebo controls. The most pronounced effect was observed for chlordecone. In follow-up studies reported here, lower doses of chlordecone (0.167 to 16.7 mcg/day) were tested in ovariectomized female (NZBxNZW)F1 mice. Chlordecone was administered in continuous-release pellets implanted subcutaneously. Mice treated with pellets without chlordecone served as controls. All doses of chlordecone decreased the time to onset of clinical SLE, defined for the purposes of this experiment as significant renal impairment. The decrease in time to onset in the highest dose group (16.7 mcg/day) was statistically significantly. Titers of serum anti-dsDNA were increased in a dose-related manner from 0.167 to 8.3 mcg/day chlordecone, with a drop-off in titers at the 16.7 mcg/day dose. Chlordecone did not produce detectable estrogenic effects in this dose range, as assessed by measurement of uterine hypertrophy. Also, when higher doses of methoxychlor, chlordecone, and o, p-DDT from previous experiments were compared, their effects to accelerate lupus were not in concordance with their estrogenic effects on the uterus. This apparent absence of concordance may indicate that these pesticides affect SLE through a mechanism unrelated to estrogenicity, or that estrogenic effects may be responsible, but occur at lower doses in the immune system as compared with the uterus. Supported by ES07375.

1814 OMEGA-3 FATTY ACIDS FROM FISH OIL SUPPRESS IGA NEPHROPATHY INDUCED BY THE MYCOTOXIN DEOXYNIVALENOL.

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Primary IgA nephropathy (IgAN) is an autoimmune disease which has as its hallmarks, elevated serum IgA level and kidney mesangial IgA deposition. Fish oil can retard the progression of IgAN in humans and in a mouse model induced by feeding the trichothecene deoxynivalenol (DON, vomitoxin) In this study the effects of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), the two main omega-3 polyunsaturated fatty acids in fish oil, on DON-induced IgAN were observed. Mice were fed for 18wk with AIN-93G diet containing 1g/100 g corn oil and 6g/100g oleic acid (CONTROL) or 1g/100g corn oil and 6g/100g oleic acid and 10ppm DON (CONTROL+DON) or 1g/100g corn oil and 2.5g/100 g DHA and 3.5g/100g oleic acid and 10ppm DON (DHA+DON) or 1g/100g corn oil and 2.7g/100g EPA and 3.3g/100g oleic acid (EPA+DON) or 1g/100g corn oil and 2.3g/100g DHA/EPA and 3.7g/100g oleic acid and 10ppm DON (DHA/EPA+DON). DON significantly increased serum IgA, serum IgA immune complexes and kidney mesangial IgA deposition. DHA, USEPA and DHA/EPA treatment significantly attenuated all three immunopathological parameters as compared to CONTROL+DON group. Furthermore DHA, USEPA and DHA/EPA significantly reduced IgA secretion by spleen cells suggesting that systemic IgA production was depressed. Taken together, the results suggest that both DHA and EPA can impair DON-induced IgAN. (Supported by NIH grants DK58833 and ES03358)

1815 SUPPRESSION OF DEOXYNIVALENOL-INDUCED IL-6 BY FISH OIL AND RELATIONSHIP TO MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION.

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Dietary fish oil has been shown to block progression IgA nephropathy in human clinical studies as well as in a murine model induced by the trichothecene mycotoxin deoxynivalenol (DON, vomotoxin). Among possible mediators of this disease, interleukin-6 (IL-6) may play a pivotal role in IgA elevation and disease aggravation. Therefore, IL-6's potential as a target for the therapeutic effects of fish oil was assessed in both *in vivo* and *in vitro* models employing DON. Mice were fed with corn oil (CO)- or fish oil (FO)-containing diet for 8 wk and then acutely exposed to DON. Serum IL-6 was significantly elevated in CO-fed mice administered DON and this effect was suppressed in FO-fed mice. IL-6 mRNA levels of spleen were similarly affected. Prostaglandin E2, another proinflammatory mediator reportedly by FO, was measured but no significant differences were found between CO- and FO-fed groups. Mitogen-activated protein kinases (MAPKs), crucial signal-transducing mediators in induction IL-6 and other inflammatory genes, are activated by DON *in vivo* and *in vitro*. DON-induced phosphorylation of ERK1/2 and JNK1/2 in spleen were significantly suppressed by dietary FO. In contrast, DON-mediated activation of p38 MAPK was slightly enhanced in FO-fed mice. To further confirm *in vivo* data, two omega-3 polyunsaturated fatty acids that are specific components of FO, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), were added to RAW 264.7 macrophage cells that were treated with lipopolysaccharide (LPS) and DON. DON superinduced LPS-driven IL-6 production but this was suppressed by EPA and DHA. DON-activated ERK1/2 and JNK1/2 phosphorylation were also reduced when cells were pre-treated with EPA and DHA. Taken together, FO retarded IL-6 production and ERK1/2 and JNK1/2 activation in spleens of mice exposed to DON. Moreover, omega-3 PUFA similarly repressed DON-mediated ERK1/2 and JNK1/2 activation, which appeared to contribute to decrease DON-induced IL-6 superinduction in macrophage cells (supported by Public Health Services Grants DK 588833, ES 03358 and ES 09521).

1816 EFFECTS OF DIETARY OMEGA-3 FATTY ACIDS ON DEOXYNIVALENOL-INDUCED GLOBAL GENE EXPRESSION *IN VIVO*.

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Dietary omega-3 fatty acid supplements have been reported to inhibit immune and inflammatory responses. Deoxynivalenol (DON), a trichothecene mycotoxin, upregulates inflammatory gene expression *in vivo*. Interestingly, chronic DON administration induces IgA nephropathy in mice, which can be attenuated by omega-3 fatty acid supplementation. In this study, cDNA arrays were used to characterize the effects of omega-3 fatty acid supplements on DON modulation of global gene expression in lymphoid tissue. Mice were fed diet containing corn oil (CO) or a diet containing 1% docosahexaenoic acid and 1% eicosapentaenoic acid (DHA/EPA) for 12 wk. Mice were treated with 25 mg/kg DON p.o. and 2 hr later, spleen samples were collected. The mRNA was extracted from spleen samples for analysis on mouse cancer 1.2 cDNA arrays (Clontech). Spot intensities were normalized using a local linear regression model. Of 1200 genes on the array, 386 genes were significantly increased or decreased above average expression levels in all treatment groups. Genes were arranged into an ontology tree using GeneSpring software to help compare expression profiles between treatment groups. IL-6 expression was highly expressed in DON-treated CO-fed mice and this was drastically repressed in DON-treated DHA/EPA-fed mice. Furthermore, basal IL-6 expression was much lower in DHA/EPA-fed mice than CO-fed mice. Eight other genes were found to have a greater than 0.9 correlation to the expression profile of interleukin 6. These included interleukin 1 alpha, small inducible cytokine subfamily (member 2), FBJ osteosarcoma oncogene, pigment epithelium-derived factor, fos-like antigen 2, DNAJ-like 2 heat shock protein, cysteine rich protein 61, and Jun oncogene. The results suggest that DON affects expression of multiple genes that may contribute to its immunotoxic effects and this expression profile can be affected by diet. (supported by Public Health Services Grants DK 588833 and ES 03358)

1817 DEVELOPMENTAL EXPOSURE TO DI-n-BUTYLTIN DICHLORIDE (DBTC): IMMUNOTOXIC AND NEUROTOXIC EVALUATION.

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Organotins are incorporated as stabilizers in PVC water supply pipe. Particularly when new, mono- and di-substituted methyl- and butyltins leach from the pipe and are thus of regulatory concern to EPA. These contaminants have adverse effects on both the immune and nervous systems under laboratory conditions. The toxicity of other members of this class, tributyltin and dioctyltin, is greater in the immature immune system; thus, DBTC was evaluated following combined gestational and lactational exposure in offspring of Wistar rats exposed by gavage with 0 (olive oil), 2.5 or 5.0 mg DBTC/kg/d from gestational day 6 until weaning on post-natal day (PND)21. Separate groups of pups born to normal dams were exposed to the 10 doses of the same concentrations by gavage, 3 time/wk from PND3 through PND22. Immune system endpoints included body, spleen and thymus weights, antibody responses to a T-dependent antigen and delayed hypersensitivity responses. Potential neurotoxicity was evaluated by measuring brain weight. Immune function was not affected by either exposure regimen, although lower body weights and increased spleen and thymus weights were noted on PND38 in nonimmunized rats at the highest dose, suggesting that an earlier decrease in lymphoid organ weight may have occurred. Normal immune function, particularly in the directly-dosed pups, was an unexpected finding, given the demonstrated immunotoxicity of organotins in adult rats. Direct exposure to DBTC caused a dose-related delay in brain weight gain on PND38 at both exposure levels. Brain weights were similar in control and low dose pups on PND48; however, there was essentially no increase in brain weight in higher dose-group pups between PND30-PND48, in spite of a 25% increase in body weight. Based on these data, it appears that the developing nervous system may be more sensitive to DBTC than is the immune system. (This abstract does not reflect EPA policy.)

1818 DEVELOPMENTAL IMMUNOTOXICITY OF COCAINE AND KETAMINE IN POSTNATAL RATS.

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The abuse of cocaine (COC) with ketamine (KET) among pregnant women was shown to be high. Transplacental exposure is not the only route by which a newborn may be exposed to these agents, they can distribute into breast milk. Chronic COC exposure is associated with immunological modulation in humans and animal models. The effect of sub-chronic exposure to COC and KET alone and in combination on the developing immune system was assessed in postnatal male rats. To simulate the route of exposure during lactation, newborn male rats were orally treated with saline, COC (20 mg/kg), KET (50 mg/kg) or KET followed 15 min later by COC (50/20 mg/kg) from day 1 to 21 of life. Male rat pups were sacrified 30 min following the last treatment. Total circulating leukocyte and lymphocyte counts were decreased with relative neutrophilia while; spleen/bodyweight ratios and IgM antibody response to sheep erythrocytes (SRBCs) were increased in animals treated with COC. Moreover, COC produced a shift in T-helper 1/T-helper 2 (Th1/Th2) balance promoting Th2 type response. On the other hand, KET treat-

ment did not produce any significant change of any of these parameters. However, when co-administered with COC these immunotoxic effects were antagonized. Studying the possible mechanisms of COC-induced immunotoxicity revealed that COC caused a significant increase in serum corticosterone concentration. Co-administration of KET effectively blocked the stimulatory effect of COC on serum corticosterone without any significant change of plasma and tissue concentrations of norcocaine (NC). The results of this study indicate that exposure to COC in early postnatal period can induce immunotoxic reactions which were antagonized by KET most likely through neuroendocrinal mechanisms.

1819 LIFETIME EXPOSURE TO TRICHLOROETHYLENE (TCE) MODULATES IMMUNE FUNCTION.

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Trichloroethylene (TCE) is an industrial solvent used in the cleaning and degreasing of metal components in various machine industries. Not only is it commonly inhaled during occupational situations, but its widespread use has resulted in groundwater contamination leading to human exposure *via* drinking water. It has been reported, in murine studies, that TCE can both exacerbate autoimmune disease and suppress immune function. While these studies have addressed effects in adult rodent models, none have explored immunological effects during developmental stages. To determine the immunological effects of TCE in B6C3F1 mice, exposure to TCE in drinking water (1000 ppb or 10, 000 ppb) began when pairs were mated (female C57 and male C3H mice) and continued through weaning (21-day old) or adulthood (56-days old). The vehicle control group was administered emulphor-treated water. Endpoints assessed included splenic and thymic weights and cellularity, natural killer cell (NK) activity, antibody plaque forming cell (PFC) response, lymphocyte proliferation, and T-cell immunophenotypes. At 21 days of age, alterations were evident. Body weight and length were significantly decreased by the 10, 000 ppb treatment. NK cell activity and T- and B-cell proliferation were not altered. IgM antibody responses to sRBC challenge were suppressed in both male and female pups by 10, 000 ppb TCE and by 1000 ppb TCE in the male pups only. Additionally, there was a distinct decrease in splenic CD4+CD8- T-cells resulting in a concomitant decrease in the CD4+:CD8+ ratio. At 56-days of age, the most striking effect was noted with increased NK cell activity in both treatment groups. Currently studies are being conducted to verify the PFC response and T-cell immunophenotypes in the adult mice. These data suggest that lifetime exposure to TCE modulates both innate and adaptive immune responses and this should be considered when assessing health risks to TCE.

1820 IMMUNOTOXICOLOGICAL ASSESSMENT OF A P38 MAP KINASE INHIBITOR.

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The p38 MAP kinase signaling pathway plays a pivotal role in the initiation and progression of inflammation. Compounds that inhibit this kinase inhibit the production of inflammatory cytokines and are potential therapies for inflammatory diseases. A p38 kinase inhibitor was tested to evaluate its effects on the net health of the immune system using the mouse sheep red blood cell (SRBC) antibody forming cell response and host resistance model to influenza virus. Female B6C3F1 mice were administered the p38 kinase inhibitor by oral gavage for 28 days at doses of 0, 3, 30 and 300 mg/kg/day. Animals were immunized with SRBC and the number of splenic cells producing antibody (AFC) to SRBC was quantitated through the production of hemolytic plaques. Cyclophosphamide was administered as a positive control. In the influenza host resistance model, Balb/c mice were administered the p38 kinase inhibitor at doses of 30 and 150 mg/kg/day or anti-TNFα antibody at 250 μg/week, 7 days prior to a mouse-adapted influenza virus intranasal infection. Administration of the compound continued post-infection for 21 days. Dexamethasone was used as a positive control. Analyses included the measurement of the clearance of infectious influenza virus on Day 2, 6, 8, 10 and 21, and influenza specific IgG in the lung on Day 2, 10 and 21. Results of the studies were as follows: A decrease of 48% and 70% in the antibody forming cell response was observed with the p38 kinase inhibitor at a dose of 30 and 300 mg/kg/day, respectively. The compound did not affect any of the parameters evaluated in the influenza host resistance model. A significant decrease in AFC response was observed with cyclophosphamide and dexamethasone significantly reduced viral clearance and IgG production. A small decrease in influenza specific IgG production was noted with the anti-TNFα antibody on Day 21. In conclusion, although modulation of the immune response was observed with the p38 kinase inhibitor this did not affect the ability of the host to clear or eliminate a viral infection.

1821 EFFECT OF DIESEL EXHAUST PARTICULATE (DEP) ON BACILLUS CALMETTE-GUERIN (BCG) LUNG INFECTION IN MICE.

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The effect of exposure to diesel exhaust particulate (DEP) on Bacillus Calmette-Guerin (BCG) lung infection in mice was studied. C57Bl6 female mice were infected with BCG (2.5x10^4 bacteria/mouse) by tracheal aspiration with or without co-administration of DEP (100 mcg/mouse). Five weeks later, mice exposed to DEP+BCG had about four-fold higher BCG load in lungs than mice exposed only to BCG ($p<0.05$). DEP treatment alone had no effect on the total number of lung lymphocytes or numbers of T, B or NK cells recovered from lungs. In contrast, BCG infection significantly increased recovery of all types of lymphocytes from lungs. Co-exposure to DEP+BCG further increased the recovery of lymphocytes from lungs of BCG infected mice. The pulmonary lymphocyte subpopulation expressing the greatest levels of mRNA for IFN gamma after BCG infection was CD4+ T cells and expression levels were similar in mice exposed to BCG or BCG+DEP. Recovery of IFN gamma secreting T cells was significantly higher ($p<0.05$) from lungs of BCG and BCG + DEP infected mice as compared to control or DEP only exposed mice, but BCG and BCG+DEP groups of mice were not significantly different. These results indicated that co-exposure to DEP+BCG did not significantly affect the level of IFN gamma response of mice to BCG infection. However, *in vitro* studies demonstrated DEP treatment inhibited IFN gamma induced nitric oxide secretion by mouse alveolar macrophages. Thus, DEP exposure did not alter the IFN gamma response to BCG infection, but reduced a key microbiocidal response of macrophages to IFN gamma. Reduced responsiveness of DEP exposed macrophages to IFN gamma may contribute to a less efficient clearance of BCG from the lungs of BCG infected mice.

1822 ROLES OF REACTIVE OXYGEN SPECIES, HEME OXYGENASE-1, AND NITRIC OXIDE IN DIESEL EXHAUST PARTICLE-MEDIATED PULMONARY IMMUNE RESPONSES TO *LISTERIA MONOCYTOGENES* IN RATS.

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This study examines the hypothesis that diesel exhaust particles (DEP) suppress pulmonary immunity to *Listeria monocytogenes (Listeria)* through the induction of reactive oxygen species (ROS), heme oxygenase-1 (HO-1) and altered cytokine production by alveolar macrophages (AM) and lymphocytes. Cells were isolated from Brown Norway rats intratracheally inoculated with saline or 100, 000 *Listeria* at 7 days post-infection. The *Listeria*-infected AM showed increased production of IL-6, IL-10, IL-12, and TNF-α over the saline control in response to lipopolysaccharide (LPS), whereas the *Listeria*-infected lymphocytes showed increased production of IL-2, IL-10, and IFN-γ when challenged with concanavalin A (ConA) or heat killed Listeria (HKLM). DEP or DEP extract, but not the washed DEP, inhibited AM secretion of IL-6, IL-12, and TNF-α and lymphocyte production of IL-2 and IFN-γ, but enhanced AM production of IL-10. The effect of the DEP extract on cytokine production was preceded by a time-dependent induction of ROS and ROS-induced HO-1 protein and activity in AM. α-Naphthoflavone (ANF), a CYP 1A1 inhibitor, partially inhibited DEP-induced ROS and HO-1 expression and reversed the DEP effect on cytokine secretion. L-NAME (N-nitro-L-arginine methyl ester), a NO synthase inhibitor, inhibited the DEP-induced ROS generation and HO-1 induction, but augmented the DEP-induced IL-10 production by *Listeria*-infected AM, suggesting that NO down-regulates IL-10 production. Similar to DEP extract, hemin induced HO-1 expression, an increase in IL-10 and a decrease in TNF-α production by AM. In comparison, DEP extract at a level that induced less HO-1 than hemin, showed greater effect on IL-10 secretion. These results show that both HO-1 and NO play a role in AM production of IL-10, and that due to its organic content, DEP suppress the host immune responses by inhibiting the innate and T cell-mediated immunity and augmenting AM production of IL-10 (NIH HL-62630).

1823 EXACERBATION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION BY ULTRAFINE CARBON BLACK PARTICLE EXPOSURE.

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Exposure to particulate matter (PM) may exacerbate preexisting respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis and pneumonia, however few experimental studies have addressed the effects of PM on

lower respiratory tract (LRT) viral infection. Respiratory syncytial virus (RSV) is a major etiological agent for LRT infections in infants, the elderly, and the immunocompromised, and may lead to chronic wheezing and the development of asthma in children. In this study we examined the effects of ultrafine particle exposure on RSV-induced pulmonary inflammation, chemokine and cytokine expression, and airway hyperresponsiveness in a mouse model of RSV. Female BALB/c mice were instilled *via* the trachea (i.t.) with 1x106 pfu RSV or with uninfected culture media. On day 3 of infection, mice were i.t. instilled with either 40 μg ultrafine carbon black (CB) particles or with saline vehicle. Endpoints were examined on days 4, 5, 7, and 14 of RSV infection. Viral titer and clearance in the lung were unaffected by CB exposure. Neutrophil numbers were elevated on days 4 and 7, and lymphocyte numbers were higher on days 4 and 14 of infection in CB-exposed, RSV-infected mice. CB exposure also enhanced RSV-induced airway hyperresponsiveness to methacholine, BAL total protein, and virus-associated chemokines MCP-1, MIP-1α, and RANTES. Immunohistochemistry and laser capture microdissection revealed that MIP-1α expression was localized to the alveolar epithelium, where ultrafine particles deposit in the lung. These data demonstrate a synergistic effect of particles on RSV infection, and suggest a mechanism for increased pneumonia in human populations after PM exposure.

1824 VARIED EXPOSURE REGIMES TO METHYL MERCURY (MEHG) DURING POSTNATAL DEVELOPMENT LEADS TO DIFFERENT IMMUNE RESPONSES.

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Infantile autism (IA) is a neurodevelopmental syndrome with 1-5 cases found in every 10, 000 children. The spectrum of autism disorders includes a range of impaired communication and language development. Also reported are diverse immunological effects including decreased T-cell proliferative function and activation, increased serum IL-2 levels, decreased CD8+ cells, decreased NK cell function, and development of anti-neural autoantibodies. Several studies indicate that the etiology of IA is multi-factorial and includes exposure to environmental chemicals. In particular, Thimerosal, an adjuvant in vaccines that contains ethyl mercury, has been implicated in IA. This association has been criticized due to a lack of supportive experimental dose-response data. Consequently, this project was designed to compare dose-responsive immunological effects between Thimerosal and methyl mercury, a known immunotoxicant. In this phase of the study, B6C3F1 pups were exposed to MeHg (10 or 50 ug/kg) and two different exposure regimes were examined during postnatal development. Pups were exposed either on postnatal days (PND) 7, 10, and 12 or weekly on PND 7, 14, and 21. On PND 22, T-cell proliferation and the IgM plaque forming cell response were enhanced after exposure on PND 7, 14, and 21, but not affected after exposure on PND 7, 10, and 12. Weekly exposure to 50 ug MeHg/kg prior to weaning resulted in selective increases in CD8+ thymic T cells, whereas 10 ug MeHg/kg exposure on PND 7, 10, and 12 increased splenic CD4+ T-cell subpopulations. The exposure regime utilized during developmental periods must be considered when assessing mercury exposure, as this affects responses in immune parameters. This understanding may be useful when evaluating vulnerable periods of mercury exposure in children. Future studies will compare effects of Thimerosal with MeHg, and include assessment of cytokine levels, autoantibody production, serotonin levels and cognitive function.

1825 MERCURY (Hg) ACCELERATES AUTOIMMUNE DISEASE IN MICE.

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Inorganic mercury (iHg) has a range of immunotoxic effects, including induction of autoimmunity in certain inbred strains of rats and mice. However, no associations between Hg and autoimmune disease have been shown in humans. We hypothesize that expression of frank disease may require interactions between Hg and other susceptibility factors, genetic or acquired. To test this hypothesis, we examined the effects of iHg pretreatment on the pathophysiology of experimental autoimmune disease in two well characterized models, the graft versus host disease (GVHD) model of chronic systemic lupus erythmatosis (SLE) and the cardiac myosin peptide (CMP) model of autoimmune myocarditis (AIM). We induced SLE by transfer of maternal splenocytes into C57Bl/6xDBA/2-F_1 hybrid offspring; and we induced AIM by immunization of A/J mice with purified murine CMP. In GVHD, both donors (female DBA/2) and hosts (female B6D2-F_1) were exposed to 20 or 200 mcg/kg $HgCl_2$ by s.c. injection every other day for 15 days; on day 20, GVHD was initiated by splenocyte transfer and disease was evaluated over 4 months. In the AIM model, male A/J mice were pretreated similarly with $HgCl_2$ at 10 or 100 mcg/kg; on day 20, mice were administered 100 nmol CMP s.c. and pertussis toxin i.p. Disease was monitored by observation, interim analyses of serum autoantibodies, and histopathological evaluation of the heart at sacrifice. The results are consistent with the hypothesis that Hg can interact with other risk factors to induce frank autoimmune disease, at low doses of Hg and in strains of mice (DBA/2, C57Bl/6) that are not susceptible to Hg immunotoxicity. *Research supported by the Arthritis Foundation and NIH (NIEHS, NHLBI).*

1826 EFFECT OF INORGANIC MERCURY ON PRIMARY MOUSE AND HUMAN MONOCYTE FUNCTION.

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Transition metal ions are toxic to human tissues and organs. Although they can be toxic to immune cells, they can also induce gene expression (including metallothioneins [MTs]) in the absence of toxic events. The consequences of non-toxic metal exposure on immune function are largely unexplored. Innate immune activation involves the acute phase response and respiratory burst of mononuclear cells (including monocytes) in response to extracellular signals. We have reported that *in vitro* treatment of immortalized human monocytes with zinc, cadmium, or mercury, at levels at least tenfold lower than that required to exert detectable cellular injury, significantly inhibits monocyte activation potential. We now report that that *in vitro* pre-treatment of primary human and mouse monocytes, isolated from peripheral blood, with low, non-cytotoxic levels of zinc (40 μM) or mercury (2 μM) have significantly decreased activation potential (assessed by reactive oxygen production and interleukin-1β [IL-1β] mRNA expression in response to phorbol myristate acetate [PMA]). In addition, pre-treatment of mice with low, non-toxic levels of inorganic mercury (0.2 - 1 μmole Hg/kg body weight, i.p.) had no effect on monocyte viability, but significantly inhibited the ability of primary peripheral blood monocytes to undergo LPS- or PMA-induced respiratory burst and differentiation into adherent macrophages. These data indicate that low level exposure to inorganic zinc or mercury inhibits primary monocyte activation potential (and, therefore, innate immune function) in the absence of cytotoxicity. The connection between low, non-toxic metal ion exposure and innate immune function, combined with the critical role of innate immunity in resistance to infection and tumour formation, highlight the potential importance of environmental metals in these pathological events.*Supported by grants from the NIEHS (ES11288) and CIHR*

1827 *IN VITRO* EXPOSURE TO SODIUM ARSENITE INCREASED INTRACELLULAR CA^{2+} LEVELS IN PHYTOHAEMAGGLUTININE STIMULATED HUMAN T LYMPHOCYTES.

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Arsenic exposure inhibits murine and human T lymphocyte proliferation. Free intracellular ($[Ca^{2+}]i$) levels have been implicated as a regulatory signal for proliferation and death cell processes. This prompted us to investigate whether sodium arsenite ($NaAsO_2$)induced changes in $[Ca^{2+}]i$ of human stimulated human T lymphocytes and if this alteration was associated with the inhibitory effects of arsenic on T cell proliferation. We used mononuclear cells from healthy human subjects to measure free $[Ca^{2+}]i$ levels by spectrophotofluorometry using Fluo-3AM label. Proliferation was determined by [methyl-^{3}H]-thymidine incorporation. *In vitro* exposure to $NaAsO_2$ (1.0 to 7.5 μM) dose-dependently increased free $[Ca^{2+}]i$ in both PHA-stimulated lymphocytes and non-stimulated cells. However the effect was more pronounced in mitogen-activated cells simultaneously treated with $NaAsO_2$ at concentrations producing inhibition of T cell proliferation. In cells pretreated with a calcium ionophore (A23187) at concentrations able to increase $[Ca^{2+}]i$ to levels comparable to those induced by $NaAsO_2$, PHA-induced proliferation was similarly inhibited. These results suggest that increased free $[Ca^{2+}]i$ levels play an important role in the inhibitory effect of arsenic on human T cell proliferative response. Partially supported by CONACYT 34508-M to ESCA.

1828 ARSENIC-INDUCED ALTERATIONS IN CONTACT HYPERSENSITIVITY.

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Chronic exposure to arsenic contaminated drinking water has been associated with neoplasias in multiple organ systems, including the skin, liver, bladder and lung. Previous studies in our laboratory indicate that arsenic alters the levels of growth

promoting and inflammatory cytokines in the skin. These cytokines regulate the migration and maturation of Langerhans cells (LC) during allergic contact dermatitis. Therefore, we hypothesized that arsenic may modulate the response to cutaneous sensitizing agents by altering cytokine production, and subsequently, LC mobilization/migration, and T-cell proliferation. To investigate this hypothesis, we examined the induction and elicitation phases of dermal sensitization, using the local lymph node assay (LLNA) and mouse ear swelling test (MEST). Mice exposed to 50 mg/l arsenic in the drinking water for four weeks demonstrated a reduction in lymph node cell proliferation and ear swelling following sensitization with 2, 4-dinitrofluorobenzene (DNFB), compared to mice that received plain water. Cultured lymphocytes from arsenic treated animals sensitized with fluorescein isothiocyanate (FITC), exhibited reduced proliferative responses following antigen-specific stimulation *in vitro*. In contrast, lymphocytes from non-sensitized, arsenic treated mice showed no alteration in mitogen-stimulated proliferation as compared to controls, suggesting that arsenic-induced suppression of cell proliferation is antigen specific and not a general anti-proliferative effect. LC and T-cell populations in the draining lymph nodes of DNFB-sensitized mice were evaluated by fluorescence activated cell sorting. LC numbers were reduced in the cervical lymph nodes, suggesting that LC migration may be altered following arsenic exposure. These studies suggest that arsenic exposure may modulate antigen-presentation and cell migration leading to suppression of dermal sensitization.

1829 ALTERATION ON IMMUNE CELLS SUBPOPULATIONS AND LYMPHOCYTE PROLIFERATION BY ARSENIC EXPOSURE IN INFANT POPULATIONS.

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Besides its carcinogenic effects, As has been described as immunotoxic. In animal models and human cells *in vitro*, As can modulate some transcription factors, cytokine secretion, and cell proliferation among other cellular activities (Germolec et al. 1996; 1998; Vega et al. 1999; 2001). Several human populations in the world are exposed to As *via* drinking water with high incidence of dermatological, vascular, neurological and carcinogenic affections. The effects of As exposure have been oriented to carcinogenic mechanisms and are mainly described in adults, no studies have been conducted with the aim of immunotoxic effects on humans neither in infant populations. We consider important to evaluate children populations since they could be more susceptible to As exposure effects. To address this question, we evaluated some parameters within the immunological status in an infant population exposed to As *via* drinking water. METHODS: PBMC from 80 children (6 to 10 years old) from an As exposed area were collected and stimulated with PHA for 72 h. Proliferation was evaluated by [^{3}H]-T incorporation, CD3, CD4, CD8, CD19 and CD57 subpopulations were determined by flowcytometry, IL-2, IL-4, IL-10, and IFN-γ secretion were evaluated by ELISA, As concentrations in urine were determined by HGAAS. RESULTS: Response to PHA lymphocyte stimulation and CD4/CD8 ratio were negatively associated with As exposure. Data obtained in this study indicate that the activation process on T cells could be altered, and thus represent an immunodepression status that could lead to an elevated incidence of opportunistic infections in children population. Study partially supported by CONACYT 34508-M to ESCA.

1830 FUNCTIONAL ACTIVITY OF TH1 AND MACROPHAGES FROM CHILDREN ENVIRONMENTALLY EXPOSED TO ARSENIC.

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Arsenic exposure has been associated with toxic effects at different levels including the immune system. Data from animal studies and scarce evidence from human adults, have shown that arsenic alters some immune system cells and affect T lymphocyte proliferation. However little is known about effects on children, a high risk group for the immunotoxicity of several chemicals. We studied the effect of arsenic exposure on Th1 and macrophage activation of children exposed to arsenic in drinking water (3.5 to 295.8 micrograms/L). We studied 87 children aged 6-10 years and having total urinary arsenic concentrations ranging from 12.3 to 1, 411 micrograms/L, determined by HGAAS. Th1 function was evaluated by measuring the capability of PHA-stimulated T lymphocytes to activate macrophages. Macrophage function was evaluated in the absence of T cells by direct activation with rhIFN-γ plus lipopolysaccharide. Macrophage activation was evaluated by superoxide anion and nitric oxide production. Superoxide anion production in directly activated macrophages was positively associated with total arsenic urinary concentrations whereas a negative association was observed with nitric oxide production. No significant association was found with macrophage activity in response to Th1 stimulation. These data suggest that macrophage activation in children is a relevant target for arsenic exposure. This effect could be related with a decreased resistance to those infections where macrophages play a protective role. Work supported by CONACYT 34508-M to ESCA.

1831 LEAD INTERACTION WITH ANTIGEN PRESENTING CELLS: A MECHANISM UNDERLYING Pb ALLO-ENHANCEMENT.

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Adverse health effects due to lead (Pb) exposure continue to be a concern. Pb is immunotoxic as evidenced by increased susceptibility to pathogens in intoxicated animals. To explore mechanisms of Pb immunotoxicity, we utilized the mixed lymphocyte reaction (MLR). In this model, irradiated murine splenocytes of haplotype H-2^b serve as stimulators and are co-cultured with non-irradiated responder splenocytes of haplotype H-2^d. Proliferative expansion of murine alloantigen-reactive CD4$^+$ T cells is called an allogeneic response. In the presence of low levels of Pb, this response is markedly increased, a process termed Pb allo-enhancement. Although it has been thought that CD4$^+$ T cells are direct targets of Pb immunotoxicity, the possibility that allo-enhancement is mediated by the interaction of Pb with antigen presenting cells (APCs) has not been ruled out. In MLR, 1μM Pb enhances the proliferation of CD4$^+$ T cells but not when T cells are stimulated independently of antigen. We hypothesize that APCs are direct targets for Pb immunotoxicity and that its effects on CD4$^+$ T cells are indirect through modulation of antigen presentation. This hypothesis was tested in MLR using fixed or irradiated C57BL/6 (H-2^b) splenocytes as allogeneic stimulators and BALB/c (H-2^d) splenocytes as responders. Pb treatment enhanced responder splenocyte proliferation when stimulated by irradiated stimulators. Although fixed stimulators still elicited an allo-proliferative response, Pb treatment had no effect on the level of this proliferation. These findings suggest a role for Pb in altering antigen processing/presentation at the level of APC. Experiments also were conducted to identify APC populations that are responsive to Pb allo-enhancement. Allo-enhancement was observed using splenic adherent cells, peritoneal exudate cells, enriched B cells, and an I-A^b expressing cell line, PMJ-2, as stimulators. These findings suggest that APCs are targeted in Pb immunotoxicity and lay the groundwork for identifying its mechanism of action. Supported by R29 ES 07365 and T32 ES 07026.

1832 METALLOTHIONEIN INTERACTIONS AT LEUKOCYTE PLASMA MEMBRANES.

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Metallothionein (MT) is a small stress protein that can be induced by exposure to heavy metal cations, and oxidative stressors, and can contribute to changes in immune function. Previous work from our laboratory has shown that exogenous MT can affect cell proliferation, macrophage and T lymphocyte function, and humoral immunity to T-dependent antigens. An anti-MT monoclonal antibody (clone UC1MT) has been shown to enhance humoral immunity to T-dependent antigens by blocking the suppressive effects of metallothionein. We have found that metallothionein can be detected on the surfaces of splenocytes from congenitally autoimmune mice at higher levels than normal littermate controls. In the studies described here, we continue to explore the interaction of extracellular metallothionein with leukocyte plasma membrane. Our findings show that metallothionein can be detected on the surface of splenocytes from adjuvant-treated animals, but not on the surface of the splenocytes from unimmunized animals or animals immunized in the absence of adjuvant. Similar findings could be produced *in vitro* with primary human leukocyte cultures. Incubation of human leukocytes with LPS or PHA for 72 h induces increases in the levels metallothionein that can be detected on the surface of these cells. T cells are the lymphocyte populations with the highest increases in surface MT after exposure to mitogen. These results suggest that MT released as a consequence of immune activation or toxicant exposure will bind to plasma membranes, and can alter the course of the immune response. These findings suggest that extracellular MT interactions with immune effector cells may prove to be a useful therapeutic target for the manipulation of undesirable immune effects caused by toxicants and other stressful agents. Supported in part by NIH grant ES 07408.

1833 INHIBITORY EFFECTS OF NICOTINE ON INFLAMMATION AND LEUKOCYTE MIGRATION.

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Cigarette smoking is a major cause of morbidity and mortality worldwide; additionally, smokers take longer to recover from injuries. Conversely, smokers have a lower risk for some inflammatory diseases and nicotine may have a therapeutic potential in some inflammatory diseases. We have reported that nicotine, a major component of cigarette smoke, suppresses the antibody response and T cell function. Using the turpentine-induced sterile abscess model, where inflammation is accompanied with increase in body temperature (fever), we show that chronic treatment of LEW rats with nicotine suppresses the fever response as well as the accumulation of leukocytes at the site of turpentine injection. Thus, in addition to adaptive immunity, chronic nicotine also suppresses the innate immune responses (i.e., inflammation and fever). To understand the mechanisms underlying the anti-inflammatory effects of nicotine, PBMCs from control and 3-wk nicotine-treated animals were analyzed for migratory responses to chemotactic stimuli in the transwell system. Our results show that, cells from nicotine-treated animals exhibit significantly lower migration in response to the neutrophil chemoattractant, formyl-methionyl-leucyl-phenylalanine (fMLP), or the lymphocyte chemoattractant, monocyte chemoattractant protein-1(MCP-1). Thus, chronic nicotine exposure inhibits the migration of leukocytes toward the site of inflammation. This effect might stem from the inability of leukocytes to respond to chemoattractants. Because inflammation is critical for wound healing process, the property of nicotine to moderate leukocyte migration might explain the suppressive effects of smoking in some inflammatory diseases and wound repair, and the therapeutic potential of nicotine in moderating some inflammatory diseases. Supported by a grant from NIDA (DA04208).

1834 ROLE OF P38 MAP KINASE IN REGULATING THE INHIBITORY EFFECTS OF UVB LIGHT ON CYCLOOXYGENASE-2 EXPRESSION IN MOUSE MACROPHAGES.

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Ultraviolet light of high energy and shorter wavelengths (UVB, 290-320 nm) is toxic to the skin. In normal skin, UVB light can induce an inflammatory response while in diseased tissue, it exhibits anti-inflammatory and immunosuppressive activity. It is well recognized that cells in the skin release lipid mediators, including prostaglandins (PGs) and leukotrienes that promote inflammation. The synthesis of PGs is dependent on the activity of cyclooxygenase (COX), an oxidoreductase that converts arachidonic acid into the common PG precursor, PGH_2. An inducible form of the enzyme known as COX-2 is expressed in the skin during inflammation. In earlier work we reported that UVB light is a potent inhibitor of lipopolysaccharide (LPS)-induced COX-2 expression in macrophages, a cell type known to mediate inflammation in diseased tissue. In the present studies, we examined the mechanism. Using RAW264.7 macrophages, we found that UVB light (2.5-25 J/cm^2) rapidly phosphorylates and activates the c-Jun-NH_2-terminal kinase (JNK), p44/42 mitogen-activated protein (MAP) kinase [extracellular signal-regulated kinase 1/2 (ERK1/2)], and p38 MAP kinase. PD-98059, an inhibitor the ERK1/2 kinase, and SP600125J, an inhibitor of the JNK kinases, were effective inhibitors of UVB light-induced ERK1/2 and JNK phosphorylation, respectively, in the macrophages. However, the inhibitors did not alter UVB light-induced inhibition of COX-2 expression. Unexpectedly, SB-203580 [(4-(4-Fluorophenyl)-2- (4-methylsulfinylphenyl)-5-(4-pyridil)imidazole], a p38 kinase antagonist, was found to block UVB light-induced p38 phosphorylation and inhibition of LPS-induced COX-2 in the macrophages. Taken together, these data indicate that activation of p38 MAP kinase plays an important role in mediating UVB light phototoxicity. Selective modulation of p38 MAP kinase may be an important target for treating epidermal inflammatory diseases. Support: NIH ES06897, ES03647 and ES05022

1835 COMPARISON OF THE ALLERGENIC POTENCY OF ALPHA-HEXYLCINNAMALDEHYDE (HCA) AND 2-MERCAPTOBENZOTHIAZOLE (MBT) IN SIX STRAINS MICE IN MURINE LOCAL LYMPH NODE ASSAY (LLNA).

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Keyword LLNA The Murine Local Lymph Node Assay (LLNA), which has been accepted by the OECD since 2000 as a stand alone alternative to the Guinea Pig Maximization Test (GPMT), has been largely used for assessing the allergic contact dermatitis potential of chemicals. According to OECD Draft Guideline No. 429 [November 2000] the animal species of choice are CBA/Ca or CBA/J mice. To test and compare the suitability of these and other mouse strains for assessing the allergenic potency of a chemical, two positive-control chemicals (HCA and MBT) were tested at three doses in the following six mouse strains at RCC: CBA/CaOlaHsd, CBA/Ca (CruBR), CBA/Jlbm (SPF), CBA/JNCrj, Balb/c and NMRI. For each strain, test groups of four mice were topically treated with HCA or MBT at 5%, 10% or 25% (w/w) in acetone:olive oil, 4:1 (v/v) on three consecutive days. A control group for each strain of mouse was treated with vehicle only. Five days after the first topical application the proliferation capacity of the lymph node cells was determined by their incorporation of 3HTdR, which was measured in a beta-scintillation counter, compared with that recorded in negative control groups. The experimental results are briefly summarized in Table 1. The test results indicate that CBA/CaOlaHsd mice should be the first choice for assessing allergenic potency. Balb/c mice are also a potential test strain for LLNA tests. CBA/Ca (CruBR) and NMRI strains of mice are not suitable for assessing the allergenic potency of chemicals.

Strain	HCA			MBT		
	5% (w/v)	10% (w/v)	25% (w/v)	5% (w/v)	10% (w/v)	25% (w/v)
CBA/CaOlaHsd	5.0	10.5	44.7	5.3	8.6	6.6
CBA/Ca (CruBR)	1.4	1.3	2.6	1.2	2.3	4.1
CBA/Jlbm (SPF)	1	1.8	8.5	1.3	8	5.4
CBA/JNCrj	2.3	4.5	8.2	1.7	3.6	0.6
Balb/c	6	3.8	9.6	6.1	9.6	7.5
NMRI	0.8	1.9	3.5	1.2	1	1.2

1836 INFLAMMATORY RESPONSE AND FREE RADICAL FORMATION IN SKIN OF B63CF1 MICE WITH DIMINISHED LEVELS OF GLUTATHIONE AFTER PHENOL EXPOSURE.

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A number of phenolic compounds that are utilized in industry (e.g., for production of resins, paints, lacquers, cosmetics and pharmaceuticals) are toxic to skin (i.e., can cause rash, dermal inflammation, contact dermatitis, leucoderma, and/or cancer promotion). The biochemical mechanisms of dermal toxicity of phenolic compounds are not well understood. We observed alpha-phenyl-N-*tert*-butylnitrone (PBN) spin-trapped free radicals generated in the skin of female B6C3F1 mice after topical exposure to phenol (3.5 mmol/kg, 100 µL, 1 h). The exposure also resulted in oxidation of GSH and protein thiols, and decreased levels of total antioxidant reserves and vitamin E in the skin. We also compared the effects of phenol in mice with normal or diminished levels of GSH. The magnitude of phenol-induced PBN-spin-trapped radical adducts in skin of mice with diminished levels of GSH (either pre-treated with DL-buthionine sulfoximine, BSO, or 1, 3-bis(2-chloroethyl)-1-nitrosourea, BCNU) was remarkably higher as compared to those in mice treated with phenol alone. Topical exposure to phenol also resulted in increased inflammatory cell infiltration in the skin of mice pre-treated with BSO or BCNU. To identify mediators involved in skin inflammation after phenol and phenol plus BSO exposure, we used JB-6 mouse epidermal cells. Using ELISA, we found that phenol and BSO plus phenol induced increases in IL-1β and prostaglandin E_2 production in the cells as early as 1 h post-treatment. Real time PCR of ICAM-1 revealed mRNA expression starting at 3 h after exposure of the cells to phenol and BSO plus phenol. Additionally, western blot analysis showed higher expression of COX-2 in cells exposed to BSO plus phenol. In the skin, we also observed mRNA expression of COX-2 and IL-1β in mice treated for 2 h with phenol or BSO plus phenol. These data indicate that mediators, such as IL-1β, ICAM-1 and prostaglandin E_2, are involved in early stages of skin inflammation in response to phenol and phenol plus BSO.

1837 EVALUATION OF THE PHOTOTOXIC AND PHOTOALLERGIC POTENTIAL OF METHYL N-METHYL ANTHRANILATE.

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Methyl N-methylanthranilate, which occurs naturally in many citrus oils, is used in both fragrances and flavors. Earlier studies reported that methyl N-methylanthranilate was phototoxic in hairless mice at a concentration of 50% in methanol and in humans at a concentration of 5% in hydrophilic ointment. Further studies were conducted to determine if a no-effect level for phototoxic effects in humans could be established. Phototoxicity was evaluated using a 24-hour occluded application of

methyl N- methylanthranilate to naive sites on the back followed by immediate exposure of the test sites to UVB and UVA from a Solar Simulator. Phototoxic effects were observed in 14/35 female volunteers with 1% methyl N-methylanthranilate in 75% ethanol/25% diethyl phthalate; no phototoxic effects were observed in 29 volunteers with 0.1%, 0.3% or 0.5% in 75% ethanol/25% diethyl phthalate. A study to determine the photoallergic potential of methyl N-methylanthranilate was conducted in 26 female volunteers using a modified human photomaximization procedure (six 24-hour occluded induction applications with each application followed immediately by UVB/UVA exposure from a Solar Simulator; after a 2-week rest period, a 24-hour occluded challenge application was immediately followed by exposure to UVA/UVB); phototoxicity was also evaluated during the induction phase of this study. No photoallergic or phototoxic reactions were observed with 0.5% in 75% ethanol/25% diethyl phthalate. Based on the findings in these studies, it can be concluded that the NOEL for methyl N-methylanthranilate for phototoxic effects in humans is 0.5%; and under the conditions of the above study, methyl N-methylanthranilate is not photoallergic in humans at a concentration of 0.5%.

1838 GENE EXPRESSION IN RAT SKIN FOLLOWING CUTANEOUS EXPOSURE TO XYLENE, SODIUM LAURYL SULFATE AND LIMONENE.

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In the US, occupational skin disease is the second most significant cause of occupational disease, after accidents. Some of this disease is due to exposures to occupational chemicals such as solvents, fuels and surfactants. Understanding the mechanisms of acute irritation will assist in assessing risks to exposures as well as potential therapy and prophylaxis. Gene expression studies may provide useful information about normal processes in the skin and the responses of the skin to exogenous chemicals. We exposed rats, cutaneously, to m-xylene (pure liquid), sodium lauryl sulfate (1% & 10% aqueous solution) and d-limonene (pure liquid) for one hour and measured transcriptional responses at the end of the exposure and three hours later for comparison with untreated skin samples. Total skin RNA was isolated and analyzed using the Affymetrix RatTox U34 array. We found that 120 of approximately 850 genes were detected as present with Affymetrix software. The largest number of these genes was in the metabolism (19) and oxidative/cellular stress responsive (9) categories. Other transcripts present in untreated skin were categorized as cellular structure, signaling, hormones, extracellular matrix, differentiation/cell division, transporters/ligands and a receptor. We found that limonene treatment caused the largest change in mRNA levels with a total of 34 transcripts increased and 4 transcripts decreased at one or four hours. 10 % sodium lauryl sulfate caused 5 transcripts to increase and 17 to decrease at one or four hours. Xylene treatment resulted in 6 increased transcripts and 14 decreased transcripts at one or four hours. Differences in the skin responses to these 3 treatments may reflect different mechanisms of irritation. These changes in gene expression suggest some proteins that should be quantified as we investigate the time course of the irritant cascade. (supported by CDC/NIOSH R01 OH03654)

1839 VEHICLE COMPOSITION INFLUENCES THE PHARMACOLOGIC EFFECTS AND KINETICS OF CAPSAICIN IN HUMAN SKIN.

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Less-than-lethal pepper spray products are prepared by solubilizing capsaicinoids in various solvents, including alcohols and propylene glycol. Previous studies showed that capsaicin activity in human skin (erythema) was 3X greater in propylene glycol than isopropyl alcohol. This study investigated the uptake and elimination of capsaicinoids in human skin as a possible kinetic basis for the responses. Twelve sites on the volar forearms of twelve human subjects were exposed for 1, 5, 10 and 15 min to a single, nonoccluded 150 mcg dose of capsaicin prepared in either 70/30 (v/v) isopropyl alcohol/water (IPA), 80/20 (v/v) mineral oil/isopropyl alcohol (MO) and 80/20 (v/v) propylene glycol/isopropyl alcohol (PG). Skin capsaicinoid concentrations were quantified in stratum corneum harvested with ten 1.3 diameter adhesive discs applied and removed from each skin site either immediately after exposure (1, 5, 10 or 15 min) or 3, 6, 11 or 24 hrs after removal of a 15 min exposure. The samples from each skin site were combined, extracted and analyzed for capsaicin and dihydrocapsaicin concentration by LC-MS and the kinetic parameters Cmax, Tmax, AUC and Thalf determined. Both capsaicinoids were detected in stratum corneum within 1 min of exposure. Cmax for capsaicin in PG (5.6 mcg) was similar to MO (6.6 mcg), but 3X less than IPA (16.1 mcg). Differences in AUC between the vehicles reflected the rank order of Cmax. Dihydrocapsaicin concentrations were ~60% of capsaicin in all solutions and all skin samples at all time points, independent of vehicle. Tmax and Thalf of the capsaicinoids were similar in PG, MO and IPA. Thalf of capsaicin and dihydrocapsaicin in human stratum corneum was ~24 hrs. Thus, alcohols influenced the extent of capsaicinoid uptake, but not the rate of uptake or elimination kinetics in human skin *in vivo*. Therefore, the elevated potency of capsaicin in PG is likely due to a vehicle influence on the receptor-mediated pharmacologic effects of capsaicin.

1840 EVALUATION OF DERMAL ABSORPTION OF AQUEOUS TOLUENE IN F344 RATS USING REAL-TIME BREATH ANALYSIS AND PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING.

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Toluene is a ubiquitous chemical commonly used for its solvent properties in industry and manufacturing, and is a component of many paint products. Because of its widespread use, there is potential for both occupational and non-occupational dermal exposure to toluene. To understand the significance of these exposures, the dermal bioavailability of toluene was assessed in F344 male rats using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to toluene at a 0.5 or 0.2 mg/ml aqueous concentration using a 2.5-cm diameter occluded glass patch attached to a clipper-shaved area on the back of the rat. Immediately following exposure, individual animals were placed in a glass off-gassing chamber and exhaled breath was monitored as chamber concentration in real time using an ion trap mass spectrometer. The exhaled breath profile clearly demonstrated the rapid absorption of toluene, with peak chamber concentrations observed within 1 hr from the start of exposure. The PBPK model describing the exposure and off-gassing chamber was used to estimate a dermal permeability coefficient (Kp) to describe each set of exhaled breath data. Regardless of exposure level, a single Kp value of 0.074 +/- 0.005 cm/hr provided a good fit to all data sets. These rat studies using aqueous toluene will form the basis for comparing the dermal bioavailability of toluene in various paint products and may ultimately aid in understanding human health risk under a variety of exposure scenarios. (Supported by NIOSH grant 1-RO1-OH03658-01A2).

1841 A MATHEMATICAL MODEL OF THE PERMEATION KINETICS OF THE MEMBRANE-COATED FIBER TECHNIQUE ACCOUNTING FOR PARTITION, DIFFUSION AND BOUNDARY LAYER FACTORS.

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A mathematical model was developed to describe the permeation kinetics of the membrane-coated fiber (MCF) technique, which is used for *in vitro* assessment of dermal absorption of chemical mixtures. In addition to the basic percutaneous absorption factors, partition coefficient and membrane diffusivity, a boundary layer adjacent to the membrane was considered in the model. The cumulative amount permeated into the membrane was expressed as a function of permeation time in an exponential equation. The two constants introduced into the model, clearly defined with the physiochemical parameters of the system, can be obtained by regression of the experimental data sampled over a limited time. The partition and diffusion coefficients, as well as, the thickness of the boundary layer were calculated from the two constants. The mathematical model adequately described the permeation kinetics of the MCF technique. All of the theoretical predictions were supported by the experimental results. The measured partition coefficients were correlated well with the published octanol/water partition coefficient (R2=0.88). The thickness of the boundary layer was 5.2μm in a donor solution stirred at 400 rpm. The contribution of the boundary layer to the permeation kinetics is $2K$ times larger than that of the membrane, where K is the partition coefficient of a given compound. These results suggest that the permeation rate of a hydrophobic compound could be controlled by the boundary layer even though the diffusivity of the compound in the membrane is lower than that in the donor solution. Supported by NIOSH R01-OH 03669 and 07555.

1842 PHYSIOLOGICAL MODELING OF THE DERMAL ABSORPTION OF OCTAMETHYLCYCLOTETRASILOXANE (D4).

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Studies on human dermal absorption of octamethylcyclotetrasiloxane, D4, through axilla skin *in vivo* and through abdominal skin *in vitro* recently have been completed. A mathematical model describing the dermal absorption of D4 was developed and combined with an inhalation PBPK model for this material. The model

includes volatilization of the chemical from the skin surface, evaporation of chemical out of the skin after the skin surface has been cleared of the chemical, and a deep skin compartment. An *in vivo* dermal absorption study of D4 in the rat provided evidence that a model structure including elimination from the skin by evaporation is appropriate. Concentrations of D4 in exhaled air and blood plasma from human, *in vivo* exposures were used to estimate the model parameters. Following either inhalation or dermal exposures, D4 blood plasma concentrations increased with time relative to exhaled air concentrations. The PBPK model for both dermal and inhalation exposures required the inclusion of a pool of unavailable D4 created in the liver, transported in the blood, and cleared in the fat to describe this behavior. Model calculations indicated that during the human, *in vivo* dermal exposures, more than 90% of the applied dose evaporated from the skin surface before it could be absorbed into the skin. Of the D4 absorbed into the skin, the majority was eliminated by evaporation before systemic absorption could occur. For men and women, respectively, about 0.1 and 0.5% of the applied dose of D4 entered the cutaneous blood within 24 hours of the exposure. M. Reddy is supported in part by NIEHS F32 ES11425-02.

1843 MEASUREMENT OF THE *IN VITRO* RATE OF PERCUTANEOUS ABSORPTION OF [^{14}C]DIOCTYL TEREPHTHALATE (DOTP) THROUGH HUMAN SKIN.

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Dioctyl terephthalate (DOTP) is a potential replacement for ortho-phthalate plasticizers. The rate of percutaneous absorption of [carboxyl-^{14}C]bis(2-ethylhexyl)-1, 4-benzenedicarboxylate through dermatomed sections of human skin was measured *in vitro*. An excess of DOTP was applied to sections of human skin contained in glass diffusion cells. Solubility of DOTP in receptor solution was determined not to be a rate-limiting step in skin absorption. The total recovery of the test substance was measured by determining the percentage ^{14}C-labeled test substance remaining in test system components for each test substance cell. The total mean (± SD) ^{14}C-labeled DOTP recovery was 104% ± 6%. The majority of ^{14}C was recovered from the donor cell and only 0.056% ± 0.032% was associated with the skin. The measured absorption rate (mean ± SD) was 0.103 ± 0.052 μg/cm^2/hr, and the permeability constant was $(8.39 \pm 2.17) \times 10^{-8}$ cm/hr. The integrity of each skin specimen was determined by measuring its permeability to tritiated water (^3H_2O) in Phase 1. The mean (± SD) ^3H_2O absorption rate for all skin specimens was 2.04 ± 0.95 mg/cm^2/hr, in agreement with historical and published values. The mean damage ratio, calculated from the rates of ^3H_2O before and after exposure of the skin to the test substance, was similar to the negative control (unexposed) values, indicating that exposure to the test substance for 29 hr did not significantly damage human skin. Applying the criteria of Marzulli, Brown, and Maibach (1969), DOTP absorption through human skin is classified as "extremely slow". These data allow the estimation of uptake in man following dermal exposure to the test substance, assuming that the skin absorption rate in man is similar to that observed in this *in vitro* study. For example, if excess test substance were to be in contact with an area of skin equivalent to both hands (approximately 720 cm^2, 70-kg human) continuously for 1 hr, the calculated internal dose would be 1.06 μg/kg.

1844 *IN VITRO* PERCUTANEOUS ABSORPTION OF ACRYLAMIDE AND STYRENE IN HUMAN SKIN.

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Polyacrylamide is formed from polymerization of acrylamide (ACR) monomers and is used in hair, nail and skin care products. Polystyrene is manufactured from styrene (STY) monomers and is used in personal care products as a thickening agent. Although most of the ACR and STY are converted to polymers, residual monomers are an impurity in the polymers formed. Polymers will not penetrate skin, but, the monomers may be substantially absorbed. ACR poses a concern because of neurotoxic effects in humans and carcinogenic, reproductive, developmental and neurotoxic effects in animals. Long term exposure to STY was reported to cause neurotoxic and carcinogenic effects in rodents. We initiated studies to measure the extent of ACR and STY absorption in human skin relevant to exposures from consumer products. Human skin was dermatomed (200 μm thick) and mounted in diffusion cells perfused with HHBSS + 4% BSA, pH 7.4. Formulations remained on skin for 24 h and the amount of ACR and STY absorbed in skin layers and receptor fluid was measured and expressed as the % of the applied dose absorbed. [14C]ACR (specific activity; 5 mCi/mmol) was applied to skin in an O/W emulsion at radiochemical doses of 0.01, 0.1 and 0.6 μCi. ACR was also applied in a 2% polyacrylamide gel cream at a dose of 0.01 μCi. ACR was rapidly absorbed from both formulations into receptor fluid with peak absorption occurring at 6 h. At 24 h, ACR absorption into receptor fluid from the O/W emulsion at the 0.01, 0.1 and 0.6 μCi dose levels was about 42, 46, and 53%, respectively. With the polyacrylamide gel cream, ACR absorption into receptor fluid was 33%. Receptor fluid values represent ACR levels available for systemic absorption. With both formulations, only about 4-7% ACR remained in skin after 24 h. [14C]STY (20 mCi/mmol) was applied to skin in the O/W emulsion at a radiochemical dose of 0.5 μCi. Only 1.2% of the applied dose of STY was absorbed into receptor fluid with 0.12% remaining in skin. The low absorption of STY is due predominately to the high volatility of this monomer.

1845 COMPARISON OF *IN VITRO* MODELS OF PERCUTANEOUS ABSORPTION.

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As part of a Colgate-Palmolive sponsored program to validate an *in vitro* percutaneous absorption model, three reference materials with extensive *in vivo* human and *in vitro* (animal and human) data were evaluated in three *in vitro* models. The objective of this study was to determine if the results are consistent with data published in the literature. The test methods used in this study were based upon guidance by the US Food and Drug Administration, and are consistent with the Office of Economic Cooperation and Development Draft Test Guidelines (2000). The *in vitro* models evaluated were human donor skin, engineered human tissue (MatTek Corporation, Model EPI-606X) and slaughterhouse-derived pig skin. The reference materials in each model were testosterone (petrolatum vehicle), benzoic acid (petrolatum and acetone), and caffeine (ethanol). The octanol/water partition coefficients (log P_{ow}) for these materials are 3.32, 1.87, and -0.07, respectively. Each reference material was evaluated at least twice in each model. The tissues were mounted in flow-through diffusion cells (PermeGear, Inc., 0.64 cm^2 surface area), qualified for barrier function by $^3H_20$ passage, followed by the application of a finite dose of ^{14}C-labeled test material (~4 μg/cm^2). The study duration was 24 hours. The recovery of caffeine and testosterone was similar (typically > 90%) in all models (a slightly lower recovery was observed in one trial with testosterone in pig tissue [mean = 81%]). Recovery of benzoic acid was notably lower, probably due to sublimation, with the highest recovery in the petrolatum preparation, as compared to the acetone preparation. In human and pig skin, the rank order for skin penetration was benzoic acid (petrolatum) > testosterone > benzoic acid (acetone) > caffeine. In Model EPI-606X, the rank order of penetration was benzoic acid (petrolatum) > testosterone > caffeine > benzoic acid (acetone). The results of these studies were reproducible and consistent with data published in the literature.

1846 METHYLEUGENOL SKIN ABSORPTION IN HUMAN AND FUZZY RAT SKIN.

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Methyleugenol (ME) is used as a fragrance ingredient in perfumes, soaps, detergents, creams, and lotions. Methyleugenol is an allylbenzene that is structurally related to safrole, isosafrole and estragole. Concerns about ME safety have been raised by National Toxicology Program carcinogenicity testing in rodents. Therefore, we initiated studies to measure ME dermal absorption in human (*in vitro*) and fuzzy rat (*in vitro* and *in vivo*) skin. *In vitro* [^{14}C]ME (approx. 0.5 μCi/cell) skin absorption was measured using either ethanol or an emulsion dosing vehicle. *In vitro* absorption was measured for 24 h by using flow-through diffusion cells (0.64 cm^2) with a receptor fluid consisting of HHBSS (pH 7.4). *In vivo* ME rat skin absorption was determined from an ethanol vehicle applied to skin for 24 h. In human skin (n=3), the percentage of applied dose absorbed (%ADA) from an ethanol vehicle over 24 h was 9.3 ± 1.82 (mean ± SEM) with approximately 0.9% remaining in skin. In rat skin *in vitro* (n=3), the %ADA from an ethanol vehicle over 24 h was 34.2 ± 2.82 with approximately 1.3% remaining in skin. Absorption of ME from an emulsion vehicle resulted in similar receptor fluid and skin levels when compared to levels measured in human or rat skin with an ethanol vehicle. Occlusion of the diffusion cells resulted in significantly higher *in vitro* absorption of ME with receptor fluid %ADA values of 49.7 ± 16.9 and 77.3 ± 6.0 for human and rat skin, respectively. ME rapidly penetrated both human and rat skin *in vitro* with approximately 7 and 30%, respectively, of the absorbed dose found in the receptor fluid within 6 h. Use of a charcoal trap attached to the top of the diffusion cell improved the recovery of the volatile ME. *In vivo* absorption of ME in the rat resulted in systemic absorption of 19.8 ± 0.1 %ADA with 0.7 %ADA remaining in skin. These studies indicate there is considerable ME absorption in skin. Systemic ME absorption should be expected after dermal application of ME-containing consumer products.

1847 THE INFLUENCE OF STORAGE TIME AND ARTIFICIAL SWEAT ON THE ERCUTANEOUS ABSORPTION OF EXPLOSIVES FROM SOILS.

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We determined the influence of sample storage time on the percutaneous absorption of C-14 labeled hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX), 2, 6-dinitrotoluene (26DNT) and 2, 4, 6-trinitrotoluene (TNT) from two soil types, Yolo having 1.9% carbon and Tinker having 9.5% carbon content. RDX soil samples stored at -20C for 27 months and 62 months were compared to freshly spiked soil samples. Similarly, 26DNT samples stored 35-36 months and TNT samples stored 18 months were compared to freshly spiked samples. Approximately 10 ug/cm2 of radiolabeled compound was applied in 10 mg/cm2 of soil to freshly excised pig skin pretreated with artifical sweat (5 ul) and mounted in skin penetration-evaporation chambers. Radiolabel recovered from the dermis and tissue culture media (receptor fluid) was summed to determine percent absorption from the soils. For each compound, percent absorptions of label were highest from Yolo soil. Storage did not significantly alter percutaneous absorption values for RDX, as values were all less than 1%, regardless of soil type or age. Similarly, 26DNT absorption was 1-2% for Tinker soil and 16-18% for Yolo soil, regardless of soil age. TNT absorption was approximately 0.5% from Tinker soil and 3-4% from Yolo soil for fresh and stored samples. HPLC analysis of 26DNT in receptor fluid at maximum flux indicated no metabolism or breakdown. For TNT, extensive conversion to monoamino derivaties and other metabolites was observed. The absorption of 26DNT from low carbon soil was reduced from 16-18% to near zero without sweat pretreatment, indicating that skin surface moisture was a critical variable in determining topical bioavailability.

1848 THE INFLUENCE OF SWEAT ON THE PERCUTANEOUS ABSORPTION OF CHLORPYRIFOS FROM NYLON CARPET FIBERS.

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Enhanced absorption of chlorpyrifos following exercise and a correlation between moisture and pesticide transfer (Williams et al., 2002) have been observed. Chlorpyrifos was chosen as a surrogate for semi-volatile chemicals used indoors. The percutaneous absorption of 14C-ring-chlorpyrifos from nylon carpet fibers was measured in porcine skin penetration-evaporation cells from nylon carpet fibers. Prior to application, synthetic sweat was applied to the skin surface in half of the cells. Radioactivity was measured in receptor fluid, dermis, epidermis, tape stripping samples, and vapor trap samples from a 24-hour period. Chlorpyrifos was successfully measured from nylon carpet fibers in the penetration-evaporation cells. The sum of radiolabel recovered from the dermis and receptor fluid was considered to represent the absorbed dose. There was no significant difference ($p > 0.05$) in percutaneous absorption or evaporative loss between cells that received the synthetic sweat application and cells that were run "dry" (1.5 ± 0.45 and 28.1 ± 4.96 percent for percutaneous absorption and evaporative loss, respectively). There was significantly more ($p < 0.05$) radiolabel recovered from tape stripping (5.4 ± 2.12 vs. 2.8 ± 0.59 percent) and in the epidermis (4.5 ± 0.78 vs. 3.1 ± 0.34 percent) from cells that received the synthetic sweat application. The percutaneous absorption of chlorpyrifos was found to correlate with an empirical model previously developed with nitro-compounds from soil. The synthetic sweat treatment facilitated transfer of chlorpyrifos from a treated substrate to the skin surface, but did not effect the rate or magnitude of percutaneous absorption in this study. This work has been supported in part through the Colgate-Palmolive/SOT Award for Student Research Training in Alternative Methods

1849 DERMAL DISPOSITION OF TRIAZINE IN CUTTING FLUID MIXTURES.

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Triazine is often added as a biocide/preservative to cutting fluids formulations used in the metal machine industry. Workers involved in metal machining are not only exposed to components in these cutting fluids, but biocides such as triazine which have been implicated in occupational contact irritant dermatitis (OCID). Little is known about how these cutting fluids and their ingredients influence the dermal disposition of triazine. The purpose of this study was to assess C^{14}-triazine membrane transport when topically applied to inert silastic membranes and porcine skin in *in vitro* flow-through diffusion cell system as aqueous mineral oil (MO) or aqueous polyethylene glycol (PEG) mixtures. C^{14}-triazine mixtures were formulated with 3 commonly used cutting fluid additives; namely, 0 or 5% linear alkylbenzene sulfonate (LAS), 0 or 5% triethanolamine (TEA), and 0 or 5% sulfurized ricinoleic acid (SRA). Triazine partitioning from the formulation into the stratum corneum (SC) was significantly reduced by LAS, while SRA significantly reduced the pH of the formulation. Triazine absorption ranged from 2.24 to 3.9% dose in porcine skin and 12.61 to 18.63% dose in silastic membranes. In silastic membranes, the complete mixture significantly reduced triazine absorption in MO-based mixtures, while in PEG-based mixtures triazine absorption and apparent permeability were significantly increased. In porcine skin, triazine permeability was significantly increased for both MO- and PEG-based complete mixtures and the trend was for greater triazine absorption in more complex PEG-based mixtures. Interestingly, SRA or TEA alone significantly reduced triazine absorption in MO-based mixtures, and this interaction appears to be more additive than synergistic. Although the physiochemical experiments suggest otherwise, triazine readily permeates a homogenous lipid membrane such as the SC, while triazine permeability and absorption was significantly enhanced by the complete mixture especially in PEG-based mixtures. Supported by NIOSH Grant R01-OH-03669.

1850 ABSORPTION OF 14C- RDX FROM SOILS THROUGH HUMAN SKIN.

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Cyclotrimethylenetrinitramine (RDX), a munition compound has been detected in water and soil as an environmental contaminant at production waste disposal sites and at certain military installations. The bioavailability of a chemical from soils depends on soil composition. We studied dermal absorption of 14C- RDX from two types of soils, Yolo (low carbon, 1.9%) and Tinker (high carbon, 9.5%), in human skin *in vitro* in flow-through diffusion cells. Soils (10 mg/cm2) containing a dose (10 mg/ cm2 / 0.05 m Ci) was applied to the skin and collected as diffused receptor fluid for every 6 hr up to 24 hrs. The soil content on the skin was washed with soap water and water with cotton swabs, and radioactivity present in washings was determined. The RDX absorbed in the skin (stratum corneum, epidermis and dermis) was also determined. Our results show that a total of approximately 2 .71 % (Yolo) and 2.24% (Tinker) of applied dose from soils was absorbed in the skin (receptor fluid and skin) in 24 hr. The absorption of RDX in the receptor fluid was about 1. 4 % (Yolo) and 0.66% (Tinker) soils in 24 hrs. The total recovery of applied dose (receptor fluid, skin and washings) was about 87 % (Yolo) and 94% (Tinker). The RDX absorption from soils in the skin was low when compared to RDX in acetone (6%) (Reddy et al., 2002). This shows that the bioavailability of RDX from soils is reduced considerably. The estimated levels of RDX absorption from soils can be used to evaluate health risks associated with dermal exposure (Supported by COE, abstract does not reflect US Army policy).

1851 DERMAL ABSORPTION OF TOLUENE FROM ENAMEL PAINT IN F344 RATS.

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Toluene is a component of many paint products and there is potential for both occupational and non-occupational dermal exposure to toluene in various matrices. To understand the significance of these exposures, the dermal bioavailability of toluene was assessed in F344 male rats using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to toluene present in a commercial enamel paint using a 1.7-cm diameter occluded glass patch system attached to a clipper-shaved area on the back of the rat. Immediately following exposure, individual animals were placed in glass off-gassing chambers and exhaled breath was monitored as chamber concentration using an ion trap mass spectrometer (MS/MS). The exhaled breath profiles from treated animals clearly demonstrated the rapid absorption of toluene. Peak chamber concentrations, representing exhaled breath, were observed within 1 hr from the start of exposure. The PBPK model describing the exposure and off-gassing chamber was used to model the exhaled breath data. A dermal permeability coefficient (Kp) of 0.073 cm/hr was found to describe each set of exhaled breath data. In comparison, the Kp value determined for enamel paint was identical to the Kp value for aqueous toluene (0.074 cm/hr) although toluene concentrations differed significantly (25 mg/ml versus 0.5 mg/ml). To evaluate the impact of paint

constituents on the dermal bioavailability, additional dermal studies were conducted using reformulated enamel paint with the titanium dioxide and xylene cosolvent replaced by toluene. PBPK model simulation of the exhaled breath data from these exposures required a Kp value roughly half the value from the intact paint (0.032 cm/hr) although the toluene concentration was more than 12 times greater. These data suggest the permeability of toluene is influenced by the exposure concentration and less so by the exposure matrix. (Supported by NIOSH grant 1-RO1-OH03658-01A2).

1852 SKIN PENETRATION AND EVAPORATION OF p-MENTHANE-3, 8-DIOL IN ETHANOL AND IN LOTION FORMULATION AFTER TOPICAL APPLICATION TO EXCISED PIG AND RAT SKIN: A MODEL FOR HUMAN DERMAL ABSORPTION.

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p-Menthane-3, 8-diol (3-8DIOL), a plant based product, was recently introduced as a topical insect repellent in the commercial product, "OFF! Botanicals" Lotion. The objective of this study was to provide an estimate of the potential for its systemic absorption in man. Carbon-14 labeled repellent formulated in the lotion or ethanol solution was applied to excised pig skin in an *in vitro* test system predictive of skin absorption in man. Twenty-four hours after application, radiolabel recovered from the dermis and receptor fluid was summed to determine percent absorption. At a dose of approximately 80 μg/cm^2 of 3-8DIOL in the lotion, a value of 3.5±0.8% was obtained with pig skin (N=6). The corresponding value for 3-8DIOL in ethanol was not significantly different (3.0±1.2%, N=6, $p>0.05$, ANOVA). For reference purposes, the pig skin absorption of piperonyl butoxide (PBO) at 100 μg/cm^2 and N, N-diethyl-m-toluamide (DEET) at 500 μg/cm^2 were significantly higher (15±6% and 23±3%, respectively, N=6, $p<0.05$, ANOVA). For additional reference, absorption of all compounds was found to be higher with excised rat skin ($p<0.05$, ANOVA) than with excised pig skin. Most of the applied dose of 3-8DIOL was found to evaporate from pig skin (77±8% for the lotion and 87±1% for ethanol solution), thus contributing to the relatively small percutaneous absorption values observed. Although methodological differences (such as contact time, etc.) need to be considered further, the absorption of DEET and PBO determined in the pig *in vitro* system is greater than what was determined previously in humans. This provides confidence that using the pig-derived dermal absorption value for 3-8DIOL does not underestimate systemic exposure and thus it would be appropriate for human exposure assessments.

1853 INDUCTION OF ADIPOSE DIFFERENTIATION RELATED PROTEIN AND NEUTRAL LIPID DROPLETS ACCUMULATION IN KERATINOCYTES BY SKIN IRRITANTS.

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Skin irritation is a complex phenomenon, and keratinocytes, owing of their anatomical location and production of inflammatory mediators, play an important role in it. We have recently identified by DD-PCR the upregulation by skin irritants of adipose differentiation related protein (ADRP) in reconstituted human epidermis. ADRP is a lipid storage droplet-associated protein, governing the deposition and release of lipids from droplets. The purpose of this study was to characterize in a human keratinocyte cells line (NCTC 2544) SDS-induced ADRP expression, to identify the biochemical events that lead to ADRP expression, and finally, to understand the function of ADRP in SDS cytotoxicity. SDS induced a dose and time related production of ADRP, which was associated with lipid droplets accumulation. Lipid accumulation following SDS treatment was likely to be due to intracellular redistribution rather that lipid neosynthesis, as indicated by equivalent 14C-oleate incorporation into di- and tri-acylglycerols. Other skin irritants, namely benzalkonium chloride, tributyltin, and phorbol 12-myristate 13 acetate, induce lipid droplets accumulation as well, indicating a common effect probably related to the essential role of lipid droplets in eukaryotic cells. SDS-induced ADRP expression and lipid droplets accumulation could be modulated by staurosporine, a broad spectrum protein kinases inhibitor, and by BAPTA, a calcium chelator, suggesting a role of calcium and protein phosphorylation in SDS-induced lipid accumulation. Modulation of SDS-induced ADRP expression by specific antisense oligonucleotide or by BAPTA resulted in increased cytotoxicity, indicating a protective role of ADRP and lipid accumulation in the process of cell damage induced by skin irritants.

1854 DERMAL ABSORPTION AND TOXICITY STUDY OF ACETONE-BASED SKIN COATINGS IN MINIATURE SWINE.

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Objective: This study was performed to evaluate both the systemic absorption of acetone and the potential for dermal toxicity from acute and chronic application of a skin coating material made from an acetone solution of polyvinylidene fluoride (PVDF) and acrylic polymers. Methods: Yucatan miniature pigs weighing 16-20 kg were topically administered a single dose of an acetone/PVDF/acrylic coating (3 animals per sex) or acetone alone (1 animal per sex) for acute evaluation. Acetone levels in blood were evaluated at regular intervals between 0 and 240 minutes. After a 3-4 day washout period, skin was abraded and a chronic 7-week study was completed with 2 daily applications (minimum of 6 hours between applications) of test material or acetone for 5 days per week. Trough blood acetone levels were taken before the first application of each week. Peak levels were taken after the second application on the last day of each week. Body weights and food consumption were recorded weekly. Clinical chemistry and hematologic parameters were evaluated. At necropsy, skin and major organs were removed for histopathological examination. Results: No evidence of toxicity was observed in any of the treatment groups. In the acute study, pigs either showed no perceptible elevation of acetone levels or slightly increased levels that would be considered non-toxic to humans. In addition, there was no evidence for elevated blood acetone levels after chronic treatment. There were no significant microscopic differences between any treatment or control groups. The most significant histopathological finding was minor disruption of the keratin layer, an observation that was also seen in untreated areas of skin. Conclusion: Repeated dosing of PVDF/acrylic coating formulations containing acetone are non-toxic and non-irritating.

1855 ACUTE TOXICITY ASSESSMENT OF BREAKFREE CLP®: A SMALL ARMS CLEANING COMPOUND.

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BreakFree CLP® ("BreakFree") is a weapons cleaning compound that is in use by the Armed Forces. BreakFree is a complex mixture made up of polyalphaolefin oil (65%), synthetic oils, esters and other synthetic proprietary ingredients (27%), isoparaffinic hydrocarbons (5%), and dibasic ester (3%). Like so many commercial mixtures, there is very little information available on the toxicity of BreakFree. Studies were conducted to characterize the dermal toxicity of BreakFree following single or repeat application. BreakFree was applied neat to the shaved backs of male and female CD-1 mice, 50 μL/application, 3 times/week for 2 weeks. Mice were then sacrificed 24 hours and 2 weeks after initiation of dermal applications. Final body, liver, and kidney weights, and blood chemistry and hematology profiles were compared with those of animals treated with deionized H_2O or acetone (negative controls) or 2.5% croton oil in acetone (positive control). Gross observations at 2 weeks included moderate dermal irritation (skin irritation) for BreakFree-treated animals and marked dermal irritation and scabbing in croton oil-treated animals. Final relative body and kidney weights were significantly lower for BreakFree-treated animals at 24 hours. There was evidence of epidermal acanthosis, and dermal inflammation in both 2 week BreakFree- and croton oil-treated animals, but differed in that serocellular crusts and multifocal ulceration was apparent for croton oil-treated skin. Blood concentrations of total protein, sodium, and alanine aminotransferase were significantly higher for BreakFree mice. Our findings indicate that repeat, unprotected handling of BreakFree could result in significant dermal irritation with possible histopatholgical damage to the epidermis and dermis. Blood chemistry profiles are suggestive of possible liver toxicity, but need to be confirmed.

1856 DERMAL PERMEATION OF THE SULFATED FATTY ACID, RICINOLEIC ACID, IS INHIBITED BY COMPLEX MIXTURE ADDITIVES.

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Performance of many cutting fluid formulations is dependent on its lubricant properties, which can often be improved by adding a sulfated fatty acid such as sulfated recinoleic acid (SRA). SRA like many of the other formulation ingredients are potential dermal irritants, yet little is known about its permeability in skin, and if other cutting fluid additives influence its dermal permeation. The purpose of this study was to assess H^3-SRA permeation when topically applied to inert silastic

membranes and porcine skin in *in vitro* flow-through diffusion cell system as aqueous mineral oil (MO) or aqueous polyethylene glycol (PEG) mixtures. H^3-SRA mixtures were formulated with 3 commonly used cutting fluid additives; namely, 0 or 2% triazine (TRI), 0 or 5% linear alkylbenzene sulfonate (LAS), and 0 or 5% triethanolamine (TEA). Formulation additives had little or no effect on SRA partitioning from the formulation into the stratum corneum (SC) in MO-based mixtures; However, in PEG-based mixtures the additives significantly decreased partitioning into the SC. The pH of SRA control and SRA+LAS mixture remained in physiological range (7.0 - 7.4), but all other mixtures were more basic pH (9.3 - 10.3). In silastic membranes, SRA absorption ranged from 1.22 to 12.84% dose and permeability and absorption were significantly reduced to one level by LAS and then another level by other additives or combination of additives in either MO- or PEG-based mixtures. In porcine skin, absorption ranged from 0.1 to 0.57% dose, and again formulation additives significantly decreased SRA absorption and permeability in both MO- and PEG-based mixtures. The observed decreasing trend of SRA permeation in both silastic and skin membranes is suggestive that this interaction is more physicochemical in nature than chemical-biological. The presence of other formulation additives that increased the pH of the mixture resulted in more charged SRA molecules that are not absorbed across the skin as readily. Supported by NIOSH Grant R01-OH-03669.

1857 PERCUTANEOUS ABSORPTION OF 2, 6-DI-*TERT*-BUTYL-4-NITROPHENOL (DBNP) IN ISOLATED PERFUSED PORCINE SKIN.

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DBNP (2, 6-di-*tert*-butyl-4-nitrophenol) has been reported as a potential contaminant in submarines. This yellow substance forms when lubrication oil mist containing the antioxidant additive 2, 6-di-*tert*-butylphenol passes through an electrostatic precipitator and is nitrated. Percutaneous absorption of ^{14}C-DBNP was assessed in the isolated perfused porcine skin flap (IPPSF). Four treatments were studied (n=4 flaps/treatment): 40.0mg/cm^2 in 100% ethanol; 40.0mg/cm^2 in 85% ethanol/15% water; 4.0mg/cm^2 in 100% ethanol; and 4.0mg/cm^2 in 85% ethanol/15% water. DBNP absorption was minimal across all treatment groups, with the highest absorption detected being only 1.08% applied dose in an aqueous ethanol group. The highest mass of ^{14}C-DBNP absorbed was only 0.5µg. The majority of the applied dose remained on the surface of the skin. This suggests that there is minimal dermal exposure of DBNP when exposed topically to skin. Supported by GEO-CENTERS, INC., Subcontract GC-3291-044-01-099 under SPAWAR SYSCEN NHRC Contract No. N66001-98-D-2600, D.O. 0044.

1858 ABSORPTION THROUGH PORCINE SKIN EXPOSED TO VARIOUS DOSES OF JET FUEL MARKER COMPONENTS DETERMINED WITH GC-FID USING HEAD SPACE SPME FIBER.

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Jet fuels (mixture of aliphatic and aromatic hydrocarbons) have been found to be potentially toxic to skin. In the past, we have studied the percutaneous absorption and mixture effects of selected individual hydrocarbons of jet fuels. Recently, dose related IL-8 release from human epidermal keratinocytes by jet fuel aromatic hydrocarbons has been studied. The present study is an ongoing approach to simultaneously observe the dose related percutaneous absorption of a number of aliphatic and aromatic hydrocarbons. Mixtures containing undecane (4.1%), dodecane (4.7%), tridecane (4.4%), tetradecane (3%), pentadecane (1.6%), naphthalene (1.1%) and dimethyl naphthalene (1.3% of jet fuels) in hexadecane was used to dose porcine skin diffusion cells. Treatments (n=4 cells) were 1X, 2X and 5X concentrations. Perfusate samples were analyzed with GC-FID using head space solid phase micro-extraction fiber technique. We have standardized the assay to have good linear correlation for all the tested components in media standards. Dosed components from perfusate were detected even with the lowest dose except for tetradecane which was detected with the highest dose only. Data indicates a dose dependent increase in absorption for naphthalene, dimethyl naphthalene and lower molecular weight aliphatic hydrocarbons. Absorption parameters including diffusivity, permeability and steady state flux were determined. This approach provides a baseline to access component interactions among themselves and with the diluent (solvents). Supported by USAFOSR F49620-01-1-0080.

1859 THE CYTOTOXICITY OF JET FUEL AROMATIC HYDROCARBONS AND DOSE-RELATED INTERLEUKIN-8 RELEASE FROM HUMAN EPIDERMAL KERATINOCYTES.

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Many aromatic hydrocarbons are known carcinogens with the ability to readily penetrate the skin with high absorption flux and cause skin irritation. In order to evaluate the *in vitro* cutaneous toxicity of individual aromatic hydrocarbons and their potential in inducing skin inflammation, we evaluated the LD_{50}, highest non-cytotoxic (5% mortality) dose (HNTD) and IL-8 release of 9 aromatic hydrocarbons in human epidermal keratinocytes (HEK). LD_{50} ranged from 1.8 mM (0.03%) for cyclohexylbenzene to 82.9 mM (0.74%) for benzene with a rank order potency of cyclohexylbenzene> trimethylbenzene≥xylene>dimethylnaphthalene>ethylbenzene>toluene>benzene. The HNTD value ranged from 0.1 mM (0.001%) for cyclohexylbenzene to 48.2 mM (0.43%) for benzene. There was a dose-related differential response in IL-8 release at 24 hr. Toluene, xylene, trimethylbenzene, cyclohexylbenzene and dimethylnaphthalene significantly decreased IL-8 release at the HNTD, while IL-8 release did not continue to decrease or significantly increase (cyclohexylbenzene and dimethylnaphthalene) at LD_{50}. IL-8 significantly increased with both doses of methylnaphthalene and naphthalene. The presence of hexadecane and mineral oil greatly attenuated the cytotoxicity to HEK cells elicited by individual aromatic hydrocarbons. This work was supported by the US Air Force Office of Scientific Research F49620-01-1-0080.

1860 CYTOTOXICITY OF THE JP-8 JET FUEL COMPONENTS m-XYLENE, 1-METHYLNAPHTHALENE, AND n-NONANE IN KERATINOCYTES.

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Cell culture methods are being developed to assess the dermal toxicity of volatile chemicals. Such tests are useful in the ranking of chemicals for irritancy, but they are not useful for quantitative risk assessment for two reasons. First, the amount of volatile chemical in the exposure media may decrease with time. Second, EC50 are reported as the concentrations in the media and not the cells. We have recently developed an *in vitro* approach for toxicity testing of volatile chemicals that avoids these problems. This system was used to expose keratinocytes grown on a collagen matrix to culture medium containing m-xylene, 1-methylnaphthalene (1-MN), or n-nonane. Partition coefficients were measured and used to estimate the chemical concentration in the keratinocytes. The EC50 for m-xylene at 1, 2, and 4 hours were 1248.46 ± 78.01, 1028.88 ± 11.12, and 860.8 ± 84.6 µg m-xylene per gram of keratinocytes, respectively. The EC50 for 1-MN in the keratinocytes at 1, 2, and 4 hours were 6494.3 ± 460.1, 4319.82 ± 372.61, 2201.06 ± 196.27 µg 1-MN per gram keratinocytes, respectively. Although marginal cytotoxicity was observed at 1 hr, the EC50 for n-nonane at 2 and 4 hours were 915.6 ± 155.5 and 980.7 ± 139.5 µg n-nonane per gram cells, respectively. These results suggest a time- and dose-dependent effect of m-xylene and 1-MN of keratinocyte viability, with little cytotoxic effect by n-nonane. This study supports the potential use of our exposure system for determining equivalent external doses for toxic endpoints.

1861 SIX MONTH SAFETY AND IMMUNOLOGY STUDY IN BABOONS OF ALLOGENEIC BABOON MESENCHYMAL STEM CELLS LABELED WITH FLUORESCENT DYE.

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The purpose of this study was to determine the safety and immunologic consequences of administering allogeneic baboon mesenchymal cells (MSCs) by routes of clinical significance. MSCs are rare cells found in bone marrow and other tissues that have the capacity to be expanded to large numbers for tissue repair. MSCs from male donors were labeled with fluorescent dye for tracking purposes and injected into female baboons as follows: group 1 control animals (n=3) received vehicle (95% plasmalyte:5% recipient plasma); group 2 (n=3) received DiI-labeled

MSCs (5 million MSCs/kg) intravenously followed by DiO-labeled MSCs (5 million MSCs/kg) by intramuscular (IM) injection from the same donor on day 42 (6 weeks later); group 3 (n=3) was treated similarly to group 2 except that the second IM injection of MSCs was from a 3rd party donor. No signs of toxicity were seen throughout the study as evaluated by body weights, food consumption, clinical pathology, organ weights, and histopathology data. Antinuclear antibodies, sedimentation rate, Ig panel (IgG and IgM), and most peripheral blood cell subsets (CD14+ monocytes, CD56+ NK cells, CD19+ B cells) were comparable between the treatment groups. The modest decrease in the percentage of CD3+CD8+ cells in group 2 and 3 animals was offset by a modest increase in the percentage of CD3+ CD4+ cells in weeks 5, 11 and 27 in these groups, thereby maintaining an unchanged total number of lymphocytes. Under conditions of this study, allogeneic MSCs administered either intravenously or intramuscularly to immunocompetent recipients did not induce toxicity in baboons.

1862 PAROTID GLAND BASOPHILIC FOCI IN MICE ADMINISTERED THE PEPTIDE AC2993 (SYNTHETIC EXENDIN-4) BY SUBCUTANEOUS INJECTION FOR PERIODS OF 13 TO 26 WEEKS.

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AC2993 (synthetic exendin-4; a 39-amino acid peptide) is under development for glucose control in patients with type 2 diabetes mellitus. As part of the development program, the peptide was evaluated in toxicity studies in mice with twice daily administration and exposure durations of 13 and 26 weeks. A histopatholgical alteration in the parotid gland described as basophilic foci was noted. The alteration was characterized as enlarged cells with increased cytoplasmic volume and finely vesicular basophilic contents. In the 13-week study, one control mouse exhibited a trace incidence. In this study the incidences in the combined sexes at a severity of trace to mild at 3, 34 and 380 mcg/kg/dose were 22 of 42, 39 of 42 and 35 of 42, respectively. In the 26-week study, no basophilic foci were observed in the control animals. The incidences in the combined sexes at a severity of trace to moderate at 9, 58 and 380 mcg/kg/dose were 43 of 47, 35 of 39 and 43 of 49, respectively. The severity appeared related to duration of exposure, but not to the dose level of AC2993 administered. No toxicities could be associated with this microscopic alteration in the salivary gland. Jackson and Blackwell reported parotid basophilic foci in rats exposed to doxylamine for 90 days (1) but not in mice (2). They also did not define any associated toxicities. Chiu and Chen reported on the spontaneous occurrence of this tissue alteration in rats and mice (3). 1. Jackson, C.D. & Blackwell, B-N (1988). Subchronic studies of doxylamine in Fischer 344 rats. Fund. Appl. Toxicology. 10, 243-253. 2. Jackson, C.D. & Blackwell, B-N (1988). Subchronic studies of doxylamine in B6C3F1 mice. Fund. Appl. Toxicology. 10, 254-261. 3. Chiu, T, & Chen H.C. (1986). Spontaneous basophilic hypertrophic foci of the parotid glands in rats and mice. Vet. Pathol. 23, 606-609.

1863 A REVIEW OF MORTALITY PATTERNS IN CD-1 MOUSE TUMORIGENICITY STUDIES CONDUCTED OVER THE PERIOD OF 1985 TO 2001.

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The interest and available information on the mouse as a model for tumorigenicity assessment have tended to be marginalised due to the possibility that the assessment would be confined to the rat model only. Currently the consensus is that both species should be used until international agreement has been reached. However, interest in the mortality patterns of mice has been renewed because of the increasing trend towards conducting mouse tumorigenicity studies over 2 years, rather than over 18 months or to a percentage survival point. In this review, the mortality data have been analysed from the control groups of up to 120 tumorigenicity studies (dietary and oral gavage) using the Charles River Crl:CD-1 ® (ICR) BR mouse. These studies used low protein maintenance diet (Special Diets Services) and were conducted over the period of 1985 to 2001. The analyses did not reveal any major differences for the terminal mortality (at Weeks 80 or 104) for the routes of administration (values given below) or housing conditions, or in the patterns over 2 years. Also, the analyses did not indicate that single housing increased the terminal mortality values. Review of the terminal mortality against time indicated a slight but increasing trend towards higher values in the females. In conclusion, there were no major differences between the terminal mortality or mortality patterns over 2 years for the routes of administration or housing conditions, but the analyses of terminal mortality against time indicated a slight but positive trend towards higher values for the females.

Terminal mortality (%)

Week	Sex	Dietary			Oral gavage		
		Mean	SD	n	Mean	SD	n
80	Males	28	7.7	80	25	9.0	40
	Females	22	7.0	78	21	7.2	41
104	Males	53	11.7	18	50	11.5	18
	Females	54	7.9	15	57	8.2	18

SD: Standard deviation; n: Number of studies

1864 EVALUATING HEALTH IMPLICATIONS OF LUBRICATING OIL ON ORTHOPEDIC MEDICAL IMPLANT DEVICES.

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Certain orthopedic medical devices may contain small amounts of machine lubricating oil (up to 50 to 100 mg/per device) due to the manufacturing process. Machine lubricating oils consist primarily of a base oil, similar to mineral oil, and small amounts (< 1%) of various additives that enhance performance of the oil. We evaluated the potential for adverse health effects due to exposure to machine lubricating oil on orthopedic medical implant devices using a hierarchical approach, based on availability of data. Studies suitable for identifying effect levels for relevant health endpoints were only available for the base oil and two of the additives (butylated hydroxytoluene and Bimox M). For another additive, dioctylamine, we identified a health effect level for a surrogate compound, cyclohexylamine. For the remaining additives we did not locate any suitable toxicology studies either for the additive itself or an appropriate surrogate. For these additives we derived a health protective screening level based on FDA's threshold of regulation for substances used in food contact articles, which, in the absence of other toxicity information, makes the conservative assumption that the substances are carcinogens. Because exposure to machine lubricating oil on orthopedic medical implant devices is likely to be most similar to i.p. exposures, we extrapolated effect levels derived from oral exposures to i.p. effect levels, based on a comparison of oral vs. i.p. LD50s in mice. We found that potential exposure levels ranged from approximately 1.5-fold below the estimated health effect level, for an additive of unknown identity (based on FDA's threshold of regulation), to approximately 200 million-fold below the health effect level identified for Bimox M. Thus, based on this analysis, exposure to machine lubricating oil on orthopedic medical implant devices is not likely to be associated with adverse health effects.

1865 BLOOD CONCENTRATION AND TISSUE DISTRIBUTION OF 14C-DI(2-ETHYLHEXYL) PHTHALATE (DEHP) IN JUVENILE AND ADULT COMMON MARMOSET.

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This study was conducted to characterize the blood concentration, tissue distribution and excretion of DEHP in juvenile and adult primates and to assess the effect of repeated DEHP-treatment on those parameters. Male and female (3 animals/sex/group) common marmosets aged 3 or 18 months (nive and pretreated repeatedly with 100 or 2500 mg/kg DEHP for 15 months) received a sigle dose of 100 or 2500 mg/kg DEHP by gavage which contained 14C-DEHP labeled in the phenyl moiety. The Cmax was achieved 1 to 4 hours after administration. Sex-related differences in Cmax and AUC were observed, and age dependent differences were seen in males and females. No significant age-related differences in Cmax were seen in males and females, but the AUC was higher in older females compared with

younger females. Repeated exposure resulted in lower pharmacokinetic parameters suggesting induction of enzymatic metabolism and/or excretion of DEHP. The difference was more evident in females than in males and at 2500 mg/kg than 100 mg/kg. In all experimental conditions, two hours after a single administration of 14C-DEHP, the tissue-to-plasma ratios for bile were 14.0 to 42.6 and for the kidneys were 1.7 to 3.0, probably associated with excretion. There were no apparent dose-dependent differences. Tissue-to-plasma ratio were 0.09 to 0.23 for testes and were <1.00 for all other organs except for 1.8 for seminal vesicle from older nive males treated with 100 mg/kg 14C-DEHP. The major excretion route was in feces (36-65% of the dose); however, substantial urinary excretion (10-22% of the dose) was noted. These data may serve to improve the pharmacokinetic models for humans predicting potential reproductive effects.

1866 TESTICULAR TOXICITY STUDY OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP) IN JUVENILE COMMON MARMOSET.

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Rats exposed to DEHP during the pre- and peri-adolescent period have been shown to be sensitive to testicular effects, hence this study was conducted to characterize the potential effects of DEHP on the reproductive organs in the juvenile primate. Male marmosets aged 3 months (5-6/group) were treated orally with 0 (corn oil), 100, 500, and 2500 mg/kg DEHP once per day for 15 months until sexual maturity. The reproductive organs (testis, epididymis, prostate, seminal vesicle), pituitary, thyroid, pancreas, liver, adrenal, kidney, and spleen were weighed and were examined microscopically. Testicular 3-beta hydroxysteroid dehydrogenase(3-beta HD) activity was determined histochemically. The testis was also subjected to electronmicroscopic examination and to a determination of mRNA expression of PPAR-alpha (peroxisome proliferator-activated receptor). Mean body weights of each DEHP-treated group were comparable to that of the control group. There were no differences in the mean organ weights in each DEHP-treated group. One male in each DEHP-treated group showed low values in testis, epididymis, prostate, and seminal vesicle weights corresponding to the low body weights. The animals exhibiting low testis, epididymis, prostate, and siminal vesicle weights had reterded growth in general. No treatment-related findings were observed microscopically in any organs except for above changes. No remarkable changes were observed electronmicroscopically in the testis. There were no differences in a 3-beta HD activity or mRNA expression of PPAR-alpha between animals in the control group and those in the each DEHP-treated group. In conclusion, exposure of juvenile marmosets (primates) to high dose levels of DEHP prior to and during adolescence (a sensitive period for testicular toxicity in rats) showed no evidence of testicular effects.

1867 COMPARATIVE TOXICITY STUDY OF 3-AMINOPHENOL IN NEWBORN AND YOUNG RATS.

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Comparison of the toxicological profiles and toxicity levels of chemicals in newborn animals with the routine test results using young animals will provide valuable information for risk assessment of chemicals in infants. Repeated dose toxicity of 3-aminophenol was examined on oral administration to newborn and young rats and susceptibility was analyzed in terms of the no observed adverse effect level (NOAEL) and the unequivocally toxic level. In the 18-day newborn rat study starting at postnatal day 4, tremors and depression of body weight gain were observed, as well as hypertrophy of thyroid follicular epithelial cells and increases of relative liver and kidney weights at 240 mg/kg. Increase of relative liver weights in males and decrease of blood sugar in females without any histopathological changes at 80 mg/kg were not considered to be adverse effects. No chemical-related changes were observed at 24 mg/kg. Abnormalities of physical development and reflex ontogeny in the newborn were not observed. In the 28-day study starting at 5 weeks of age, depression of body weight gain, tremors, anemia, and liver, kidney and thyroid toxicity were observed at 720 mg/kg. Although slight pigmentation in renal proximal tubular epithelium was observed in females at 240 mg/kg, this was not considered to be an adverse effect because of the lack of changes in related toxicological parameters. It was concluded that the NOAEL is 80 mg/kg/day in newborn rats and 240 mg/kg/day in young rats, with unequivocally toxic levels of 240 mg/kg/day and 720 mg/kg/day, respectively. Based on these two endpoints, the susceptibility of newborn rats to the chemical was approx. 3 times higher than that of young rats, consistent with our previous results for 4-nitrophenol and 2, 4-dinitrophenol.

1868 EVALUATION OF CO2/O2 ANESTHESIA DURING THE JUGULAR BLEEDING PROCEDURE IN RATS.

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The jugular bleeding procedure has been utilized at Schering-Plough for the collection of blood samples from rats, required during the conduct of toxicology studies for both clinical pathology and toxicokinetic evaluations. To refine the procedure, the feasibility of CO2/O2 anesthesia during jugular bleeding was assessed. Twenty rats (10 males and 10 females) were bled for hematology and serum chemistry, respectively, under CO2/O2 anesthesia (60:40 ratio) on Day 0 and unanesthetized on Day 4. Twenty additional rats (10 males and 10 females)were bled for hematology and serum chemistry, respectively, unanesthetized on Day 0 and under CO2/O2 anesthesia on Day 4. The predominant clinicopathologic changes associated with CO2/O2 anesthesia were consistent with hemodilution and/or intravascular volume expansion and included minimal decreases in red blood cell mass (erythrocyte count, hemoglobin concentration and hematocrit), platelet numbers and serum total protein (albumin and globulin) concentration in anesthetized animals on Day 4 compared to controls on Day 0. Additional findings in anesthetized rats, compared to unanesthetized rats included minimal leukocytosis, characterized by proportional increases in lymphocyte, neutrophil and monocyte counts, and minimally lower serum aspartate aminotransferase activity. Equivocal were several changes including higher mean corpuscular volume and lower mean corpuscular hemoglobin concentration and higher serum electrolyte (phosphorus, sodium, potassium and chloride) concentrations in CO2/O2-anesthetized rats. All effects on clinical pathology parameters attributed to the anesthesia procedure were minor and at no time resulted in values that were considered to be out of the range of physiologic variation. It can be concluded that CO2/O2 anesthesia is a suitable means of restraint for rats in toxicology settings and should have no meaningful impact on interpretation of clinical pathology parameters.

1869 CANINE PURKINJE FIBER ACTION POTENTIAL DURATION: INFLUENCE OF STIMULATION FREQUENCIES AND EFFECTS OF DMSO CONCENTRATIONS.

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Purkinje fibers are highly sensitive to the influence of action potential duration (APD)-prolonging agents and are believed to be the source of early after depolarization, the stimulus that commonly triggers Torsade de Pointes (TdP) arrhythmias. Canine Purkinje fibers are routinely used as a screening model to detect the ability of agents to prolong the QT interval and cause TdP. However, the ideal stimulation frequency and solvent concentration to be used in these types of experiments are controversial. We examined the effects of dl-sotalol, a positive standard, on canine Purkinje fiber APD under different stimulation frequencies to identify the ideal stimulation frequency for these type of experiments. We have also studied the effects of different concentrations of DMSO, a commonly used solvent for these types of studies, on APD in these same tissues. Standard microelectrode techniques were utilized to record transmembrane action potentials from canine Purkinje fibers at 0.5, 1 and 3 Hz stimulation frequencies. Action potential durations were measured at 30, 50 and 90% repolarization (APD_{30}, APD_{50} and APD_{90}). Dl-sotalol (50μM), prolonged APD_{50} and APD_{90} without any significant effects on APD_{30}. While dl-sotalol-induced prolongation of APD_{50} and APD_{90} was observed at all the three stimulation frequencies, the effect was minimal at 3 Hz and maximal at 0.5 Hz. At concentrations of 0.01, 0.05, 0.1, 0.5 and 1%, DMSO was found to not produce any significant effects on APD_{30}, APD_{50} or APD_{90}. However, 5% DMSO caused significant abbreviation of APD_{50} and APD_{90}. In conclusion, a significant prolongation of APD_{50} and APD_{90} by dl-sotalol at 1 and 0.5 Hz stimulation frequencies indicate the suitability of these frequencies for APD prolongation studies. Furthermore, DMSO, up to a concentration of 1%, can safely be used as a solvent in this type of studies.

1870 COMPARISON OF LASER MICRODISSECTION TECHNIQUES FOR CELL COLLECTION AND MOLECULAR ANALYSIS.

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Laser microdissection allows for fine-dissection, at the light microscopic level, of specific cell populations of interest which can then be processed and analyzed by various molecular techniques. Currently, two major types of laser dissection techniques are available: near-infrared laser capture microdissection (e.g. LCM, Arturus Pixcell II), and ultraviolet laser light beam cutting (e.g. LMD, Leica AS). In this study we evaluated both laser techniques in order to determine which process was

more time-efficient in collection of cells and to determine which process obtained better quality RNA. Samples of male rat kidney and liver were collected under RNAase-free conditions and frozen in OCT blocks. Sections (8um thick) of liver and kidney were cryosectioned and stained with hematoxylin in an RNAase free environment, to aid in the identification of hepatocytes in the liver and tubular epithelial cells in the kidney. Approximately 10, 000 cell profiles were obtained each from the liver and kidney. Cells were processed by standard methods and total RNA isolated using commercially available kits. Quantity and integrity of RNA were determined using the Agilent 2100 Bioanalyzer (RNA 6000 Nano LabChip). RNA obtained by both techniques was assessed for quaility by measuring mRNA expression of selected genes with Q-RT-PCR (Taqman). The laser dissection process took approximately 60 min/organ with the Arcturus Pixcell II and 3 min/organ with the Leica AS system to collect the defined sample size. The quantity and integrity of RNA obtained from the two laser systems were similar in nature and of high quality. Results of this study indicate that the Leica laser light beam cutting technique provides a more time-efficient method for the collection of large numbers of cells as compared to the Pixcell II (20-fold reduction in time), and that the RNA obtained by this method is amenable for subsequent molecular analysis.

1871 INDUCTION AND CHARACTERIZATION OF GRANULOMAS INDUCED BY INTRATHECAL OPIATES IN DOGS.

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Chronic intrathecal (IT) infusions of morphine sulfate have been shown to produce aseptic inflammatory masses (granulomas) in both humans and laboratory animals. We examined the role of opiate receptors in granuloma formation by infusing other opiates delivered at the maximum tolerable dose (MTD). Purpose-bred beagle dogs were implanted with chronic IT catheters terminating at the L1-L2 region. Solutions were delivered *via* vest-mounted infusion pumps with an infusion rate of 40 ul/hour for 28 days. One group of dogs was infused with saline or high concentration (HC) morphine sulfate (12.5 mg/ml;12 mg/day). Additional dogs were infused with methadone HCl, hydromorphine HCl, or the mu antagonist naloxone HCl with dose escalation every 24 hours to determine the acute MTD. To examine the role of dose versus concentration, low concentration (LC) 1.5 mg/ml morphine was infused at 334 ul/hr (12 mg/day). The MTD for methadone and hydromorphone was 3 mg/day. No effects were seen for naloxone up to 10 mg/ml. Arousal, muscle tone and motor coordination were assessed daily. Dogs receiving the MTD of HC morphine, methadone or hydromorphone developed hind limb muscle stiffness and motor dysfunction, tactile hypersensitivity, allodynia, and in some cases paralysis. Histopathology revealed aseptic granulomatous masses localized at the catheter tip of dogs receiving HC morphine, methadone and hydromorphone producing significant spinal cord compression. These masses, evolving from the dura-arachnoid, contained extensive accumulation of macrophages (CD68, iNOS positive cells), lymphocytes (CD3) and neutrophils. Inflammatory cells expressing TNF-alpha were limited to granulomas and did not impinge upon spinal cord tissue. Infusions of LC morphine, naloxone or saline displayed no significant adverse events or pathology. These data suggest IT opiate induced granuloma formation is dependent upon opioid receptor activation and that formation is a factor of infusate concentration rather that total dose infused. (Supported by DA02110 (TLY), UCSD Cancer Center Lopicolla Foundation (JWA))

1872 TOXICITY OF THE FIBROBLAST GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITOR, PD176067, IN 11-MONTH OLD FEMALE RATS.

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PD176067 is a reversible and selective inhibitor of the basic fibroblast growth factor receptor tyrosine kinase, and was in development as an anti-cancer agent. In a previous study, bidaily oral administration to 7-week old rats resulted in vascular mineralization and physeal changes. To determine if these changes are specific to young, rapidly growing animals, PD176067 was administered to approximately 11-month old rats. Female Wistar rats (5/group), received PD176067 by gavage (BID) for 14 days at total daily doses of 2.5, 5, and 10 mg/kg (15, 30, and 60 mg/m^2, respectively), and were necropsied on Day 15. Clinical signs of toxicity were seen at ∝5 mg/kg and one death occurred at 10 mg/kg. Physeal dysplasia occurred in all drug-treated animals and was characterized by dose-related increased thickness of the zones of chondrocyte proliferation and hypertrophy, and marked thickening of the zone of ossification. Cartilage hyperplasia was characterized by proliferation of chondrocytes along margins of the physis and body of the sternebra. Bone marrow hypocellularity occurred at ∝5 mg/kg. Serum phosphorus levels increased 47% and 166% at 5 and 10 mg/kg, respectively. Mineralization of cardiac myocytes, aorta, various arteries, renal tubules, and gastric mucosa and muscularis was seen at 10 mg/kg. PD176067 produced morphologically similar lesions in young and adult rats. Systemic mineralization was the most toxicologically significant change, and is consistent with hyperphosphatemia and calcium-phosphorus deposits.

1873 LIPOSOME BASED FORMULATION OF SN-38 (LE-SN38): A FOUR-CYCLE TOXICITY EVALUATION IN BEAGLE DOGS.

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SN-38 (7-ethyl-10-hydroxy camptothecin) is the active metabolite of CPT-11 (Irinotecan), a semi-synthetic antitumor derivative of camptothecin. Although SN-38 is 100-1000 times more cytotoxic than CPT-11, it has not been developed as anticancer drug due to its poor solubility in pharmaceutically acceptable solvents. We have developed a liposome based formulation of SN-38 (LE-SN38) with enhanced therapeutic efficacy in animal tumor models. In the present study, we have evaluated the toxicity of LE-SN38 in beagle dogs after intravenous (iv) bolus administration once every three weeks for four complete cycles (q3 weeks x 4). Eight groups (3 dogs /group) of male and female dogs received a single 0, 0.4, 0.8, or 1.2 mg/kg SN-38 (as LE-SN38) iv bolus dose on days 1, 22, 43, and 64. All of the animals survived to scheduled necropsy (days 84-85). No drug related effect on body weights was observed during the study with the exception of one male dog at 1.2 mg/kg dose group, which lost 17.4% of its initial body weight between week 1 and 2. Changes in group mean hematology parameters such as decrease in reticulocyte, lymphocyte, monocyte and eosinophil counts were noticed at approximately 72 hours after drug administration. Also, decreases in platelet, neutrophil, and WBC values were observed in LE-SN38 treated groups after the third or fourth dose cycle. However, these changes were reversible and reached within normal range after 20 days of drug administration. Clinical chemistry parameters were unchanged after LE-SN38 administration at all dose levels. There was no drug related changes in adrenals, brain, heart, kidneys, liver, lung, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid: parathyroid and uterus weights of males or females. Also, no direct, drug related microscopic changes were noticed in all 50 organs collected after LE-SN38 administration. The results obtained after the initial dose of LE-SN38 at 1.2 mg/kg/dose indicated that this dose level might have been close to a maximum tolerated dose. This study was performed at Southern Research Institute, Birmingham, Alabama.

1874 TOXICITY OF THE FIBROBLAST GROWTH FACTOR INHIBITOR, PD 176067, IN JUVENILE AND ADULT DOGS.

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PD 176067 is a reversible inhibitor of basic fibroblast growth factor receptor (FGFR) tyrosine kinase that was in development for the treatment of cancer. To assess its toxicity, PD 176067 was administered by oral gavage to young adult (∽ 14 months) and juvenile (∽ 4 months) beagle dogs (1 dog/sex/dose) twice daily up to 2 weeks at 10, 40 and 100 mg/kg/day. Clinical signs and mortality occurred in both age groups at ≥40 mg/kg. Clinical signs included ataxia, hypoactivity, generalized tremor, abnormal gait, diarrhea, dehydration, salivation, fecal changes and reduced food consumption. Carpal swelling in the juvenile male at 40 mg/kg correlated with increased metaphyseal radiodensity and thickened growth plate. At 10 mg/kg, there were minimal clinical signs (fecal changes, reduced food consumption) and no significant findings in serum biochemistry or histology. At ≥40 mg/kg, serum osteocalcin, bone formation marker, decreased by >50% and serum phosphorus increased by >50% in both age groups. Urinary markers of bone resorption were elevated by ≥50% in juveniles at ≥40 mg/kg. Vascular and/or parenchymal mineralization occurred in heart, coronary artery, lung, kidney, parietal pleura and pancreas at ≥40 mg/kg in both age groups. Additionally, sporadic gastrointestinal mucosal ulceration and hyperplasia, hemorrhage and necrosis were observed at ≥40 mg/kg in both age groups. Physeal dysplasia characterized by chondrocyte proliferation and hypertrophy was more prominent in juvenile animals than adult. These findings show that PD 176067 causes widespread systemic mineralization of soft tissues in dogs independent of age and does not appear to be secondary to alterations in bone physiology.

1875 EXPERIENCES WITH VAGINAL OR PENILE ADMINISTRATION IN TOXICITY STUDIES.

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There are several instances where pharmaceuticals are administered by the vaginal route. These include spermicidal agents and anti-infective agents. There are also cases where pharmaceuticals come in contact with penile tissue. This may be by accidental exposure or by intention, for example materials for erectile dysfunction. It is, therefore, important that these are subjected to appropriate safety tests. There are a number of factors to consider when designing suitable studies. These include species, duration and the volume to be administered. Experiences at this laboratory are discussed.

1876 RARE GASTRIC MUCOSAL DAMAGE FOLLOWING ACUTE ADMINISTRATION OF MELANOCORTIN RECEPTOR LIGANDS IN FISHER 344 RATS.

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Since the melanocortin system is believed to play a role in the central control of feeding, it is possible that melanocortin receptor ligands could be used to treat human obesity. Eleven structurally similar ligands were evaluated in female F344 rats to determine the safety of this class of compounds. Rats were gavaged daily with 100, 250, or 500 mg/kg of compound in 10% Acacia/0.05% Antifoam in purified water (pH > 2) for 4 days (n = 3/dose group). In treated rats, notable clinical observations included a dose-dependent decrease in mean body weight and food consumption. Analysis of terminal blood demonstrated a dose-responsive inflammatory leukocytosis with superimposed stress leukogram as well as increased serum alanine and aspartate aminotransferase activity in all treatment groups. Important compound-related gross and histologic observations occurred in the gastric mucosa. With 6 compounds, fluid-filled stomachs with multiple, focal, small (1-2 mm), raised, sometimes ulcerated, white foci in the nonglandular portion of the gastric mucosa were observed. Histopathological evaluation of the mucosa revealed discrete, raised, intraepithelial vesicles with dense accumulations of neutrophils at the core. Advanced lesions penetrated the stratum basale and were associated with edema, lymphohistiocytic inflammation, and neovascularization of the immediate submucosa. Ruptured vesicles resulted in ulcers and occasionally gastric perforation. No other mucosal surfaces of the gastrointestinal tract were affected, including the glandular mucosa of the stomach. Although the pathogenesis of this rare lesion is unknown, it was not likely pharmacologically-mediated due to the gastroprotective effect of melanocortin-related systems. The risk associated with formation of this lesion in species without a nonglandular portion of the stomach mucosa, such as humans, is likely minimal.

1877 A COMPARATIVE TOXICITY EVALUATION OF MITOXANTRONE AND ITS LIPOSOME BASED FORMULATION IN BEAGLE DOGS.

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Mitoxantrone (MTO), an anthraquinone is an anti-neoplastic agent and is used for the treatment of leukemia, lymphoma, breast and prostrate cancer. In clinical trials, mitoxantrone has shown to cause moderate gastrointestinal toxicity, alopecia and a dose limiting cardiotoxicity. To overcome these side effects we have developed a novel liposome based Mitoxantrone (LEM) that has shown significantly improved safety profile, pharmacokinetics and efficacy profile compare to free MTO in mice (Gokhale, et al. Anticancer Research 21:3313-3322, 2001). The improved safety profile in mice prompted us to evaluate the toxicity of LEM in a more relevant large animal model. The current study demonstrates a comparative toxicity of free MTO and LEM in male and female dogs. MTO (0.129 and 0.258 mg/kg/dose) or LEM (0.258, 0.580, and 0.869 mg/kg/dose) was administered intravenously to beagle dogs on Days 1, 23, 43, and 65. Animals that received MTO at 0.258 mg/kg/dose had gastrointestinal toxicity, cutaneous sores and swelling of limbs whereas only at highest 0.868 mg/kg/dose of LEM showed the similar level of toxicity in dogs. The dogs receiving the LEM at 0.258 and 0.580 mg/kg/dose, had no gastrointestinal toxicity. The animals receiving LEM at 0.258 mg/kg/dose level, had reduced toxicity associated with testicular degeneration, bone marrow hypocellularity and lymphoid depletion compared to dogs that received the same dose of MTO. The toxicokinteic data showed that plasma concentration of mitoxantrone in dogs was about 6 fold higher in LEM group compared to MTO. The results of the present study, demonstrates that LEM is more than two fold less toxic and has longer half life (t1/2) compared to MTO. Thus, liposome based formulation of mitoxantrone may be safe and have better therapeutic index as compare to MTO. Currently, phase I clinical trials are in progress. The study was performed at Southern Research Institute, Birmingham, Alabama. *Authors equally contributed.

1878 EFFECTS OF THE ADDITION OF LICORICE EXTRACT TO TOBACCO ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF CIGARETTE SMOKE.

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Licorice extract (LE) is used as a flavoring material in cigarettes. As such, it is useful to determine if it has an effect on the chemical and biological activity of mainstream smoke. LE was incorporated into cigarettes at a targeted normal use level (1.25%; 1X), and at multiples of the use level, (3.75%; 3X), and (12.5%; 10X). A cigarette without LE served as a control. Smoke constituents analyzed included those proposed by the CPSC and those classified as carcinogens by the IARC. The results obtained for genotoxicity (Salmonella Reverse Mutation) and cytotoxicity (Neutral Red Uptake) were comparable to the control cigarette. Cigarette analysis showed that 10X displaced tobacco as indicated by a reduction in smoke nicotine. On an equal TPM basis, there was a statistically significant decrease in B(a)A (10 %) for the 1X level, and an increase in phenol (10 %) and catechol (5 %) for the 3X level. These values were within the normal range of variability of the assay and were not considered to be LE related. At the 10X level it appeared that LE has the potential to alter the measured smoke constituents (decreases in nicotine (6%), CO (14%), nitrogen oxides (16%), HCN (18 %), acetaldehyde (6%) and NNN (11 %); increases in formaldehyde (43 %), phenol (27 %), catechol (9 %), B(a)P (19 %), and indeno(1, 2, 3-cd)pyrene (14 %)). In the 90-d rat nose-only inhalation study, COHb was slightly lower in the LE groups. Histopathologic changes were comparable to those seen in previously reported 90-d cigarette smoke inhalation studies; addition of LE had no effect. The overall assessment suggests that LE did not increase the measured smoke constituents (beyond normal variation) nor biological activity of the cigarette smoke, even at 3X the normal use level. The 10X level changed some smoke constituents, but these changes did not affect the biological activity as measured by the tests used in this study.

1879 A SUMMARY OF THE TOXICOLOGICAL AND CHEMICAL DATA RELEVANT TO THE TOXICOLOGICAL EVALUATION OF DRY ICE EXPANDED TOBACCO (DIET).

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RJRT has developed a tiered testing strategy to evaluate the potential for tobacco processes, ingredients, and other technological developments to increase or reduce the biological activity resulting from burning tobacco. The foundation of this strategy is comparative testing, typically including chemical and biological components. Expansion processes have a long history of use in food and tobacco manufacturing. DIET is an example of a tobacco expansion process currently used in the manufacture of cigarettes to increase the filling capacity of tobacco. As part of RJRT stewardship program, test cigarettes containing DIET were compared to control cigarettes containing tobacco expanded with a traditional expansion agent (Freon 11). Principal components of this program included chemistry studies using mainstream cigarette smoke (determination of selected constituent yields), *in vitro* studies using cigarette smoke condensate (Ames study in Salmonella typhimurium and sister chromatid exchange study in Chinese hamster ovary cells) and *in vivo* studies (13-week inhalation study of mainstream cigarette smoke in Sprague-Dawley rats and 30-week dermal tumor promotion study of cigarette smoke condensate in SENCAR mice). Comparisons between test and control cigarettes demonstrated that the inclusion of DIET in cigarettes had no significant impact on mainstream smoke chemistry. Also, biological tests indicated that the cigarettes containing DIET were not different from the cigarettes containing tobacco expanded using Freon 11. Collectively, these data demonstrate that the use of DIET in the manufacture of cigarettes is acceptable from a toxicological perspective.

1880 FOUR-WEEK TOXICITY STUDY OF A SURROGATE MURINE ANTI-CD11A ANTIBODY IN MICE.

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Surrogate antibodies offer a potential solution to the limited preclinical safety testing possible with humanized monoclonal antibodies with restricted species crossreactivity. CD11a is a subunit of lymphocyte function antigen-1 (LFA-1), a cell surface protein expressed on leukocytes. The interaction between LFA-1 and its ligand,

intercellular adhesion molecule-1 (ICAM-1) mediates intercellular interactions important for immune responses and inflammation. Raptiva™ is a humanized anti-human CD11a monoclonal antibody that inhibits the binding of LFA-1 to ICAM-1, and is being developed for psoriasis. A chimeric rat/mouse anti-mouse CD11a antibody (muM17) was developed as a surrogate antibody for Raptiva. This study evaluated potential adverse effects of muM17 administered to C57BL/6 mice. Male and female mice were administered vehicle or muM17 (3, 10, or 30 mg/kg) *via* subcutaneous injection weekly for 4 weeks; all animals were terminated on Day 29. The 3 mg/kg/week muM17 dose was determined by previous studies as approximately equivalent to the clinical dose of Raptiva (1 mg/kg/week). Toxicokinetic analysis confirmed exposure in muM17-treated mice, and no anti-muM17 antibodies were detected. There were no treatment-related effects on mortality, clinical signs, body weight, clinical chemistry parameters, or macroscopic observations. Increased spleen weights and histologic findings of increased cellularity in spleens and reduced cellularity in lymph nodes were observed in treated mice in all dose groups. These findings are consistent with those demonstrated in LFA-1 deficient mice and studies using other anti-CD11a antibodies, and are likely a result of altered lymphocyte trafficking to the lymph nodes and spleen. In summary, muM17 was well tolerated in mice up to 30 mg/kg/week for 4 weeks, and treatment-related changes in the spleen and lymph nodes are likely the result of the pharmacological activity of the antibody.

1881 DEVELOPMENTAL TOXICITY TESTING OF FOUR VACCINES.

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There are currently no regulatory requirements for preclinical developmental toxicity studies with vaccines, but this is a current area of interest[1]. Potential risks result either from vaccine interaction with the maternal immune system or from the binding of maternal antibodies on embryonic or fetal tissues. The following strategy was adopted to screen four new vaccines against viral or bacterial diseases for developmental toxicity. A preliminary study was performed in the rat, mouse and rabbit to evaluate the induced maternal immune response and to compare pre- and post-natal antibody titres in the offspring. Twelve females of each species were vaccinated before and during gestation. Half of the pregnant dams were submitted to caesarean section; the other half were given a booster vaccination at the end of gestation and sacrificed on day 11 post-partum. Serum antibody titres were determined in the dams, fetuses and pups. The most appropriate species was then selected for a main developmental toxicity study. On the basis of acquired experience (particularly for post-natal examinations), the rat was the preferred species followed by the mouse and rabbit. As would be expected from the literature, the highest rate of prenatal antibody transfer was usually observed in rabbits. Nonetheless, the fetal antibody titres to two vaccines attained maternal levels in mice. In rats, the fetal antibody titres were consistently lower than maternal levels, but had equalised by post-natal day 11. Consequently, the mouse was selected for the main study with two vaccines, and the rabbit for the other two. The main studies comprised a single vaccinated group of 40 mice or 36 rabbits and a sham-treated control group. Where feasible, the vaccine was administered at the human dose level without scaling for body weight. Twenty-five mice or 20 rabbits were subjected to caesarean examination and fetal examinations. The remaining litters were used for post-natal assessments. No indications of developmental toxicity were found with the four vaccines tested. [1] http://www.fda.gov/cber/gdlns/reprotox.pdf.

1882 EVALUATION OF THE SUBCHRONIC, REPRODUCTIVE, AND DEVELOPMENTAL TOXICITY OF A FLUOROALKYLETHYL ETHOXYLATE SURFACTANT.

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The objective of these studies was to evaluate the subchronic, reproductive, and developmental toxicity of a fluoroalkylethyl ethoxylate surfactant in rats. Test substance was administered by gavage at 0, 25, 100, or 500 mg/kg/day (90-day subchronic toxicity and one-generation reproduction), and at 0, 50, 100, or 400 mg/kg/day on gestation days 6-20 (developmental toxicity). The NOEL for subchronic toxicity was 25 mg/kg/day based on clinical signs, reduced body weight and nutritional parameters, thyroid follicular hypertrophy, and chronic progressive nephropathy at ≥100 mg/kg/day. Other adverse effects at 500 mg/kg/day were reduced red blood cell mass, increased liver enzyme activity, and reduced grip strength. Non-adverse liver hypertrophy and splenic effects were observed at ≥25 and ≥100 mg/kg/day, respectively. Except for effects on the thyroid at 500 mg/kg/day and kidney at ≥100 mg/kg/day, other effects were reversible after a 1- or 3-month recovery. No NOEL was determined for reproductive toxicity, based on reduced fertility in P1 rats at ≥25 mg/kg/day, which was of similar magnitude at all dose levels. Body weights, food efficiency, and number of implantation sites were reduced in P1 rats at 500 mg/kg/day, and viability and weights were reduced in F1 pups at ≥100 mg/kg/day. The reduced pup viability was relatively less severe and required much higher doses than the structurally related perfluorooctane sulfonate. Red blood cell and spleen effects were similar to those observed with ethylene glycol ethers, but there were no developmental or testicular effects as seen with glycol ethers. The mechanism for reduced fertility is not known. In the developmental toxicity study, the NOELs were 50 and 100 mg/kg/day for maternal and fetal toxicity, respectively, based on reduced maternal body weight and nutritional parameters, and clinical signs at ≥100 mg/kg/day and reduced fetal weight and increased fetal variations at 400 mg/kg/day. No fetal malformations were observed.

1883 A COMPARISON OF MULTIPLE TOXICITIES FOLLOWING DEVELOPMENTAL EXPOSURE TO PESTICIDES: NEUROTOXICITY, IMMUNOTOXICITY, AND REPRODUCTIVE TOXICITY.

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The NAS report (Pesticides in the Diets of Infants and Children, 1993) called for significant research effort into the long-term effects of perinatal pesticide exposure on the nervous, immune, and reproductive systems. In response, the USEPA and NIEHS collaborated on a series of multidisciplinary studies. The exposure paradigm was designed to encompass the development of all three organ systems (in the rat, mid-gestation through weaning and/or sexual maturity). Broad batteries of tests were used to evaluate the structure and function of each organ system in the offspring. Tissue levels of chemical and general pathology were also measured Where possible, littermates were used for the various endpoints, to draw stronger conclusions regarding relative organ system vulnerability. Five pesticides from different chemical classes, targeting different organ systems, were evaluated: methoxychlor (organochlorine), carbaryl (carbamate), chlorpyrifos (organophosphate), tebuconazole (triazole), and heptachlor (cyclodiene). A comparison of these data reveals two critical findings. First, no single organ system was consistently more sensitive than another. Developmental/reproductive measures were most sensitive to the effects of methoxychlor and carbaryl. The nervous system was most sensitive to tebuconazole, chlorpyrifos, and heptachlor, whereas the immune system was most altered by low doses of methoxychlor and heptachlor. Second, the lowest effective doses for methoxychlor and heptachlor were lower than those reported in the literature. Thus, adverse health effects following developmental exposure may occur at lower doses than previously expected. These multidisciplinary studies provide valuable and unique data; furthermore, this shows that these organ systems may be evaluated in a single combined study. This strongly supports the need for more thorough evaluation of the nervous, immune, and reproductive systems during development. This abstract does not necessarily reflect EPA policy

1884 MULTIDOSE TOXICITY STUDIES OF TNK-TPA IN NORMAL BEAGLE DOGS.

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To evaluate the safety of new thrombolytic TNK-tPA in dogs.TNK-tPA was administered intravenously, daily at 1.5mg/kg, 4.5mg/kg and 13.5mg/kg to Beagle dogs(2 dogs/sex/group) for 2 weeks. Daily intraveneous administration of TNK-tPA for 2 weeks produced expected effects on blood coagulation system in a dose-related manner (subcutaneous hemorrhage at the sites of injection, increased pothrombin time, actvated partial thromboplastin time, and decreased fibrinogen, plasminogen). At 13.5mg/kg the following additional effects were observed: mild decreases in red blood cell count, hemoglobin, and hematocrit, higher serum levels of asparate aminotransferase, and total cholesterol, hemorrhage and adipic degeneration in the livers. These findings were not unexpected based on the pharmacologic activity of TNK-tPA, and were consistent with the preclinical pharmacokinetic data (unpublished) indicating that the liver was the major organ for TNK-tPA clearance.

1884a EVALUATION OF INTRAVENOUS EXPOSURE TO DI-(2-ETHYLHEXYL)PHTHALATE IN MALE NEONATAL RATS.

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Di-(2-ethylhexyl)phthalate (DEHP) was administered intravenously for 21 consecutive days in neonatal rats to determine the potential for residual effects in sexually mature rats at 90-days of age. DEHP was administered to three- to five-day-old

Sprague-Dawley rats by daily injections of 60, 300, or 600 mg/kg/day or by daily oral gavage of 300 or 600 mg/kg/day for 21 days. Histopathological evaluation and organ weight measurements were performed on some animals after 21 days of dosing (primary group) and later on the recovery group animals that were held without further treatment until sexual maturity at approximately 90 days of age. No effects of any type were observed in animals treated intravenously with 60 mg/kg/day. Testicular changes, consisting of a partial depletion of the germinal epithelium and/or decrease in diameter of seminiferous tubules, were present in all animals of the 300 and 600 mg/kg/day groups after the 21-day dosing period. Testes weight decreased and liver weight increased in these animals. Testes changes were dose-related and generally more severe among animals dosed orally versus intravenously. In the recovery animals, a residual DEHP-induced decrease in seminiferous tubule diameter was present in the testis of several animals dosed orally at 300 and 600 mg/kg/day, but not in animals dosed intravenously. There was no germinal cell depletion or Sertoli cell alteration observed in any dose group at any time. Notably, no effects on sperm count, sperm morphology or sperm motility were observed at 90 days of age in any of the groups.

1885 BEEFING UP — REVISED BODY WEIGHTS AND SKIN SURFACE AREA ESTIMATES.

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Exposure parameters such as body weight and surface area are important inputs for both deterministic and probabilistic risk assessments. Currently, USEPA guidance for body weight is obtained from the 1997 Exposure Factors Handbook (EFH). The EFH developed body weights from data available in the second NHANES (National Health and Nutrition Examination Survey II, 1976-1980). Since that time additional information has become available from the third NHANES study (1988-1994). The goal of this analysis was to use the NHANES III data set to develop age-specific body weight estimates for male and female US residents along with ethnic group sub-populations. Maximum likelihood and minimum variance unbiased estimates were computed assuming that the body weights follow a lognormal distribution, and age-specific body weight percentiles were estimated to support future probabilistic risk assessments. Since body surface area can be calculated from an allometric relationship to body weight, revised surface area estimates were also developed. Our analysis indicates that Americans are growing heavier: the average body weight for adults (18-74 yrs) has increased from 71.8 kg to 82.11 kg. The mean body weight for white males (82.67 kg) is slightly greater than black males (82.00 kg). Black females (75.98 kg) have a greater mean body weight than white females (68.88 kg). Although these differences among ethnic groups and genders are qualitatively the same as in previous data from NHANES II, the extent of the differences has increased slightly. Use of updated body weight and surface area estimates will provide a more accurate and contemporary estimation of risk.

1886 ESTIMATION OF CHEMICAL-SPECIFIC INTERINDIVIDUAL UNCERTAINTY FACTORS USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) ALGORITHMS.

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In non-cancer risk assessments, an interindividual uncertainty factor of 10 is used as the default value. The numerical value for the pharmacokinetic component of the interindividual uncertainty factor (IUF) has been suggested to be 3.17. Steady-state algorithms, derived from PBPK models, can be used to derive the chemical-specific IUF, provided the distributions of the input parameters are known. The objective of this study was to estimate the magnitude of the pharmacokinetic component of the IUF for toluene (TOL), styrene (STY), m-xylene (XYL) and 1, 4-dioxane (DIO) using steady-state algorithms derived from PBPK models. The PBPK algorithms were used to calculate the steady-state arterial blood concentration (Ca) and rate of the amount metabolized (RAM) of chemicals in humans during chronic exposures. Following the specification of the characteristics (mean, SD) and distributions of input parameters such as the pulmonary ventilation rate (345.3, 102.1 L/hr; normal), cardiac output (342.8, 245.6 L/hr; lognormal), fractional hepatic blood flow (0.25, 0.06; normal), metabolic parameters [Vmax (mg/hr): XYL (192.6, 95.9), TOL (111.2, 55.5), STY (155.4, 78.0), DIO (147.1, 74.4); Km (mg/L): XYL (0.20, 0.04), TOL (0.55, 0.11), STY (0.36, 0.07), DIO (3.0, 0.6); lognormal] and blood:air partition coefficient [XYL (32.5, 1.6), TOL (19, 3), STY (62, 10), DIO (2905, 613); normal], 10, 000 iterations of Monte Carlo simulations were conducted to generate the output. The chemical-specific pharmacokinetic uncertainty factor was computed as the ratio of the simulated 95th and 50th percentile values of Ca and RAM. The resulting uncertainty factors for XYL, TOL, STY and DIO based on Ca were 2.52, 1.93, 2.61 and 2.23, respectively. The pharmacokinetic uncertainty factor for XYL, TOL, STY and DIO was 1.5, if the calculations were based on the rate of metabolism. These results suggest that the chemical-specific IUF calculated for TOL, STY, XYL and DIO, on the basis of mechanistic considerations, are within the currently used default value of 3.17.

1887 CHILDRENS HEALTH RISK CHARACTERIZATION FROM EXPOSURE TO TETRACHLORETHYLENE.

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In 1995 the US Environmental Protection Agency (EPA) established an agency-wide policy that calls for the consideration of the risks to infants and children in all risk assessments. We summarize the current state of the science regarding how children may differ from adults in their response to tetrachloroethylene exposures. Ideally, the uncertainty factors used to explain variability in a population could be data-derived numbers. To begin, a review of how a childs exposure may differ from an adults exposure was conducted. The primary routes of exposure include inhalation of vapor and ingestion of contaminated drinking water. Exposure through breast feeding was also considered as an exposure pathway for nursing infants. Further evaluation was carried out to determine how a childs toxicokinetic and toxicodynamic responses may differ from those of an adult. This included a review of differences in metabolism as well as other susceptibility factors that are specific to the life stage of a child. In an ideal situation, the data would allow for a quantitative understanding of these differences. However, the current knowledge base allows for only a qualitative discussion of the differences that may exist between children and adults. Attempts are made to understand these differences and data gaps are identified to better assess potential risks of tetrachloroethylene exposure to children. Disclaimer: The views presented in this abstract are those of the authors and do not necessarily represent views and/or policy of their affiliations.

1888 EMPIRICALLY IDENTIFYING AN INTERSPECIES UNCERTAINTY FACTOR FOR MILD ACUTE INHALATION EXPOSURES.

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This analysis was conducted to evaluate the uncertainty in acute inhalation risk assessment and to better understand the interspecies variability in response to mild acute exposures. When using animal data in threshold-based risk assessments, the primary approach applies a 10-fold uncertainty factor (UF) to the animal data to estimate an average human response. We identified 318 data sets, for 66 hazardous air pollutants for which NOAELs and LOAELs were reported for mild effects from acute inhalation exposures. There were 208 animal-to-human data comparisons available for mild eye or respiratory irritation for ten chemicals. We evaluated the interspecies relationship for both LOAELs and NOAELs for each chemical. First we evaluated them assuming the effects were independent of exposure time. The animal-to-human ratios for LOAELs were 5.0 at the 50th percentile, and 100 at the 95th percentile. In contrast, the animal-to-human ratios for NOAELs were 4.0 at the 50th percentile, and 200 at the 95th percentile. The value of the standard UF (10) was approximately the 65-70th percentile. Exposure time was adjusted using a modification of Haber's Law, $C^n * T = K$, where C is concentration, T is time and K is a constant. The value "n" was obtained from the literature when available. Time adjustment of the data did not improve the animal-to-human correlation. When adjusted for exposure time, the animal-to-human ratios for LOAELs were 24 at the 50th percentile, and 1300 at the 95th percentile. The time-adjusted ratios for NOAELs were 29 at the 50th percentile, and 1700 at the 95th percentile. These data suggest a very large skew in the data. Finally, it must be emphasized that this analysis is reflective only of mild acute inhalation toxicity for a limited set of chemicals. For other exposure routes, subchronic or chronic exposures or more severe toxicity, the distributions are expected to be different.

1889 REFERENCE DOSES (RfDs): FACTORS IN THE SELECTION OF HUMAN INTRASPECIES UNCERTAINTY FACTOR (UFH) WHEN USING HUMAN DATA.

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There are 14 RfDs on the USEPA's Integrated Risk Information System (IRIS) based on human studies. Data from these RfDs are taken from epidemiological, clinical and nutritional studies and their UHFs range from 1 to 10. However, there

are no definitive guidelines for the selection of UFHs on IRIS. With human data, the choice for UFH may be affected by several factors and it is important to understand the rationale for the selection of the UFHs. for each compound. One subset of chemicals on IRIS is comprised of the essential trace elements (ETEs) and their UFHs range from 1 (F and Mn) to 3 (Se, Mo and Zn). For the ETEs, the major determining factor in the selection of the UFH was the presence of human nutritional data and the setting of Recommended Dietary Allowances (RDAs). The UFHs for the other chemicals range from 1 (benzoic acid and nitrate/nitrite) to 3 (As and Ag) to 10 (aldicarb, Cd, methyl mercury and 2, 4-dinitrophenol). Rationales for assignment of UFHs of less than 10 included one or more of the following reasons; 1. adequate peer-reviewed human studies (all), 2. presence of RDAs (Mn, Mo, Se and Zn), 3. exposure to a large human population (F and Mn), 4. most sensitive species exposed (nitrate/nitrite [Although nitrite has an UFH of 1, a 10-fold modifying factor was added for its greater toxicity as nitrate data were used in the RfD derivation.]), 5. end-point not based on an adverse health effect (F and Ag), and 6. data base uncertainties (As). [The statements and opinions expressed in this abstract are those of the authors and are not necessarily those of the USEPA.]

1890 USING TOXICOKINETIC DATA FOR KINETIC COMPONENTS OF THE INTERSPECIES AND INTERINDIVIDUAL UNCERTAINTY FACTORS FOR THE BORON REFERENCE DOSE.

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Boron is a naturally-occurring element that is commonly found in food. Studies in rats fed boron in the diet during pregnancy indicate that decreased fetal body weight is the critical effect. A dose response analysis resulted in a benchmark dose (BMDL05) of 10.3 mg/kg-day. Inter- and intra-species uncertainty factors (UFA and UFH, respectively) used to extrapolate this dose from animals to humans and to the sensitive human subpopulation can be divided into toxicokinetic (TK) and toxicodynamic (TD) components. Toxicokinetics in rats and humans is markedly similar and renal elimination (filtration) accounts for differences in plasma half-life. Differences in urinary elimination of boron in pregnant rats and pregnant humans were used to quantify the inter-species TK variance as a replacement for the default value for the TK component of UFA. Differences in renal filtration (GFR) in normal pregnant women and at-risk pregnant women were used to quantify the intra-species TK variance as a replacement for the TK default value for the TK component of UFH. Together with default values for the TD components of the UF, these results suggest an overall adjustment factor for boron of approximately 50 to 60, which would replace a default uncertainty factor of 100.

1891 INTER-SPECIES DIFFERENCES IN SUSCEPTIBILITY TO PERCHLORATE: A CRITICAL CONSIDERATION FOR HUMAN HEALTH RISK ASSESSMENT.

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Due to the widespread occurrence of perchlorate as a groundwater pollutant, considerable research effort has been focused on understanding the toxicology of this chemical. A key concern involves understanding how to use rodent data to predict health effects in humans. While humans and rats exhibit similar dose-response relationships in terms acute inhibition of thyroidal iodide uptake, the two species exhibit notable differences in terms of thyroid hormone response, the downstream effect of ultimate interest. To explore this issue, data on changes in serum T3, T4 and TSH levels in response to increasing perchlorate doses were obtained from a number of studies in humans, rats, mice and rabbits. The studies were of subchronic duration (14 to 35 days). We found that the rat was strikingly more sensitive to perchlorate than any of the other species. Particularly in regard to serum TSH levels, rats exhibited a response at 0.1 mg/kg-day whereas other species remain unresponsive even at doses of 10 mg/kg-day. Perchlorate effects on serum T4 levels in the pregnant rat were also notable, although the male rat and fetal rat appeared to be less sensitive. T3 levels appeared to be less affected by perchlorate than T4 or TSH, although the rat still exhibited small decreases in T3, an effect not observed in the other species. The greater sensitivity of the rat (particularly the pregnant rat) to perchlorate compared to other species is consistent with greater rat sensitivities reported for other thyroid active agents such as amitrol or propylthiouracil. These cross-species comparisons provide strong evidence that data obtained from rat thyroid studies need to be critically judged for their quantitative relevance to humans. Direct application of rat dose-response relationships to humans would overestimate the potential human risks from perchlorate. We propose that the case of perchlorate is one of the rare situations where an inter-species uncertainty factor of less than one can be adequately supported.

1892 HIGHER SUSCEPTIBILITY OF NEWBORN RATS TO 3-METHYLPHENOL THAN YOUNG RATS.

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Considering higher susceptibility of infants to 3-methylphenol, the repeated dose toxicity study was conducted by oral administration in newborn and young rats. In the 18-day newborn study from postnatal days 4 to 21 at doses of 30, 100 and 300 mg/kg, various clinical signs including deep respiration, hypersensitivity at handling and tremors under the contact stimulus, and depressed body weight gain were observed at 300 mg/kg. At 100 mg/kg, hypersensitivity and tremors were also noted in a small number of males only at each single day during the dosing period. No other adverse effects were observed in 100 and 30 mg/kg groups. There were no abnormalities of physical development including sexual maturation and reflex ontogeny. The no observed adverse effect level (NOAEL) was considered to be 30 mg/kg/day and the unequivocally toxic level 300 mg/kg/day for newborn rats. In the 28-day study starting at 5 weeks of age, clinical signs and depression of body weight gain as observed in the newborn rats appeared in both sexes at 1000 mg/kg but not 300 mg/kg. The unequivocally toxic level and the NOAEL were 1, 000 mg/kg/day and 300 mg/kg/day, respectively. From these results, newborn rats were considered to be 3 to 10 times higher susceptible to 3-methylphenol than young rats. However, realistic no adverse effect dose for the newborn must be slightly lower than 100 mg/kg/day, at which the toxicity incidence was very low, rather than 30 mg/kg/day. Based on this speculation and the equal toxicity at the unequivocally toxic levels, the differences in the susceptibility to 3-methylphenol could be concluded to be 3 to 4 times. It is consistent with the results of our previous comparative studies on 4-nitrophenol, 2, 4-dinitrophenol and 3-aminophenol, which showed 2 to 4 times differences in the susceptibility between the newborn and young rats.

1893 ASSESSMENT OF THE SKIN ABSORPTION OF MALATHION.

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Malathion is a commonly used organophosphate pesticide on field crops, fruits, nut trees, vegetables, livestock, agricultural premises, and land. The approved uses also include mosquito and medfly control. These uses can result in human skin contact. The purpose of this study was to evaluate the human skin absorption of malathion for the purpose of assessing the risks associated with aqueous solution exposures following applications. Aerial applications can result in solubilized malathion in swimming pools and other waters that may be contacted. Human volunteers were selected and exposed to aqueous solutions of malathion at various concentrations. Participants submerged their arms or hands in a 10-gallon tank containing 20 liters of malathion in either a stagnant or moving environment. Malathion concentration in the water was determined before and after the human subjects placed their arms or hands in the water for various periods of time. Malathion was measured using Gas Chromatography. No measurable skin absorption was detected in 43% of the participants. Measurable skin absorption among some participants resulted in doses that were more than an order of magnitude less than the minimal dose necessary to cause a measurable change in blood cholinesterase. Extrapolation of these results to recreational exposure to swimming pool or surface water levels of malathion typically detected after application again are an order of magnitude below the doses needed to cause a detectable change in blood cholinesterase. These data indicate that exposure to aqueous malathion following usual aerial applications is not appreciably absorbed, and therefore, it is not a public health hazard. (Supported in part by the Department of Defense)

1894 PHYSICAL ACTIVITY PRIOR TO EXPOSURE INCREASES HUMAN ABSORBED DOSE TO SURFACE RESIDUES OF CHLORPYRIFOS (CP).

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To develop realistic and responsible estimates of human exposure and absorbed daily dosage (ADD), it is important to identify and evaluate potential determinants of exposure, and to clarify the extent of human exposure following pesticide applications. Transferable chemical residue rapidly declines following application (Krieger et al., 2001), correlated with a decline in carpet moisture (Williams et al,

2002). Skin moisture has also been shown to increase the dermal absorption of propoxur (Meuling et al., 1997). A structured activity program (SAP) was used to estimate human exposure potential (Jazzercise®; Ross et al., 1990; Krieger et al., 2000; Bernard et al., 2001). Participants were randomly divided into two groups. Group 1 participated in a warm-up exercise program in an untreated area, while group 2 remained sedentary. All participants completed the SAP in an area treated 24-hours earlier with CP. Each participant collected a morning urine specimen prior to the SAP and then for 5 subsequent days, that were analyzed for TCP. Two weeks later the study was repeated with group 2 participating in the warm-up exercise, while group 1 remained sedentary prior to the exposure period. Participants that completed the warm-up exercise (123 ± 39 and 88 ± 32 μg TCP excreted following exposure 1 and 2, respectively) had a significantly higher absorbed dose compared to those that rested prior to the SAP (84 ± 41 and 57 ± 22 μg TCP excreted following exposure 1 and 2, respectively). Estimates of absorbed dose made from environmental measurements, e.g. deposition (34 ± 4 and 31 ± 3 μg CP/cm2 for exposure 1 and 2, respectively), modified California roller (0.25 ± 0.07 and 0.23 ± 0.07 μg CP/cm2 for exposure 1 and 2, respectively), and personal dosimetry (0.6 ± 0.3 and 0.3 ± 0.1 μg CP/cm2 on shorts worn during exposure 1 and 2, respectively; (2.6 ± 1.1 and 0.7 ± 0.3 μg CP/cm2 on socks worn during exposure 1 and 2, respectively), overestimated the results of urine biomonitoring.

1895 ORGANOPHOSPHATE CUMULATIVE ASSESSESSMENT USING CARES (CUMULATIVE AND AGGREGATE RISK EVALUATION SYSTEM).

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The Food Quality Protection Act mandates that the US Environmental Protection Agency evaluate both the aggregate and cumulative risks associated with pesticide use. CARES, a computer program developed by CropLife America (CLA) is designed to perform such analyses. CARES utilizes relevant databases to evaluate potential risk from dietary, drinking water, and residential sources *via* the oral, dermal, and inhalation routes of exposure for a single pesticide or multiple pesticides with a common mechanism of toxicity. A 365-day exposure profile for each individual in a population of interest is created and used to estimate acute, short term, intermediate term, and chronic risks according to the relevant toxicity profile of the pesticide(s). CARES allows identification of the factors contributing to the highest percentiles of risk. The organophosphate insecticides were the first group of pesticides to be evaluated by EPA for cumulative exposure. The Sound Science Policy Alliance and CLA undertook a parallel assessment utilizing CARES. The toxicity endpoints used in the assessment were Relative Potency Factors based on BMD10 21-day female rat brain choinesterase inhibition studies of 30+ organophosphates. The dietary exposure results are based on the USDA/EPA Food Consumption Intake Database, and the PDP food residue data supplemented by additional studies. Residential assessments within CARES were based on EPA Residential SOP algorithms and OP specific product use data. Drinking water was not included in the CARES assessment because it had previously been determined by EPA to be a non-significant contributor to overall risk. Results at various percentiles closely paralleled the EPA-derived Margins of Exposure. Results from the CARES assessment indicate that the overall safety of the US population is not threatened by the use of OP insecticides.

1896 METHYL ISOTHIOCYANATE (MITC): RISK TO HUMANS FOLLOWING AGRICULTURAL APPLICATIONS OF METAM SODIUM (MS).

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Metam sodium (MS; sodium N-methyldithiocarbamate) is a fumigant used in agriculture as a preplant biocide. The primary degradation product and pesticidal agent, methyl isothiocyanate (MITC), evolves as a gas after MS application. MS gained public attention following the spill of 19, 500 gallons into the Sacramento River in July 1991. Subsequent exposure to airborne MITC and other MS breakdown products caused an array of irritational health effects in local residents. Drift incidents following MS agricultural applications have also resulted in irritational injuries. We evaluated the risks to humans from MITC following normative agricultural use of MS. Exposure of workers and the public was documented in a series of MITC air monitoring studies conducted mainly in the southern Central Valley. The critical acute NOEL of 220 ppb was determined in a human eye-only exposure study, based on eye irritation at 800 ppb. The subchronic NOEL of 100 ppb was based on nasal epithelial atrophy in a 4-week rat inhalation toxicity study; it was estimated from the study LOEL of 1.7 ppm using Haber's Law. The chronic NOEL of 10 ppb was calculated by dividing the subchronic NOEL by a default uncertainty factor of 10. Acute margins of exposure (MOE=exposure dose/NOEL) for occupational and application site scenarios ranged between <1 and 59, with many scenarios <10, indicating an acute health concern. The acute MOE range for ambient air scenarios was 15-2200. The seasonal MOE range for occupational/application site scenarios was 1-250, also indicating a health concern. Seasonal ambient air MOEs were 28-166, 667. Annual MOEs were <1-143 for occupational/application site exposures and 5-25, 000 for ambient air exposures. While some monitoring studies were not done using currently approved application methods, this evaluation does indicate a clear health concern for individuals exposed to airborne MITC near fields under treatment with MS.

1897 FRAMEWORK FOR ASSESSING DIETARY CHEMICAL THREATS.

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Safeguarding food supplies from the deliberate introduction of chemical agents is a major challenge, especially with the world-wide access to a range of raw and processed foods. Pre-farm, farm and slaughterhouse levels provide opportunities for chemical contamination. Trace-back and forward of contaminated products is very complex, particularly on an international level. Food production includes the following critical steps: agricultural production, storage and transport of commodities, processing, storage and transport of processed products, retail distribution, food service, and individual food preparation. In assessing a myriad of potential dietary chemical threats it is necessary that a threat assessment framework be used that considers critical salient factors. A multi-factorial framework was developed that included the following critical factors which were considered critical in identifying the most probable acute/short-term dietary threats: (1) well known historical use - name recognition - it has to be perceived as a threat by the public and the media, (2) accessibility of source material, (3) acute/short-term potency, (4) detectability - organoleptic or analytical, (5) stability and ease of handling and (6) transport stability in food. A wide-range of classes of potential chemical toxicants were assessed by an internal expert panel using this critical factors framework. The following classes were selected as the most pr obable threats on the basis of their numerical ranking: acetylcholine esterase inhibitors; (e.g., organophosphate pesticides), plant toxins - exquisitely potent and easy to work with in terms of stability and transport (e.g., ricin), pharmaceuticals - ease of availability and inherent toxicity, mushroom toxins - several classes demonstrate extreme toxicity, mycotoxins while more of a chronic issue several do demonstrate potent acute/short-term toxicity and marine toxins - while generally limited in their accessibility several are exquisitely toxic and are available (e.g., tetrodotoxin).

1898 RISK CHARACTERIZATION MODEL-1.1 AND AN ASSESSMENT AND CHARACTERIZATION FOR A RIOT-CONTROL AGENT.

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Riot-control agents are important assets in nontraditional military operations, such as peacekeeping missions. DoD directives call for these weapons to achieve an appropriate balance between the competing goals of having a low probability of causing death, permanent injury, and collateral materiel damage, and a high probability of having the desired anti-personnel or anti-materiel effects. A conceptual framework for characterizing the risks of human effects from non-lethal weapons has been developed and applied to different types of weapon systems. From this framework a probabilistic software model illustrating how exposure and risk characterization modeling can be conducted for non-lethal weapons was developed. This Rick Characterization Model-1.1 was modified for use with the riot-control agent, o-chlorobenzylidene malononitrile (CS). The toxicity of this weapon, both mild and significant, has been characterized at a detailed level. However, aggregation of information is also given in order to compare the effectiveness and toxicity of this weapon in a limited number of different situations. The Risk Characterization Model (RCM-1.1) was modified to incorporate exposure variables and available data, and dose response information of CS to determine potential risks and benefits of its use. The human physiological measures of mild toxicity were the dose concentrations required to cause transient burning and pain of exposed mucous membranes and skin. The significant toxicity from possible longer-term exposure and possible high level exposure were included, as were the possible hazards from impact from the vehicle of CS delivery. This work was funded by Department of Defense under subcontract PO P66050-DSC0142.

1899 A RISK ASSESSMENT-BASED TOXICOLOGICAL WEIGHTING OF CIGARETTE SMOKE CONSTITUENTS.

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Characterization of the potential risks from chemicals are based on risk assessment, which deals with the qualitative classification of substances and/or the quantitative delineation of risk factors or reference concentrations thereof. To prioritize cigarette smoke constituents as targets for reduction, we applied the approach outlined in the USEPA guidelines for the comparative assessment of chemical mixtures (1986) and used published inhalation unit risks and reference exposure limits, e.g., Cal EPA (2002), for cancer and non-cancer risk estimates. Smoke constituent yields for the Reference Cigarettes 1R4F and 1R5F as well as 8 market brands were determined and weighted with the cancer and non-cancer risk factors. With this model, the ranking of calculated risks suggests that the major risk contribution comes from only a few gas phase constituents, e.g., 1, 3-butadiene and acrylonitrile for cancer and acrolein for non-cancer. This weighting approach can also be used in the comprehensive assessment of the smoke chemistry of new cigarette designs. In smoke from an electrically heated cigarette with ammonium magnesium phosphate in the overwrap, the yields of most smoke constituents were significantly reduced compared to the 1R4F, while a few were increased. Application of the weighting approach demonstrated an overall reduction in the calculated cancer and non-cancer risk estimates for the electrically heated smoking system compared to the 1R4F. Although this approach is somewhat limited by the uncertainty/lack of potency data and the inherent limitation of the chemical analysis of complex mixtures such as cigarette smoke, it can supplement the evidence obtained from experimental toxicological investigations for the overall evaluation of new cigarette designs.

1900 FEASIBILITY OF TESTING INGREDIENTS ADDED TO CIGARETTES.

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The Life Sciences Research Office (LSRO), a nonprofit, biological research organization, is currently reviewing the data relevant to testing ingredients added to cigarettes in order to ascertain their potential health risks, relative to cigarettes without the additives. The US Food and Drug Administration approved many of the nontobacco ingredients for use as food additives, often with Generally Recognized as Safe (GRAS) status. The Flavor and Extracts Manufacturers Association of the United States also recognizes many of these ingredients as safe for ingestion. However, few data exist concerning the toxicology of nontobacco ingredients when inhaled, either directly or as pyrolysis products. In addition, consumers inhale nontobacco ingredients (or their pyrolysis products) in combination with the combustion products of tobacco. LSRO assembled an Expert Committee of ten scientists, representing multiple disciplines to provide advice regarding the review, and divided the work into three phases: feasibility, scientific criteria, and detailed reviews. For the first phase of the project, LSRO's staff scientists have reviewed more than 1, 800 scientific publications and met with the Committee eight times to discuss and prepare a report about the feasibility of testing nontobacco ingredients added to cigarettes. The feasibility report will cover smoke generation, smoke properties, adverse health effects of smoking, testing principles, test data, and research recommendations. LSRO solicited public comments and held an open meeting in August 2002. Nontobacco ingredients added to cigarettes could change relative risks of smoking in any of three ways: (1) a direct effect of inhaling the additive (or its pyrolysis products) transferred in the smoke, (2) a change in the physics or chemistry of the smoke, or (3) a change in human smoking behavior. Transfer and pyrolysis of ingredients in smoke is crucial to the assessment of relative risks. Please see the LSRO web site at www.lsro.org for additional details. Work sponsored by Philip Morris USA.

1902 ACRYLAMIDE: A CASE STUDY IN HAZARD ASSESSMENT OF GENETIC TOXICITY.

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Recent discovery of acrylamide (A) in processed, carbohydrate-rich foods renews concerns initiated by its use as a flocculent in drinking water and as component of some plastics that could migrate into food. Exposure to A produced by processing of food is at much higher levels than A which migrates from wrappings, but still is orders of magnitude below most experimental dose levels. A is a reported animal mutagen and carcinogen and a human neurotoxin. Dose-response relationships for these endpoints are critical for policy and regulatory decisions for A. FDA concluded that A could be carcinogenic upon migration from plastic food-wrappings. In the case of germ cell mutagenicity, USFDA faces a particularly steep challenge in that widely accepted or standard extrapolation approaches are not available. Furthermore, general insight into the genotoxic potential of A through examination of mutagenicity data will be important in evaluation of mode of action for carcinogenicity. While positive results of A have been noted at some i.p. high doses, mutagenicity assays leave the scientific question of whether lower dietary doses would cause heritable mutations. Conversely, it is unclear whether doses sufficient to cause mutation will, without causing sterility, result in mutations conveying to offspring. Exposure timing is a critical factor for mutagenesis in mouse male germ cells. Exposure is most effective after the second meiotic division; continuous exposure would be required for continuous production of mutated sperm. The underlying policy question is how to appropriately consider this uncertainty in decision-making. A preliminary hazard analysis of dietary A shows potential hazard, but the relevance to human dietary exposure is equivocal. For risk assessment, further data are needed to justify extrapolations, such as *in vitro* to *in vivo* extrapolation, data which clearly define the dietary intake, effective human exposure from food matrices, and appropriate dose-response relationships.

1903 RISK ASSESSMENT OF ORAL EXPOSURE TO DIISONONYL PHTHALATE (DINP) FROM CHILDREN'S PRODUCTS.

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Diisononyl phthalate (DINP) is a plasticizer used in children's products such as soft plastic toys. DINP may be released when children mouth PVC products. The potential chronic health risks of phthalate exposure to infants have been investigated by regulatory agencies worldwide. Manufacturers voluntarily removed DINP from teethers and rattles in 1999, but DINP is still used in toys. This paper describes a CPSC staff risk assessment of DINP exposure from children's products. It has not been reviewed or accepted by the Commission. Key input data include DINP migration rates for a representative sample of soft plastic toys and an observation study of children's mouthing behavior. The acceptable daily intake (ADI) level of 120 μg/kg-d is based on chronic liver effects. Probabilistic risk assessment methods were applied. Mouthing behavior and, thus, exposure depend on the child's age. Estimated exposures for soft plastic toys (an estimated 42% of which contained DINP) by age are: 3 to 12 months old, mean, 0.07 (95% C.I. 0.03-0.13) μg/kg-d; 95th percentile, 0.44 (0.15-0.82) μg/kg-d; 12 to 24 months old, mean 0.08 (0.04-0.14) μg/kg-d; 95th percentile, 0.53 (0.24-0.89) μg/kg-d; and 24 to 36 months old, mean, 0.03 (0.01-0.06) μg/kg-d; 95th percentile, 0.12 (0.04-0.23) μg/kg-d. The authors conclude that oral exposure to DINP from mouthing soft plastic toys containing DINP is not likely to present a health hazard to children. Exposures were also estimated for several hypothetical cases, all assuming 100% prevalence of DINP. Estimated 95th percentile exposures for soft plastic toys; soft plastic toys, teethers, and rattles; all soft plastic objects; and all toys, teethers, and rattles for these hypothetical cases were below the ADI. (The opinions expressed by the authors do not necessarily represent the views of the Commission. This abstract is in the public domain and may be freely copied or reprinted.)

1904 CHEMICAL-SPECIFIC HEALTH CONSULTATION FOR CHROMATED COPPER ARSENATE (CCA) SPILL.

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This health consultation was prepared to support assessment of the public health implications of leakage of large amounts of CCA from defective shipping containers to soil. Conclusions are applicable to other spills and releases of this chemical mixture. CCA, a wood-preserving pesticide, contains chromium(VI), arsenic(V), and copper(II) in an aqueous solution or concentrate. Concerns for long-term exposure to CCA included persistence in soil, potential runoff with consequent contamination of shellfish and fish, and leaching to sources of drinking water. Evaluation of experimental studies of CCA fate in soil and monitoring studies of wood-preserving facilities where CCA was spilled on the soil indicated that chromium(VI), arsenic, and copper can leach from soil into groundwater and surface water. Leaching potential decreased in the order chromium(VI)>arsenic>copper. CCA persisted in soil and remained leachable for years. Chromium(VI) is not likely to accumulate in fish and shellfish. Copper may accumulate in tissues of mussels and oysters. Arsenic may accumulate, primarily in a relatively nontoxic form, in tissues of fish and shellfish. Evaluation of health effects studies on CCA indicated little or no harm to workers exposed at low levels, but data were inadequate for derivation of a health guideline. Adverse effects in people and animals from high-level exposure were similar to those from the individual components of CCA. These effects included irritant or corrosive effects (all three components), dermal effects (arsenic), neurological effects [arsenic and chromium(VI)], hematological and renal effects (all three). Additional effects of concern for oral exposure to CCA include

hepatic effects (all three components) and cancer (arsenic). A weight-of-evidence analysis predicted that interactions among the components are likely to be less than additive or additive. Thus, no increase in toxicity beyond that expected from additive toxicity towards shared target organs is expected for CAA.

1905 COMPARATIVE EXPOSURE ASSESSMENT FOR THIMEROSAL MERCURY.

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Thimerosal (ethylmercurithiosalicyclic acid) is a mercury-containing preservative that has been widely used as a vaccine additive since the 1930s. Recent safety concerns and litigation over routine use of thimerosal-containing vaccines in infant immunization schedules have forced major manufactures of vaccine preparations to re-formulate their products or cease manufacturing, with unintended consequences including vaccine shortages and missed immunizations. Various agencies and organizations have developed exposure guidelines for methylmercury (but not for ethylmercury) ranging from 0.1 μg/kg/day (EPA reference dose) to 0.47 μg/kg/day (WHO daily tolerable intake). According to the immunization schedule recommended by the Centers for Disease Control and Prevention, infants may be exposed to a total mercury intake of 237.5 μg over the course of the first year and a half of life, or approximately 0.03 μg/kg/day. This is more than 3-fold lower than the EPA reference dose and more than 14-fold lower than the WHO daily tolerable intake. This is also from 2- to 70-fold lower than the daily dietary intake of mercury (1 - 30 μg). Repeated injections of thimerosal-containing gamma globulin have been reported to result in increases in blood mercury concentrations of 18 μg/L. This is only 9% to 36% of the background blood concentration found in consumers of mercury-containing seafood (50 - 200 μg/L). Thus, health threats from thimerosal-derived mercury exposure are more imagined than real and detract from far greater adverse public health consequences associated with missed vaccinations.

1906 ESTIMATED CHILDREN'S EXPOSURE TO DECABROMODIPHENYL OXIDE IN THE US.

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The EPA's Voluntary Children's Chemical Evaluation Program (VCCEP) will provide information to the public on 21 chemicals, selected based on their presence in biomonitoring or environmental samples, by requesting that chemical manufacturers or importers quantify children's potential health risks from exposure. Included in the VCCEP is decabromodiphenyl oxide (DBDPO), one of the most widely used brominated flame retardants. The majority of DBDPO is used as a flame retardant for high-impact polystyrene products such as television cabinets. DBDPO is also applied to upholstery textiles as a back coat, to make the fabric flame retardant. Information from published literature and manufacturers about the uses and concentrations in various media was combined with data on the chemical and physical properties of DBDPO, and serum concentrations of DBDPO from workers exposed to DBDPO and blood donors thought to represent the general population, to characterize children's potential exposures to DBDPO. The exposure pathways evaluated were ingestion of breast milk, mouthing of consumer products containing DBDPO, and exposures *via* the general environment. Reasonable and upper estimates (REs, UEs) of intake were calculated for three different populations: 1) breast-feeding infants of mothers who manufacture DBDPO, 2) breast-feeding infants of mothers who disassemble T.V.s, and 3) a child's and adult's lifetime average exposures associated with DBDPO in the environment. The highest estimated exposure (representing a very conservative upper bound) was the UE for breast-feeding infants of mothers who manufacture DBDPO. The lowest was the RE for the lifetime environmental exposure scenario. The calculated exposures were combined with toxicity data to calculate non-cancer hazards. Exposures for all scenarios evaluated were significantly below the reference dose for DBDPO; therefore, based on this assessment, DBDPO is unlikely to pose a health risk to infants or children.

1907 DOSE-RESPONSE INVESTIGATION OF TRICRESYL PHOSPHATES POTENTIALLY PRESENT IN AIRPLANE CABIN AIR FROM JET ENGINE OILS.

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Tricresyl phosphate (TCP), a heterogeneous mixture of organophosphate aryl congeners, has been the subject of considerable toxicological interest due to its use in some synthetic jet engine oils to provide anti-wear properties and concerns regarding "incidents" of unintended venting of exhaust or vapors into aircraft cabins during flight. To assess possible toxicologic sequelae, we completed an exhaustive review of the scientific literature and a detailed dose-response assessment of TCPs under probable human exposure conditions. A primary effect of exposure to some TCP mixtures observed historically in some species, including humans, is organophosphate-induced delayed neuropathy (OPIDN), which manifests as a delayed neurological response up to two weeks after exposure. However, evaluation of this characteristic neuropathy reveals a striking dose-response threshold, dependent on the concentration of ortho-configured components in the mixture, below which toxicological effects are not observed. Unlike other organophosphates, toxic effects attributable to cholinesterase inhibition are not likely since the components of TCP are weak acetylcholinesterase inhibitors. Using dose-response data from exposure of the most sensitive animal species (chicken) to TCPs of differing ortho content, historical data from human accidental and occupational exposures, current engine oil compositional data, and assumptions about worst-case exposure conditions, the evaluation revealed that achievement of toxicological thresholds under probable exposure conditions in aircraft cabins is extremely unlikely.

1908 EPIDEMIOLOGICAL VALIDATION OF ENVIRONMENTAL CANCER RISK ASSESSMENTS: A CASE STUDY IN POPULATIONS EXPOSED TO POLYCYCLIC AROMATIC HYDROCARBONS.

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It has been proposed that epidemiological methods could validate locality-specific environmental cancer risk assessments (RA). We assessed the feasibility of this approach in populations exposed to polycyclic aromatic hydrocarbons (PAH) in 6 aluminium smelting and 4 other communities in Quebec. Lifetime lung cancer risks predicted for these communities on the basis of PAH and benzo(a)pyrene (BaP) levels measured in the early 1990s were converted to equivalent annual incidence rates among women. We compared these predictions with statistically detectable incidence rates based on a Poisson sample size formula. We then used robust weighted linear regression to estimate an exposure-response gradient between female lung cancer rates (1989-1993) and BaP exposure levels measured during those years. The highest upper bound estimate of the lifetime risk of lung cancer was 89 x 10^{-5}. The corresponding excess incidence rate (1 x 10^{-5} x $year^{-1}$) was 9 times smaller than what could be detected statistically over the 1989-1993 period in that community. Indeed 380 years of observation would be required for that excess to become statistically significant. If however exposures were 10 times higher before 1970, the predicted excess incidence of 10 x 10^{-5} x $year^{-1}$ today would require only 4 years of data (100 times less) to be significant. A linear regression model based on "dispersion-adjusted" exposure estimates showed an exposure-response gradient (P=0.035) across the 10 communities. This gradient would match the BaP-based RA if pre-1970 exposures had been 54 (95% CI 5-103) times higher than today, akin to Alcan's own guesstimate of 7 times. Although RAs may be the only option to assess health impacts of low levels of carcinogens in small populations, epidemiological studies may have the statistical power to assess impacts of past environmental exposures one order of magnitude higher than today under certain conditions.

1909 AN ESTIMATION OF CANCER RISKS POSED BY EXPOSURE TO PARTICULATE MATTER IN AIR IN SANTIAGO, CHILE.

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The objective of this analysis was to conduct a two-dimensional probabilistic risk assessment for lung cancer from exposure to respirable particulate matter in Santiago, Chile. Small particulate air pollution in Santiago has increased dramatically since the 1980s due to an increase in the number of motor vehicles. The problem is particularly severe during the winter, when air dispersion of particulates is reduced. Lung cancer risks from inhalation of particulates were estimated using three different approaches based on the PM2.5, diesel exhaust (DE), and polycyclic aromatic hydrocarbon (PAH) composition of air. The DE concentration in air was estimated from PM10 measurements and previous analysis of the proportion of PM10 due to DE in Santiago. Annual average concentrations from 1996 were used, the most recent data set with information needed to derive all three air concentrations of interest (DE, PM2.5, and PAHs). Outdoor air concentrations were corrected to reflect personal breathing zone exposure using comparison data from children living in Santiago. For DE, cancer risks were estimated using two different inhalation unit risk (IUR) ranges, one developed by the CalEPA and the other from an exploratory analysis conducted by the USEPA. Cancer risks from PM2.5 were estimated using data from a large prospective mortality study focusing on PM2.5 (Pope et al., JAMA 287:1132-1141, 2002). Lung cancer risks for PAHs were estimated using an IUR for benzo(a)pyrene. Excess lung cancer risks from DE and

PM2.5 were both in the single-digit percent range. Predicted excess lung cancer risks from PAHs were orders of magnitude lower, suggesting that PAHs in DE and PM2.5 are not the primary source of the large lung cancer risks from particulates. Given the lag time for lung cancers, the full consequences of the recent deterioration in air quality in Santiago may not yet be apparent. However, it may be relevant to note that while mortality from most cancer types has decreased in Chile since 1970, mortality due to lung cancer has doubled.

1910 RISK COMPARISONS OF EXHAUST EMISSIONS FROM SCHOOL BUSES IN COMPRESSED NATURAL GAS, LOW-EMITTING DIESEL, AND CONVENTIONAL DIESEL ENGINE CONFIGURATIONS.

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This study compared the emissions profiles of three currently available school bus configurations: conventional diesel, low-emitting diesel and natural gas. The low emitting diesel engine used Green Diesel Technology® (catalyzed diesel particulate filter, a low nitrogen oxides (NOx) engine control module, and ultra-low-sulfur fuel). Each engine configuration was tested three times with each test consisting of three consecutive runs of the City-Suburban Heavy Vehicle Cycle. Regulated and unregulated emissions were analyzed using standard methods. Twenty-one of the 41 toxic air contaminants (TACs) listed by the California Air Resources Board as being present in diesel exhaust were not found in the exhaust of any of the vehicles tested despite very low detection limits. The conventional diesel had the highest emissions for five TACs. Natural gas exhaust had higher levels of six TACs (acetaldehyde, acrolein, benzene, formaldehyde, methyl ethyl ketone, propionaldehyde) compared to low-emitting diesel. None of the TAC emissions of the natural gas vehicle were lower than the low-emitting diesel. Low-emitting diesel had the highest emissions of nitrogen dioxide (NO2) and carbon dioxide (CO2); but had the lowest emissions of NOx, nitrogen oxide (NO), particulate matter (PM), soluble organic fraction of PM (SOF), total hydrocarbons (THC), non-methane hydrocarbons (NMHC), methane, and carbon monoxide (CO). Natural gas had the highest emissions of NOx, NO, THC, NMHC, methane and CO, but the lowest emissions of CO2 and lower emissions of NO2 than low-emitting diesel. Both the natural gas and low-emitting diesel buses had essentially no sulfate emissions. Both cancer and non-cancer potency weighted emissions were lowest for the low-emitting diesel vehicle. Overall, the low-emitting diesel technology had the lowest risk from emissions associated with EPA criteria pollutants and TACs.

1911 POTENTIAL HEALTH IMPACTS OF A MAJOR TIRE FIRE AT WESTLEY, CA IN SEPTEMBER 1999.

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The California Air Resources Board conducted air monitoring during the Westley Tire Fire. Air monitoring data can be useful in prioritizing the potential health impacts from major tire fires and identifying appropriate public health responses and recommendations. PM-10, carbon monoxide, black carbon, 1, 3-butadiene, benzene, metals and PAHs were monitored for several weeks during the tire fire at populated sites. PAHs monitored include: benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenz (a, h) anthracene, benzo (ghi) perylene and indeno (1, 2, 3-cd) pyrene. Elevated PM-10 levels were identified as posing the greatest risk to public health based in part on the air monitoring results. Airborne PAH levels monitored during the Westley tire fire did not appear to significantly increase lifetime cancer risk relative to routine exposures. Monitored levels of metals did not appear to pose a threat to public health based on comparison with California Reference Exposure Levels. Favorable meteorology and other factors ameliorated the potential public health impacts. However, a major tire fire under different circumstances could result in very serious public health impacts.

1912 RISK ASSESSMENT OF POLYCHLORINATED BIPHENYLS AT HAZARDOUS WASTE SITES.

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We recently reported on a hazardous waste site with polychlorinated biphenyl (PCB) vapors in indoor air in housing units built over soil contaminated with the PCB mixture Aroclor 1260. This poster illustrates chemistry, exposure assessment and toxicity issues associated with PCB risk assessment. Commercial mixtures of PCBs, e.g. Aroclors, consist of different combinations of the 209 individual PCB congeners. Environmental fate and transport processes change the congener composition following release into the environment. This was demonstrated by the preponderance of mono- and dichlorinated congeners in indoor air resulting from an Aroclor 1260 spill containing mostly penta- to octachlorinated congeners. Long term toxicity studies, and hence toxicity criteria, are only available for Aroclors rather than individual congeners. Our findings showed that PCB mixtures at a waste site may be quite different than mixtures on which toxicity criteria are based. USEPA currently provides three cancer slope factors ranging from 0.07 to 2.0 (mg/kg-day-1) based on bioassays of Aroclors 1260, 1254, 1242, and 1016 and the exposure route, exposure medium and congener makeup; Cal-EPA uses the same range of slope factors and slightly different selection criteria. Additionally, non-cancer toxicity is of concern at relatively low doses. For example, doses of Aroclor 1016 predicting a cancer risk above 5E-6 are associated with an unacceptable non-cancer hazard. Consideration of dioxin-like activity of individual congeners is also appropriate but consensus methods are unavailable. Exposure assessment of PCBs is complicated by the bioaccumulative properties, necessitating consideration of potential for food chain exposure and by the low-level ambient presence of PCBs in environmental media including air, soil and food and a paucity of physical chemical and physiological data needed to characterize the potential for other exposures such as infants exposed *via* breast milk.

1913 RISKS TO CHILDREN FROM EXPOSURE TO LEAD IN AIR DURING REMEDIAL OR REMOVAL ACTIVITIES AT SUPERFUND SITES: A CASE STUDY OF THE RSR LEAD SMELTER SUPERFUND SITE.

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This study explored modeling approaches for assessing potential risks to children from air lead emissions during removal of a smelter facility. The EPA Integrated Exposure Uptake Biokinetic (IEUBK) model and the International Commission of Radiologic Protection (ICRP) lead model were used to simulate blood lead concentrations (PbB) in children, based on monitored air lead concentrations. Although air lead concentrations at monitoring stations located in the downwind community intermittently exceeded the NAAQ standard for lead (1.5 μg/m3), both models indicated that exposures to children in the community areas did not pose a significant long-term or acute risk. Long-term risk was defined as greater than 5% probability of a child having a long-term PbB that exceeded 10 μg/dL, the CDC and the EPA concern level. Short-term or acute risk was defined as greater than 5% probability of a child having a PbB on any given day that exceeded 20 μg/dL, the CDC trigger level for medical evaluation. The models were also used to estimate air lead levels for short- or long-term exposures that would not exceed specified levels of risk (risk-based concentrations, RBCs) for given daily exposure durations (3 or 8 hr/day) or frequencies (1-7 days/week). RBCs for long-term exposures, based on the ICRP model, ranged from 0.3 (7 days/week, 8 hr/day) to 4.4 μg/m3 (1 day/week, 3 hr/day) and were lower than those based on the IEUBK model (1.9-18 μg/m3). RBCs short-term exposures based on the ICRP model ranged from 3.5 to 29.0 μg/m3. Recontamination of remediated residential yards from deposition of air lead emitted during remedial activities at the site was also examined. The increase in soil lead concentration due to lead deposition in the community at large was predicted to be 3.0 mg/kg; this would be less than 1% increase when compared to the cleanup level of 500 mg/kg developed for residential yards at the site. (Views are those of the authors and not necessarily of any Federal agency or department.)

1914 HUMAN HEALTH RISK EVALUATION OF STRUCTURAL SURFACES CONTAMINATED WITH METALS.

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Contaminated surfaces, such as the walls and/or floors inside a former manufacturing facility, on occasion generate concern for potential human health risks. A responsible party may seek the consensus of a regulatory environmental agency on the potential human health risks and hazards from exposures to indoor structural surfaces by workers, or children residents as a conservative screening tool. The involvement of the Human and Ecological Risk Division in such cases has resulted in the development of spreadsheets to evaluate risks and hazards and calculate site-specific chemical-specific surface screening goals. The spreadsheets utilize exposure parameters based on professional judgment, since no regulatory guidance currently exists on the subject. The exposure parameters developed include skin surface area for dermal exposures as well as for inadvertent oral exposure, contact frequency, transfer efficiency from surface to skin, and fraction of contaminant transferred from skin to mouth. Analytical chemistry results from wipe samples of indoor building surfaces were the source of the exposure point concentrations and toxicity criteria recommended by the California Environmental Protection Agency were employed. The chemicals evaluated were arsenic, cadmium, chromium, copper, cyanide, nickel and zinc. The risk evaluations did not include potential exposures *via* other sources, such as soil or groundwater. Further research quantifying expo-

sure parameters from contact with structural surfaces will reduce the uncertainty in the risk estimates and likely generate less conservative structural surface screening goals.

1915 THE EFFECT OF CENSORED DATA ON THE PERFORMANCE OF TECHNIQUES FOR CALCULATING 95% UPPER CONFIDENCE LIMITS (95% UCLS) ON THE MEAN.

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The upper 95% confidence limit on the mean (95% UCL) is often used as an upper-bound estimate of average contaminant concentration for risk assessments at hazardous waste sites. Statistical techniques used to calculate 95% UCLs often rely on underlying distributional assumptions. However, sampling datasets from contaminated sites may contain a number of values below the detection limit. For the calculation of exposure point concentrations, these values are typically assigned a proxy concentration of one-half of the detection limit. This practice results in a left-censored dataset, and in some situations the extent of censoring can be substantial. Limited guidance exists for handling censored datasets. The most common approach is to calculate a 95%UCL from the full dataset, including the censored values. The effects of this approach on distributional tests and 95% UCL techniques were evaluated using simulated lognormal datasets with known distribution parameters and fixed levels of data censoring. Censoring was found to profoundly affect the ability of standard tests to correctly identify the underlying distribution. Several methods for calculating the 95% UCL also performed poorly with censored datasets. The extent of the effect of censoring on calculation of the 95% UCL depended upon the method of calculation and the sample size and skewness of the dataset. For example, the non-parametric bootstrap method performed well with sample sizes of 50 and mildly skewed data with no censoring, but underestimated the true mean 27% of the time (rather than the target 5% value for a 95% UCL) when 50% of the data were censored. Other methods performed better with censored data. The results of this analysis can be used to guide the selection of the distributional tests and UCL techniques depending on degree of censoring of site-specific datasets.

1916 COMPARISON OF TECHNIQUES FOR CALCULATING 95% UPPER CONFIDENCE LIMITS (95% UCLS) ON THE MEAN.

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Exposure point concentrations used in risk assessments should reflect the average contaminant concentrations encountered by a receptor at a site. This parameter is typically represented by the upper 95% confidence limit on the mean (95% UCL) of an often limited site dataset. Numerous statistical methods are available for the calculation of 95% UCLs, however, they often yield disparate results. There is currently little guidance related to their appropriate application. In the present analysis, we have systematically evaluated the performance of several 95% UCL techniques using simulated distributions with known population parameters. The UCL techniques evaluated were: central limit theorem (CLT), standard bootstrap, bootstrap-t, Students-t, H-statistic (Land's method), MVUE and MLE based, and Chebyshev's methods. For each method, 95% UCLs were calculated for simulated datasets of different sample sizes and skewness. By comparing the 95% UCLs with the underlying simulation expected value, the coverage of each technique (i.e., the proportion of times the true population mean lies at or below the 95% UCL) was evaluated. The extent to which the true mean was overestimated by the 95% UCL for each technique was also determined. The analysis revealed that small sample sizes and highly skewed data are particularly problematic, and depending on the technique, can result in poor coverage or significant over-estimation of true site contamination. For example, using a simulated lognormal distribution [LN (100, 200)] and a sample size of 10, the CLT method generated 95% UCLs that were, below the true mean from which the data were drawn 26% of the time. With the same simulated dataset, the H-statistic 95% UCL had better coverage (1% of means were under predicted) but some 95% UCLs calculated with this method exceed the true mean by more than 100-fold. The results of this analysis can be used to guide the selection of the best UCL technique depending on attributes of site-specific datasets.

1917 HEALTH RISKS FOR CONSTRUCTION WORKERS IN INDUSTRIAL REDEVELOPMENT: A MAJOR RISK DRIVER?

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Redevelopment of former industrial areas may result in exposure of construction workers to historical subsurface contamination. A human health risk assessment addressing such exposures was performed for a site near a former manufactured gas plant in Kansas City, MO. This site had unique construction-phase exposure scenarios because of proposed future residential and commercial uses, including potential construction of a Federal Reserve Bank building requiring excavation of contaminated soil to 40 feet below ground surface. The primary chemicals of concern (COCs) were volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), coal tar, and a petroleum hydrocarbon (diesel). Because of the spatial heterogeneity of the soil and groundwater data for these COCs, we divided the site into six exposure zones and estimated risks for the two most contaminated zones. Exposure pathways included dermal contact with soil and groundwater; incidental soil ingestion; and inhalation of air in construction pits, trenches, and buildings. We estimated cancer risks and non-cancer hazards for construction and utility workers during the construction phase and for future residents and office workers in the post-construction phase. We also estimated risks for exposure to coal tar, which was noted in the soil boring logs, but not quantified analytically. Cancer risks for the construction and utility workers exceeded Missouri's acceptable risk of 1 x 10-5 in two exposure zones and non-cancer hazards exceeded 1.0 in one exposure zone. There were no unacceptable risks for either residents or office workers in the post-construction phase. Because estimated risks to the construction and utility workers exceeded state criteria, risk reduction measures prior to construction were recommended.

1918 DERIVATION OF AIR ACTION LEVELS FOR USE IN MONITORING DURING SITE REMEDIATION.

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Air monitoring is often conducted during site remediation to ensure that the surrounding community is not adversely affected by chemicals released during remediation. For this project, our goal was to identify air action levels, expressed in terms of air concentrations protective of subchronic exposures, that could be used as the basis of air monitoring during remediation at an inactive industrial property with a predominance of coal tar constituents. We identified the chemicals that are important contributors to risk at such properties, developed toxicity criteria for each, then calculated air action levels - levels that could be used as a basis for air monitoring and risk management actions during site remediation. An important challenge to this effort was the translation of the toxicity criteria based on subchronic exposures to shorter-term air action levels for use in decision-making. We evaluated two classes of compounds: volatile organic compounds (VOCs) and particulates. For VOCs we developed toxicity criteria for subchronic exposure based on a review of the primary literature for benzene, toluene, and xylene. For particulates we evaluated lead, benzo(a)pyrene, and naphthalene. We calculated action levels based on toxicity criteria, which were derived based on primary literature with adjustments to reflect an anticipated remediation schedule of 5 days per week, 10 hours per day. The action levels were then calculated based on frequency of continuous monitoring and the site remediation schedule. A particular action level could be exceeded during the monitoring period as long as the average value was in compliance. The point of compliance was conservatively chosen as the site boundary, where few residents would be exposed for continuous periods. We developed the following action levels for 15-minute measurement periods which are protective of subchronic exposures: benzene 0.5 ppm; toluene 7 ppm; xylenes 7 ppm; lead 4 mg/m^3; naphthalene 16 mg/m^3; and benzo(a)pyrene 1 mg/m^3.

1919 RISK-BASED ACTION LEVELS FOR FENCE-LINE MONITORING PROGRAMS.

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Risk-based action levels (RBAL) for a fence-line monitoring program should adequately address both project needs and the protection of human health. Issues to be considered and addressed in the development of risk-based action levels include the following. 1) Receptors. Off-site receptors should be identified, and appropriate receptors should serve as the basis for the fenceline air standard. 2) Timeframe of remediation. RBALs for a fenceline program should be developed within the context of potential human exposure periods. In toxicology, appropriate exposure periods to consider are: Acute (less than 24 hours up to 3 months); Subchronic (3 months up to 7 years); and Chronic (greater than 7 years). RBALs should be developed to address each of these exposure durations, depending on the intended length of the remediation. Potential noncarcinogenic health effects should be considered for each exposure duration, however, chronic exposure durations are generally not anticipated for a remediation project. Potential carcinogenic effects are most appropriately considered for the chronic exposure duration, however, they can also be considered for the subchronic exposure duration. 3) Toxicity values. Appropriate chemical-specific toxicity values from the USEPA should be employed in the RBAL calculations. This includes the use of subchronic toxicity values. For benzene, the range of toxicity values provided by USEPA for addressing the potential carcinogenic effects at a given target risk level should be used in developing the RBALs; ideally a range of standards should be developed for benzene where any air monitoring data within that range would be considered to have not exceeded the RBAL.

4) Point of Exposure. Where potential receptors are not at the fenceline, air modeling should be used to calculate an RBAL to be protective of the receptors at the point of exposure.

1920 A SOIL CONCENTRATION LIMIT FOR LEAD BASED ON ACUTE EXPOSURE IN CHILDREN.

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Lead-contaminated soil is a potential public health problem in many parts of the United States. Current regulatory limits for lead in soil are based on chronic exposure. There are some situations, however, where exposure may be brief. Risk management for these situations would benefit from an assessment of lead concentrations in soil that pose a risk from acute exposure. This analysis was conducted in order to derive a concentration limit for lead in soil protective of acute exposure for a small child. Based on information in the USEPA Child-Specific Exposure Factors Handbook, a small child was assumed to be capable of ingesting, during a one-time pica episode, 10 g of soil. For this dose of soil, a concentration limit was sought that would result in a peak concentration of lead in blood no more than 60 mcg/dL, based on clinical observations of children with acute lead intoxication. The Leggett model was selected to model blood lead (PbB) concentrations resulting from acute lead ingestion. While several models predict PbB concentrations, the Leggett model has the critical features of scaling for children with the ability to model PbB concentrations after an acute dose. The dose-dependent oral bioavailability of lead in juvenile non-human primates was used to derive the estimate of gastrointestinal lead absorption. The baseline PbB concentration (before acute exposure) was defined as 2.5 mcg/dL. PbB concentrations were modeled with and without non-linear partitioning of lead into red blood cells. Assuming non-linear partitioning, the model predicted a peak PbB concentration matching the target limit of 60 mcg/dL for acute ingestion of 10 g of soil at 7, 800 mg lead/kg soil. Assuming linear partitioning, the soil lead concentration corresponding to the target peak PbB concentration was 6, 800 mg/kg. The results of this modeling exercise may be useful in evaluating risks posed by lead contaminated soils at sites where children may be present, but are not expected to have long-term contact.

1921 EVALUATING THE BIOAVAILABILITY OF METALS IN SOILS FOR USE IN HUMAN HEALTH RISK ASSESSMENT.

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Site-specific bioavailability is an important consideration in determining potential threats to human health that are posed by metals-contaminated soils at waste sites. It is important to consider bioavailability because metals may be absorbed to a lesser or greater extent following ingestion of contaminated soils as compared to the fraction absorbed in the studies used to establish toxicity values, such as a reference dose or a cancer slope factor. USEPA's Office of Solid Waste and Emergency Response led an effort to develop additional guidance on evaluating and incorporating bioavailability adjustments into human health risk assessments. A decision framework was developed for data collection and utilization of bioavailability data at waste sites. The framework consists of a tiered approach which is intended to both improve risk estimates made at specific sites where the framework is applied, as well as to encourage the expansion of a knowledge base that can be applied to future assessments of bioavailability of metals in soils at all sites. Available bioavailability data on arsenic and other metals were also evaluated to determine if alternative default values for adjusting relative bioavailability could be derived. This paper summarizes these activities and other ongoing USEPA efforts on bioavailability.

1922 DEVELOPMENT OF CHILD-SPECIFIC HEALTH CRITERIA FOR SCHOOL SITE RISK ASSESSMENT.

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The Office of Environmental Health Hazard Assessment (OEHHA) in the California Environmental Protection Agency (Cal/EPA) is responsible for evaluating health risks for environmental chemicals. Recent legislation has required development of health criteria for School Site Risk Assessment pursuant to California Health and Safety Code 901(g). The first part of the mandate is to identify chemical contaminants at school sites which may be of concern because of child-specific exposure and child-specific physiological sensitivities. OEHHA has identified a group of candidate chemicals that will likely include contaminants found at school sites (comprising approximately 200 chemicals), and another group that has the potential for causing adverse effects on school-age children (approximately 198 chemicals). This report is available on the OEHHA website at http://www.oehha.ca.gov. The second part of the mandate is to make available child-specific numerical health guidance values for five of the chemical contaminants each year. OEHHA selected the first five chemicals on the basis of: 1) those which had been identified by Department of Toxic Substance Control monitoring studies to be present at proposed school sites, 2) those for which review articles presented evidence of possible adverse effects in three or more of the systems that are undergoing critical development during childhood, 3) chemicals that other OEHHA programs had identified as a regulatory concern, and 4) carcinogens for which existing adult 1 x 10-4 cancer risk dose might not be protective of non-carcinogenic risks to children. After completing in-depth review of the scientific literature for relevant chemicals, OEHHA has proposed child-specific reference doses (RfDs) for cadmium, chlordane, heptachlor, methoxychlor, and nickel. OEHHA's process for approval of proposed child-specific RfDs is to undergo internal peer review, public comment, and external peer review with University of California faculty. The child-specific RfDs will be presented.

1923 MINERALOGICAL DILEMMAS IN EVALUATING THE HUMAN HEALTH IMPACTS OF ASBESTOS IN COMMERCIAL MINERAL DEPOSITS.

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The term asbestos, a commercial-industrial term rather than a mineralogical term, refers to well-developed, long, thin (fibrous) particles and bundles of specific minerals that fulfilled particular industrial applications. Most commonly 6 mineral species are defined as asbestos in the US, including a member of the serpentine group (chrysotile) and 5 minerals of the amphibole group: asbestiform varieties of riebeckite (commercially called crocidolite), cummingtonite-grunerite (amosite), anthophyllite, actinolite, and tremolite. Recently, debate has focused on the potential health effects from airborne exposures to mineral particles that do not completely fit some definitions of asbestos. These particles include amphiboles that: a. produce an abundance of fibrous particles when crushed or b. display asbestiform textures, but do not have chemical compositions that fit the precise mineralogical definitions of the asbestos minerals listed above. Adding to the dilemma of classifying amphibole particles and their health effects is the difficulty of distinguishing, under high magnification, the difference between long, thin cleaved fragments formed by fracturing versus fibers formed by mineral growth. Much of the difficulty in determining the presence of asbestos in talc deposits centers on transitional fibers, which are fibrous mineral particles composed of talc and amphibole in various proportions. There is disagreement regarding the potential long-term health impacts resulting from airborne exposure to the various particles described above. Collaborative research is needed between the geosciences and biomedical/public health communities to refine the classification of asbestos minerals. This collaboration must include toxicological research that can identify the roles and processes responsible for deleterious effects to humans after exposure to amphibole-bearing materials. Such research should include mineralogical study to determine if the airborne particles can be effectively defined by chemical composition, geometry, or other means.

1924 DISSOLVED ORGANIC CONSTITUENTS IN COAL-ASSOCIATED WATERS, AND IMPLICATIONS FOR HUMAN AND ECOSYSTEM HEALTH.

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Decisions concerning the disposal or use of waters produced during the production of coalbed methane (CBM) are typically made based on water quality as expressed by the salinity and/or inorganic ionic constituency. The quality of CBM water is frequently evaluated in terms of its suitability for irrigation. Water that has acceptable salinity and sodium adsorption ratio is considered safe for surface discharge and for potential injection into a drinking-water aquifer. It is important to remember, however, that water associated with coal seams, independent of its inorganic constituents, may contain dissolved organics and other constituents at levels that may adversely affect human health and the environment. It is well known that coal, lignite, or coaly materials present in aquifers used as drinking-water supplies are associated with adverse or potentially adverse human health effects. Water produced from coal-associated aquifers has been linked, or is suspected to be linked, to goiter, Balkan Endemic Nephropathy (BEN), multiple sclerosis, and increased rates of cancer morbidity and mortality. Water-soluble organic compounds found in coals include goitrogens such as the hydroxyphenols resorcinol, 2-methyl resorcinol and 5-methylresorcinol (orcinol), and hydroxypyridines. Well waters containing poly-

cyclic aromatic hydrocarbons, aniline, aminophenols, and aromatic amines leached from low-rank Pliocene coals may be the cause of, or a contributing factor to, BEN, an incurable interstitial nephropathy that is believed to have killed more than 100, 000 people in the former Yugoslavia alone. There are few, if any, systematic and comprehensive analyses of dissolved organic compounds in CBM-produced water. Prudence suggests that the dissolved organic constituents in CBM-produced water should be systematically characterized, and their potential for harm to human health and the environment should be evaluated before potentially harmful chemicals are discharged to the environment or released to drinking-water aquifers.

1925 COMMUNICATION OF WORKPLACE HAZARDS - TOPIC CENTRE GOOD PRACTICE, SYSTEMS AND PROGRAMMES.

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The Topic Centre Good Practice, Systems and Programmes has been established in 2002 to promote the aims of the European Agency for Safety and Health at Work to collect, evaluate and disseminate relevant information on good practices at workplace level and occupational safety and health (OSH) systems and programmes in Europe. The Topic Centre, consisting of 14 European OSH institutes, is a part of the Agency's network build up on Focal Points in EU Member and Candidate States, Thematic Network Groups and expert groups. As an important part of its work, the Topic Centre aims to raise awareness of chemical safety at workplaces. In 2002, the Topic Centre disseminates and maintains Internet sites for several industries and topics, and studies the information needs of Fisheries sector and success criteria of assistance schemes for the small, and medium sized enterprises and integration of OSH, including prevention of chemical hazards into Education. In addition, material is provided to support the European OSH weeks. The Agency network provides the Topic Centre with examples and other material on workplace practices and OSH systems and programmes around Europe. The Topic Centre gathers material from the Internet and literature. Each product of the Topic Centre is validated within the Agency's network for relevance and suitability to its target group. For the promotion of chemical safety, the Topic Centre has provided evaluated and maintained the website on good practices related to dangerous substances. Articles, fact sheets and a report on communication of risks of dangerous substances have been produced. Studies on integration of OSH thinking, success criteria of assistance schemes and fisheries sector information needs have detected several new methods need to be utlised in the future for chemical safety promotion. The Topic Centre work has proven to be effective and efficient network, which can tackle communication of OSH with various aspects and methods.

1926 CATEGORIZATION OF THE ASSOCIATIONS BETWEEN EXPOSURE TO THE HERBICIDES USED IN VIETNAM OR THEIR CONTAMINANTS AND HEALTH OUTCOMES.

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Between 1962 and 1971 military forces sprayed herbicides over Vietnam. In 1991, because of ongoing uncertainty about the long-term health effects on Vietnam veterans of the herbicides sprayed, Congress passed the Agent Orange Act of 1991. That legislation directed the Secretary of Veterans Affairs to request the National Academy of Sciences, a non-profit organization that provides independent, objective advice on scientific issues, to perform a comprehensive review and evaluation of scientific and medical information regarding the health effects of exposure to Agent Orange, other herbicides used in Vietnam, and the various chemical components of those herbicides, including dioxin. In assessing the evidence of an association between exposure to the herbicides and their components and a health outcome, the committees responsible for the reports review the available literature and categorize the evidence as 1) sufficient of an association; 2) limited or suggestive of an association; 3) inadequate or insufficient to determine whether an association exists; or 4) limited or suggestive of NO association. In addition, conclusions regarding the biological plausibility of the health effects and the risk to Vietnam veterans are also made. Biennial updates are produced; the latest of those reports is Veterans and Agent Orange: Update 2002, which will be released in December 2002. The assessments in the reports are used by the Department of Veterans Affairs to make policy decisions regarding compensation to veterans for service-related illnesses. The methods used by the committee as well as the most recent conclusions made by the committee will be discussed.

1927 MORE THAN 10, 000 ANIMALS ARE REQUIRED FOR THE REGISTRATION OF A SINGLE PESTICIDE - THIS PARADIGM MUST BE CHANGED.

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More than 10, 000 mice, rats, rabbits, guinea pigs and dogs are used for toxicity studies to satisfy regulatory data requirements for the registration of a single pesticide product. Although data requirements depend upon use patterns, most of the focus of the toxicity testing is driven by concern for pesticide residues in food. On the other hand, analyses from the University. S. Department of Agriculture (PDP 2000) show that dietary exposures to pesticide residues in food are not measurable or are very small. In contrast, foods have hundreds of times more intrinsic self-defense chemicals (natural pesticides) than synthetic pesticide residues and, when tested, some of the natural pesticides are genotoxic, carcinogenic, developmental toxicants or cause other effects. Still, with few exceptions, there is a long history of food consumption without apparent harm, providing a practical demonstration of efficient detoxification mechanisms in real life. Obviously, our bodies do not distinguish between natural and synthetic chemicals, but only among chemical structures. These considerations have led to at least one approach that resulted in the reduction in the number of animals used in hazard evaluation. The University. S. Food and Drug Administration (FDA) evaluates the safety of food additives by assigning a concern level based on the additive's structural configuration and an estimation of exposure. Toxicology testing then is either expanded or reduced depending upon the concern level. Similarly, the low genotoxic potential and typically very low levels of pesticides in the diet would result in a low level of concern for many pesticides and could result in a significant reduction in the numbers of animals necessary for safety studies. A myriad of other strategies are possible and new thinking is required to address overall food safety and to "Reduce, Refine, or Replace" laboratory animals in the current regulatory requirements for registration of pesticides.

1928 EVALUATION OF HUMAN PESTICIDE NOAEL STUDIES FOR CONSISTENCY WITH US FEDERAL POLICY FOR THE PROTECTION OF HUMAN SUBJECTS (THE COMMON RULE).

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The US Environmental Protection Agency (EPA) recently suspended the use of toxicity data obtained from human studies for chemical assessments over concerns regarding ethics. One argument against the use of human pesticide toxicity data asserts that such data are obtained from studies that do not comply with the "Federal Policy for the Protection of Human Subjects, " (known as the "Common Rule"). The Common Rule provides ethical guidance for the conduct of studies using human subjects that are funded by federal agencies, including EPA. To determine whether contemporary pesticide studies funded/sponsored by registrants meet the criteria for ethical treatment of human subjects as specified by the Common Rule, we analyzed over a dozen pesticide studies that sought to identify oral no-observed-adverse-effect-levels (NOAELs). The studies chosen were identified primarily from a list of studies that were submitted to EPA's Office of Pesticides Programs since passage of the Food Quality Protection Act. Fundamental and detailed requirements of the Common Rule regarding informed consent and ethics committee review were identified, and copies of study reports, protocols, and ethics committee documentation were evaluated to determine whether the Common Rule requirements were met. We found all the studies analyzed complied with the fundamental requirements of the Common Rule, including an ethics committee review and approval of the study protocol, use of volunteer subjects for the study, and acquisition of written informed consent from the volunteers. We found a few deviations in some studies from specific detailed requirements of the Common Rule. Some of these deviations may have been due to absence of documentation available for our evaluation, and not necessarily to failure of the laboratory to fulfill the requirements. In the sub-set of studies we evaluated our results demonstrate that the fundamental ethical requirements of the Common Rule were followed.

1929 A COMPARISON OF THE NUMBER OF RISK VALUES DERIVED BY DIFFERENT ORGANIZATIONS FOR 20 PRIORITY HAZARDOUS SUBSTANCES AND FOR CHEMICALS IN THE INTERNATIONAL TOXICITY ESTIMATES FOR RISK *(ITER)* DATABASE.

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Human health risk values are used by many organizations to make important public health decisions, and are developed by various organizations from around the world. Not all organizations derive risk values for the same chemicals, and even if

they do, the values may differ due to scientific judgments, the mission of the organization, or the use of more recently published data. This analysis used risk values derived by the Agency for Toxic Substances and Disease Registry, Health Canada, the National Institute of Public Health and the Environment (The Netherlands), the US Environmental Protection Agency, and independent parties to compare trends in the type and quantity of currently published risk values among organizations. For example, we find that there are risk values available from 3 or more organizations for 10 of the 20 Priority Hazardous Substances, often with different guidance values provided. We also find that for the majority of the roughly 600 chemicals in the International Toxicity Estimates for Risk *(ITER)* database, only a single organization has a risk value available. This analysis suggests the importance of being able to conduct side-by-side comparisons to both identify the availability of and to select risk values for application in public health decision-making. This analysis provides a significant update to previously reported results, as all of the ATSDR risk values have been added to *ITER* and included in the analysis.

1930 IDLH DOCUMENTATION REVIEW.

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Immediately Dangerous to Life and Health (IDLH) values have been established by the National Institute for Occupational Safety and Health (NIOSH) to protect workers against exposure conditions that may cause severe irreversible health effects or impair escape from exposure environments. Original assessments were used primarily as a tool to recommend respiratory protection and IDLH values were often based on limited scientific data, secondary source reported data, or safety considerations. In 1994, NIOSH reevaluated the IDLH values and developed more formal criteria for determining IDLH values. A review of the toxicological basis for 35 of a total 398 of the current IDLH values was conducted to determine if the existing IDLH recommendations were consistent with current toxicological data. For this critical analysis, a qualitative rating method was developed as a tool for conducting the evaluation. Technical guidelines were established for selection of experimental studies, evaluation of each study, and evaluation of the database for each substance. Experimental study protocol and methods were recorded into a customized database designed for tracking and sorting scientific data specific to acute exposures to hazardous substances. Two overall evaluations were made for each substance. The first evaluation rated the adequacy of the database to support the development of an IDLH value and classified each as adequate, marginally adequate, or inadequate. Out of a total 35 substance databases, approximately 50% were considered marginally adequate and 50% adequate. The second evaluation rated the degree to which the current IDLH value was likely to be protective against dangerous levels of exposure. Of the current 35 IDLH values reviewed, approximately 20% were considered more than adequately protective, 63% were considered protective, and 17% were considered inadequately protective.

1931 DATABASES YIELDING RISK ASSESSMENTS AS ONGOING PROCESSES.

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Regulatory agencies currently produce risk assessment (RA) documents as text files. Much of the input data in an RA comes from previously completed agency reviews of toxicity and exposure data, which must be incorporated into each RA version. Often RAs are initiated out of newly-recognized concerns, but substantial time is often required before an RA is drafted and peer reviewed for dissemination. Integration of endpoints from reviewed studies into RAs could be an ongoing process rather than an occasional event, if reviews and associated RAs are organized into an appropriate database (DB) format. Advantages of using DB approach include transparency, flexibility, utility to data providers and users, and real-time updates of critical derived values. Transparency suggests that a minimally-filtered public version of the DB should be accessible to all stakeholders. Agencies should define default assumptions in the DB design, and what data would displace default values. Key endpoints should be linked to associated data tables. Reviews should tabulate all plausibly treatment-related findings, then justify selection of relevant effects, as is currently done. When a review is finalized and incorporated into the DB, new endpoints of regulatory interest are considered in the re-calculation of reference doses for the associated duration of exposure. Any report bearing titles or keywords indicating plausible relevance to the subject chemical should be identified in the DB, indicating whether it was reviewed by agency. Data providers would benefit from real-time updates of critical derived values and the associated endpoints and uncertainty factors. Companies could then make economic decisions regarding future testing to reduce uncertainties which apply to regulatory reference doses. Challenges in moving to a DB system include cost factors, protecting data integrity, staff acceptance, transition time, coordination with other regulatory agencies, and decisions of whether and how extensively to retrofit existing data.

1932 THE ACTIVITIES AND PERSPECTIVES OF THE KOREAN NATIONAL TOXICOLOGY PROGRAM.

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The Korean National Toxicology Program (KNTP) was established on 2001 as a multi-disciplinary program supported by the Korean Food and Drug Administration (KFDA). The objectives of KNTP are to prevent human health from toxic chemical exposure, and to provide science based toxicity information to citizens to keep any social disturbance from the invalid toxic information. The activities of the KNTP include: sponsoring chemical toxicity testing, establishing toxicity evaluation infra-structure, developing alternative testing methods, building toxicity database with integrated toxic chemical management program, organizing inter-ministrial cooperative program, and initiating toxicology research centers to evaluate toxic chemicals in depth and systemic. The toxic chemicals will be selected, tested, and monitored through the KNTP and finally utilized for assessing the risk of the chemicals. These activities to ensure valid toxic information coordinated by specialized government organization eventually will be helpful in improving public health and regulating policy cooperating with related government organization including Ministry of Labor, Ministry of Agriculture & Forestry, Ministry of Maritime Affairs & Fisheries and etc., sharing common toxic information for same chemicals. The KNTP is also interesting in establishing international cooperation program to share the toxicology program experiences with other countries and to collaborate toxicity assessment activities.

1933 PROMOTION OF CHEMICAL SAFETY AWARENESS: INTERNET DATABANK ON RISK MANAGEMENT TOOLS OF DANGEROUS SUBSTANCES.

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Databank on hazardous substances has been established on the website of the European Agency for Safety and Health at Work in Bilbao, Spain. The goal of this project is to create a user-friendly information source on substitution, practical risk management of dangerous chemicals, and Occupational Exposure Limit (OEL) systems. The project is conducted by the Topic Centre Good Practice, Systems and Programmes. The target group of the databank includes health and safety professionals and other competent persons within small and medium sized enterprises. The core of the databank is substitution and risk management data on asbestos and organic solvents. This material is collected from the Internet outside the EU Member States, and evaluated for credibility, currency, relevance, usefulness, accessibility, and ethical appropriateness. The hyperlinks are written abstracts and defined keywords to facilitate Internet search. OEL systems are also included and written descriptions in co-operation with the authorities in these countries. The databank is maintained by continuos monitoring and maintenance of the descriptions and Internet references of the entered links. The search for information on risk management of asbestos and organic solvent has provided over 200 links covering various industrial sectors. International organisations and national institutes outside the EU provide most of the material. Additionally, the databank displays descriptions and reference for OEL systems in 27 countries, and the work of the EU Scientific Committee on Occupational Exposure Limits (SCOEL). Usability of the Good Practice website has been improved by a glossary of the applied terms, and two sets of Frequently Asked Questions on the definitions and use of the website, and risk management and substances. The goal is still to further develop and maintain the databank. The user-friendliness will be developed further according to feedback from user and focus group surveys.

1934 THE RELATIVE BIOAVAILABILITY OF METALS FROM SOIL TO ECOLOGICAL RECEPTORS.

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Small mammals (e.g., short-tailed shrew and cottontail rabbit) are among the wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential exposure to metals in soil. These small mammals receive much of their metals exposure either from direct soil ingestion during foraging or preening activities, or from consumption of soil-laden earthworm. In assessing risks to these receptors from soil contamination, the standard method is to assume that contaminants have a relative bioavailability of 100% (i.e., the efficiency of metals absorption from ingested soil is equal to that which occurred in the laboratory tests conducted to determine toxicity thresholds). However, a growing body of research indicates that many chemicals - including metals - are less bioavailable from ingested soil than from soluble forms (i.e., the forms typically used in labora-

tory toxicity tests), when dosed in a similar manner. Our research is designed to determine the relative oral bioavailability in shrew for arsenic, cadmium, chromium, and lead in several soils. To date, the research has involved determining the appropriate target metals for evaluation, the appropriate target species, and conducting feasibility studies for this novel small-mammal model. Results of soil dosing experiments will be presented, along with a preliminary interpretation of the implications for use in ecological risk assessment. A brief summary of other aspects of our research (including relative oral bioavailability of metals in soil to avian receptors and human receptors, as well as dermal absorption for humans) will also be provided, with an update on ongoing and planned research.

1935 THE TOXICITY OF LEACHATES FROM A MUNICIPAL SOLID WASTE LANDFILL IS DEPENDENT ON CADMIUM AND MODULATED BY NICKEL.

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Landfills are used to dispose municipal solid wastes including food residues, used paper and both scrap metal and plastic. Although recycling in landfills is extensive, diverse materials release toxic metals and other contaminants that are incorporated into the leachate. The objective of this work was to determine the chemical source of the toxicity of leachates from the landfill of the city of Cartagena (Colombia), by using bioassays with the crustacean Artemia saline. Leachates were initially pre-filtrated and then passed through 0.45 micrometer membranes to remove most of the bacteria. This liquid was characterized physicochemically, and the concentration of several metals such as Cd, Ni, Hg, Mn, Cu and Pb were measured by atomic absorption spectroscopy. Bioassays were conducted using leachate dilutions with synthetic seawater, and the toxicity recorded as LC50 after 24 and 48 hours exposure. The LC50 values oscillated between 3.20% and 39.33% v/v. Multivariate analysis showed that toxicity was a function of both Cd concentration and chemical oxygen demand. Interestingly, the slope of the dose-response curve correlated with Ni concentration and this relationship was independent from toxicity. The results suggest that the toxicity of leachates from the landfill of Cartagena depends on the levels of Cd associated with organic matter, and also that this effect is modulated by Ni.

1936 RENAL TOXICITY IN RATS AFTER ORAL ADMINISTRATION OF MERCURY-CONTAMINATED BOILED WHALE LIVERS MARKETED FOR HUMAN CONSUMPTION.

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Some people living in Japan have traditionally eaten the internal organs of small cetaceans (toothed whales and dolphins), and a mixed package of boiled liver, kidney, lung and so on is still marketed for human consumption. Recently, we reported that these organs are highly contaminated with mercury. In this study, we investigated the absorption, distribution, excretion and biochemical parameters in serum and urine after a single oral administration of boiled whale liver containing 1980 $_{\mu}$g/g of total mercury and 23.5 $_{\mu}$g/g of methyl mercury to rats (0.2g boiled liver/100 g rat). The single administration of the boiled liver resulted in marked increases of the mercury concentration in the rat kidney, urinary volume, and the urinary excretion of N-acetyl-$_{\beta}$-D-glucosanidase, albumin and sodium, suggesting the renal toxicity caused by inorganic mercury. Furthermore, the administration increased lactate dehydrogenase activity and creatinine, potassium and phosphorus concentrations in serum. About 95% of total mercury dose was excreted in feces and less than 0.05% was excreted in the urine. Thus, the absorption rate of mercury from the boiled whale liver appears to be about 5 % or slightly higher. These data indicate that the human consumption of boiled whale liver may cause acute intoxication by the contaminated inorganic mercury.

1937 SKELETAL LEAD CAUSES AN ARTIFACT IN BONE MINERAL DENSITY MEASUREMENTS BY DEXA.

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No reports have documented the presence of low bone mineral density in patients exposed to significant sources of lead. This is surprising given the known adverse effects of lead on both bone formation and bone resorption. We believe we have discovered the reason for this discrepancy. Our findings indicate that even trace amounts of skeletal lead artifactually increase DEXA (dual energy x-ray absorptiometry) values. This finding suggests that the diagnosis of osteoporosis is under reported in patients with a history of lead exposure. Bone mineral density of bovine bone containing added amounts of lead that mimic human levels was measured with DEXA and ultrasound (US). Also, the calcaneus of 18 human subjects with differing lead-exposure histories were measured with both DEXA and US. The DEXA measurements were performed on both GE/Lunar and Hologic instruments. The range of lead levels found in the bone of adult humans causes an artifactual increase in bone mineral density. The artifact ranges from a 4% (10 ug Pb/g bone) to 11% (100 ug Pb/g bone) overestimate of bone density. The overestimate occurred with instruments from both GE/Lunar and Hologic. Ultrasound measurements of the same samples showed no such increase. A ratio of an US measurement to a DEXA measurement at the calcaneus in patients (N=18) showed a statistically significant inverse relationship with blood lead levels. We have used this relationship to calculate a correction factor for bone mineral density based on historic lead-exposure. A 4 - 11% overestimate of bone density can alter a patients t-score by as much as 1.0. and fracture risk by 150%. Thus, unless one knows the skeletal lead content of their patient, an accurate bone density cannot be determined. If this finding proves to be consistent throughout the population, then a large number of patients are being under-treated for their low bone density. This would contribute to a substantial morbidity in the post-menopausal population.

1938 PERINATAL LEAD EXPOSURE IN THE ROMAN EMPIRE: ARCHEOMETRIC EVIDENCE FROM ISOTOPE ANALYSIS.

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Lead (Pb) poisoning or 'plumbism' was a major public health issue in the Roman empire. Pb exposure in Rome has been attributed to Pb-contaminated drinking water, and Pb salts added as preservative to wine and stewed food. As excessive wining and dining was a privilege of the Roman upper class, Pb poisoning is an example of class-related disease at that time. Plumbism may well have lead to the rapid decline of the Roman empire in the first centuries. Earlier studies with Romano-British bones indicated that Pb-poisoning was not limited to Rome proper but also occurred in Romanized settlements throughout the empire. We studied Pb exposure in infants exhumated in the cemetery of the Roman settlement Praetorii Agrippinae (near Leiden, The Netherlands). Previously, we demonstrated with EAAS a high Pb concentration in femora of neonates. Although these results indicated a high perinatal exposure to Pb, uptake of Pb in bone from the surrounding soil could not totally be excluded. To discern between the origins of Pb in bone and soil, stable isotope analysis of Pb204, Pb206, Pb207 and Pb208 was performed with a single-collector sector field ICP-MS (Finnigan MAT Element). Total Pb in bone measured with ICP-MS correlated satisfactorily with EAAS-results. For 13 bone and 11 soil samples, the Pb207/Pb206 ratios were plotted against the Pb208/Pb206 ratios. Bone and soil ratios clearly form two different populations. The mean Pb207/Pb208 ratio for bone is 0.4054 ± 0.0007, for soil 0.4023 ± 0.0008 ($p < 0.01$). This strongly suggests that Pb in infant bone is from a different source than Pb in soil. This is the first study that convincingly shows that Pb in bone from a Roman cemetery is not concentrated from Pb in the surrounding soil, but must have been transmitted from the mother to the fetus. Future studies are aimed at defining the actual geographic and nutritional source of Pb in bone.

1939 A NOVEL ISOTOPIC APPROACH FOR DETERMINING THE CONTRIBUTION OF LEAD FROM BONE TO BLOOD IN CHILDREN.

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Epidemiological studies have shown that household lead (Pb) abatement yields at most a 32% decrease in blood Pb (PbB) levels of children 3 to 6 months post-intervention. To evaluate the role of bone Pb release in supporting elevated PbB levels post-intervention we implemented a novel isotopic approach. Lead concentrations and isotopic compositions of blood and feces (4 consecutive daily collections) were determined for three children with PbB levels between 15 and 30 µg/dL. In addition, Pb levels and isotopic compositions of different environmental media in the children's homes were measured before and after a cleaning intervention. Through mass balance equations of Pb and Pb isotopes in blood we derived the endogenous Pb contribution to blood. The isotopic composition of most feces, a surrogate of the sources and magnitude of Pb ingested, agreed with the isotopic composition of

the household dusts exceeding EPA clearance levels. This underscores the importance of household dust as the direct pathway of Pb exposure. In case #1, intervention produced a small decrease in the child's PbB level (from 29 to 25μg/dL), even though fecal Pb levels suggested relatively low Pb exposures post-intervention (fecal Pb ~5μg Pb/day). Lead isotope data substantiated this, showing that the bone Pb contribution accounted for more than 95% of the Pb in blood. The other two children had slightly larger relative drops in PbB levels with intervention (20 to 15μg/dL, and 18 to 13μg/dL, respectively), and had higher fecal Pb levels suggesting higher on-going exposures (fecal Pb = 20 – 70μg Pb/day) post-intervention. The isotope results for these latter cases suggest that initially (pre-intervention) bone was accumulating (i.e., scavenging) Pb from blood equivalent to 3μg/dL, and releasing Pb to blood post-intervention equivalent to 4 μg/dL. These results highlight the importance of endogenous Pb sources and provide a blueprint for the utilization of Pb isotopes to quantify the toxicokinetics of this important source of Pb to Pb poisoned children.

1940 EFFECT OF OCCUPATIONAL EXPOSURE TO MANGANESE ON STEADY-STATE SERUM CONCENTRATIONS OF IRON, ZINC, COPPER, SOD, AND MDA AMONG WELDERS.

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Recent studies suggest that exposure to Mn alters homeostasis of trace elements such as iron (Fe) and copper (Cu). This study was performed to investigate if occupational exposure to Mn in welders was associated with distorted serum levels of Fe, Cu and zinc (Zn), all of which are important to CNS function. Thirty-seven welders (ave. 38.1 years old) who have been engaged in electrical arc welding with potentially high level of exposure were selected from about 500 welders in one factory as the exposed group. Welders worked 7-8 hours per day with the employment of 2-36 years. Control subjects consisted of 50 workers (ave. 34.2 years old), who were recruited from a nearby food factory with no history of Mn exposure. Ambient Mn levels in welders breathing zone had a geometric mean of 1.45 ug/m^3, while it was 0.11 ug/m^3 in the control breathing zone. Results showed that serum levels of Mn and Fe in welders were about 4 fold ($p<0.01$) and 1.9 fold ($p<0.05$), respectively, higher than those of controls. In contrast, serum Zn levels were significantly lower in welders than in controls, while serum Cu did not differ significantly between two groups. ANOVA analyses revealed the lack of association between serum levels of all four metals and the duration of employment. However, the latter was positively associated with urinary Mn among welders ($p<0.05$). In addition, biochemical assays showed that the activity of erythrocytic superoxide dismutase (SOD) in welders was reduced by 24% compared to controls ($p<0.05$), whereas the levels of serum malondialdehyde (MDA) was increased by 44% ($p<0.05$). These findings suggest that occupational exposure to Mn among welders disturbs the homeostasis of trace elements and induces oxidative stress. These factors may contribute to the etiology of Mn-induced Parkinsonism (Support in part by P30 ES-09089).

1941 DIETARY CADMIUM ABSORPTION IS ACCELERATED IN YOUNG WOMEN WITH LOW SERUM FERRITIN LEVELS AMONG FEMALE JAPANESE FARMERS.

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Dietary absorption rate of cadmium (Cd) was investigated among 37 female farmers selected from the previously studied 596 participants, who have been exposed to Cd close to the current provisional tolerable weekly intake (PTWI). This study group consists of 7 diabetics and their 13 age-matched controls, and 5 anemic subjects and their 12 controls. With their informed consent, they were confined in an inn for 7 days in order to collect all the food duplicates consumed and their feces and urine. The absorption rate *via* gastrointestinal tract was calculated for each subject. The means and 95% confidence intervals of diabetic group and anemic group showed no significant differences from their respective controls. The anemic group and its control revealed higher absorption rate than diabetic group and its control group. Among all subjects, significantly higher Pearson's correlation coefficients were observed between Cd absorption rate and age, serum ferritin and iron levels, and both blood and urine Cd levels. However, multiple regression analysis revealed that only age and serum ferritin level were significant factors for Cd absorption rate. The mean value of Cd absorption in younger female (20s-30s) with iron deficiency was 30-40%, while elder people (60s-70s) without iron deficiency showed zero to negative value. These results demonstrate that the absorption rate of Cd is independent of the diagnostic criteria of anemia or diabetes, but decreases by aging, and increases by body iron deficiency. This research protocol was approved by the Committee on Medical Ethics in Jichi Medical School.

1942 AMINOLEVULINIC ACID DEHYDRATASE GENOTYPE DISTRIBUTION IN LEAD EXPOSED CHILDREN IN TORREON COAHUILA, NORTHERN MEXICO.

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Aminolevulinic acid dehydratase (ALAD) is a zinc containing enzyme which is part of the heme synthesis pathway. This enzyme has two alleles: ALAD-1 and ALAD-2, and has different distribution according with ethnic characteristics of populations. ALAD-2 allele has been associated, in epidemiological studies with people having higher lead concentrations in blood (PbB). The aim of this work is to assess the relationship between PbB and ALAD genotyope distribution in a children with chronic lead exposure, as well as, with an effect biomarker as is zinc protorporhyrin in blood (ZPP). Methods: 569 children were recruited from 9 primary schools around a smelter site. Selection was made taken all children in first degree in these schools. All parents children studied were informed and signed a consent form letter. PbB was made by atomic absorption spectrometry with Zeeman background correction. ALAD genotype was made according to Wetmur et al (1991). ZPP was made by direct fluorimetry in blood. Results: Geometric mean (range) of PbB in children was 10.25 μg/dL (2.0 to 47.9 μg/dL). The individuals (number; %) having different allele combination were: ALAD-1-1 (530; 93.15 %), ALAD1-2 (38; 6.67 %), and ALAD2-2 (1; 0.18 %). There were a significant relationship between PbB and ZPP. There were a significant direct relationship between the proportion of ALAD-2 and PbB. Individuals with ALA-2 allele had lower levels of ZPP ($p= 0.0512$). These results support the view that determination of ALAD genotype can be useful as susceptibility biomarker. Wetmur JG, et al (1991) Am. J. Human Genet. 49:757-763.

1943 TRANSPORT OF MERCURIC-THIOL CONJUGATES IN BASOLATERAL MEMBRANE VESICLES FROM RAT KIDNEY: EFFECT OF COMPENSATORY RENAL CELLULAR HYPERTROPHY.

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The susceptibility of developing renal injury induced by inorganic mercury (Hg) increases subsequent to the induction of compensatory renal growth. We hypothesize that this is related to increased rates of uptake of Hg into target proximal tubular epithelial cells. The goals of this study were 1) to determine the roles of specific transporters in the uptake of Hg, in the form of Hg-thiol conjugates, across the renal basolateral membrane (BLM) and 2) to assess the effects of compensatory renal cellular hypertrophy on each transporter involved in the uptake of Hg-thiol conjugates. BLM vesicles were isolated from kidney(s) of both control and uninephrectomized (NPX) rats. Non-specific binding of Hg accounted for 75 to 90% of total amount of Hg associated with membranes exposed to $HgCl_2$, which decreased with increasing Hg concentrations. The presence of sodium ions had little effect on the fraction of binding, but the presence of thiols (glutathione [GSH], L-cysteine [Cys], or *N*-acetyl-L-cysteine [NAC]) reduced binding by more than 70%. BLM vesicles from kidneys of NPX rats exhibited a much lower proportion of binding (only 20 to 45% of total). Of the Hg-thiol conjugates studied, transport of Hg-cysteine was fastest. Use of selective inhibitors of BLM carriers indicated involvement of system ASC and the organic anion transporters in the transport of Hg-$(Cys)_2$, the organic anion transporters and the sodium-dicarboxylate exchanger in the transport of Hg-$(GSH)_2$, and system ASC and the organic anion transporters in the transport of Hg-$(NAC)_2$. Transport of each Hg-thiol conjugate was higher in the BLM vesicles from the NPX rats, with specific increases noted in transport by the organic anion transporter, system ASC, and the sodium-dicarboxylate carrier. These results support the hypothesis associating increased cellular injury with increased Hg transport across the BLM. (Supported by NIH Grants ES05157, ES05980 and DK40725.)

1944 TRANSPORT OF DICYSTEINYLMERCURY IN MADIN-DARBY CANINE KIDNEY (MDCK) CELLS OVEREXPRESSING SYSTEM $b^{0,+}$.

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It has been suggested that dicysteinylmercury (Cys-Hg-Cys) can serve as a molecular homolog or "mimic" of the amino acid cystine at the site of one or more of amino acid transporters involved in the uptake of cystine. System $b^{0,+}$, which is a heterodimeric transport system comprised of $b^{0,+}$AT and rBAT, has been postulated to be one of these transport systems. The aim of the present study was to determine whether a line of renal epithelial cells (MDCK cells), which do not express system $b^{0,+}$, gain the ability to transport Cys-Hg-Cys after being stably transfected with the genes for this transport system. Kinetic analysis was performed by measuring the uptake of Cys-^{203}Hg-Cys or ^{35}S-cystine in the presence of unlabeled Cys-Hg-Cys (1, 10, 50, 100, 250, 500 nM, 1, 10 μM) or cystine (0.1, 1, 10, 100, 250, 500, 1000 μM), respectively. Substrate-specificity experiments were performed by adding 3 mM unlabeled amino acids. The uptake of cystine and Cys-Hg-Cys in the transfected cells was approximately 3-fold and 2-fold greater than that in wild-type cells, respectively. Kinetic analysis of the transport process demonstrated a 2-fold increase in Cys-Hg-Cys transport (Wild type, Vmax = 1.4 μM, Km = 0.8 μM; $b^{0,+}$, Vmax = 3.0 μM, Km = 0.5 μM). Transport of Cys-Hg-Cys was greatest during the initial hour of incubation. Uptake of Cys-Hg-Cys was inhibited by known substrates of system $b^{0,+}$ (cystine, Leu, Arg, Tyr, CycloLeu, His, Phe) as well as unlabeled Cys-Hg-Cys. Val, Ala, and Gly did not inhibit uptake. These studies demonstrated that system $b^{0,+}$ is capable of transporting Cys-Hg-Cys, and that this transport likely involves Cys-Hg-Cys acting as a molecular homolog or mimic of the amino acid cystine. This study is the first to implicate system $b^{0,+}$ in the transport of the biologically relevant thiol-conjugate of inorganic mercury, Cys-Hg-Cys, in renal epithelial cells.

1945 BASOLATERAL TRANSPORT OF THE MERCURIC CONJUGATE, CYS-HG-CYS, IN NON-PERFUSED S1, S2, AND S3 SEGMENTS OF THE RABBIT RENAL PROXIMAL TUBULE.

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Cellular uptake (bath-to-cell) of dicysteinylmercury (Cys-Hg-Cys) across the basolateral membrane was studied in non-perfused segments of the rabbit proximal tubule. Proximal tubular segments were selected and separated according to their morphological features and location in coronal sections of the kidney. Each isolated tubular segment was incubated for 20 minutes in a saline-based bathing solution containing 20μM 203Hg^{++} and 60μM L-cysteine. All segments took up Cys-Hg-Cys avidly at 38°C. Uptake was 2473 ± 31, 3340 ± 36 and 2154 ± 15 fmol (mm tubular length)$^{-1}$ (20 min)$^{-1}$ in the S1, S2 and S3 segments, respectively. In S2 segments, the only segment studied in the following experiments, the rates of uptake were reduced by about 80% when incubated at 12°C. When S2 segments were co-incubated with 200 μM PAH (para-aminohippurate) or 200 μM L-cystine, uptake of Cys-Hg-Cys was reduced to about the same level as incubating the tubular segments at 12°C. Co-incubation with PAH caused the rate of uptake to decrease to 710 ± 8 fmol (mm tubular length)$^{-1}$ (20 min)$^{-1}$ while co-incubation with L-cystine reduced uptake to 610 ± 12 fmol (mm tubular length)$^{-1}$(20 min)$^{-1}$. Since it has been observed that Cys-Hg-Cys is taken up most avidly at the basolateral membrane of the S2 segment, the segment that is known to contain the greatest activity of organic anion transport, and since PAH greatly inhibited the uptake of Cys-Hg-Cys, we conclude that Cys-Hg-Cys is transported by the organic anion transport systems. Also, since L-cystine effectively inhibited Cys-Hg-Cys uptake, we conclude that the Cys-Hg-Cys mercury conjugate can be transported by a mechanism that transports the amino acid cystine.

1946 MADIN-DARBY CANINE KIDNEY (MDCK) CELLS GAIN THE ABILITY TO TRANSPORT MERCURIC CONJUGATES OF CYSTEINE (CYS) OR N-ACTEYLCYSTEINE (NAC) AFTER BEING STABLY TRANSFECTED WITH OAT1.

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One of the main transporters implicated in the basolateral uptake of inorganic mercury (Hg^{++}) along the renal proximal tubule is the *p*-aminohippurate (PAH)- and probenecid-sensitive organic anion transport system. The primary aim of the present study was to provide direct molecular evidence that the organic anion/dicarboxylate exchanger, OAT1, is capable of transporting biologically relevant dithiol-conjugates of Hg^{++}. To that end, transport of radiolabelled PAH and the mercuric conjugates of CYS (CYS-Hg-CYS) or NAC (NAC-Hg-NAC) was studied and compared in two populations of MDCK cells; one which had been stably transfected with *hoat1*, and the other, which had been transfected with a control vector. Uptake of Hg^{++} during exposure to 5 uM CYS-Hg-CYS or NAC-Hg-NAC, or uptake of PAH alone, was significantly greater in hOAT1-expressing MDCK cells than in the corresponding control cells. The Km for the uptake of NAC-Hg-NAC in the hOAT1-expressing cells was determined to be 73.3 ± 2.9 uM. Transport of the mercuric conjugates or PAH was both time-dependent and temperature-sensitive. For example, uptake of NAC-Hg-NAC in the hOAT1-transfected cells decreased by 82% when temperature was decreased from 37°C to 25°C. Uptake of each mercuric conjugate or PAH was decreased significantly in the presence of probenecid (200 uM) or PAH (1 mM) only in the hOAT1-transfected cells. PAH also inhibited the uptake of NAC-Hg-NAC by the hOAT1-expressing cells in a concentration-dependent manner. Similarly, a decreased pattern of uptake of PAH was noted in the presence of increasing concentrations of NAC-Hg-NAC. These findings indicate clearly that OAT1 can efficiently transport certain dithiol-conjugates of Hg^{++}. Moreover, the activity of OAT1 is likely responsible for a significant component of the basolateral uptake of, and nephropathy induced by, Hg^{++} along the proximal tubule *in vivo*.

1947 ROLE OF ORGANIC ANION TRANSPORTER 1 (OAT1) AND AMINO ACID TRANSPORTERS IN THE UPTAKE OF THIOL-CONJUGATES OF METHYLMERCURY IN MADIN-DARBY CANINE KIDNEY (MDCK) CELLS.

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Several sets of recent *in vivo* and *in vitro* findings have implicated OAT1 and certain amino acid transport systems in the basolateral and luminal uptake of inorganic forms of mercury by proximal tubular cells. By contrast, very little is known about the transporters involved in the uptake of methylmercury (CH_3Hg^+) by proximal tubular epithelial cells. Therefore, the principal aim of the present study was to investigate the potential role of OAT1 and amino acid transporters in the uptake of biologically relevant thiol-conjugates of CH_3Hg^+. Transport of the CH_3Hg^+ conjugates of cysteine (CYS) or N-acetylcysteine (NAC) was studied and compared in two populations of MDCK cells; one which had been stably transfected with *hoat1*, and the other, which had been transfected with a control vector. The uptake of CH_3Hg^+ during exposure to 5 uM CH_3Hg-CYS or CH_3Hg-NAC was significantly greater in the hOAT1-expressing cells than in the corresponding control cells. The Km values for transport of CH_3Hg-NAC in hOAT1-expressing and control MDCK cells were 42.8 ±+ 2.5 uM and 118 ±+ 17.8 uM, respectively. Transport of these conjugates was also time-dependent and temperature-sensitive. In both the hOAT1-expressing and control cells, the uptake of CH_3Hg-NAC was decreased by approximately 80% at 21°C and by 95% at 4°C. Similarly, the uptake of CH_3Hg-Cys was decreased by 60% at 21°C and 88% at 4°C. In the presence of L-type amino acids (such as Leucine, Isoleucine, Cystine, Histidine or phenylalanine), the uptake of CH_3Hg-Cys was significantly inhibited in both hOAT1-expressing and control MDCK cells. However, in the presence of well-established inhibitors of OAT1 (probenecid, *p*-aminohippurate, glutarate or adipate), the uptake of these complexes was significantly inhibited only in the hOAT1-transfected cells. These findings indicate that both OAT1 and amino acid transporters are capable of transporting thiol-conjugates of CH_3Hg^+ into cells containing these transporters.

1948 P-GLYCOPROTEIN MEDIATED TRANSPORT OF CADMIUM IN CULTURE RENAL EPITHELIAL CELL LINE, LLC-PK_1

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The purpose of this study using the renal cultured epithelial cell lines, LLC-PK_1, and P-gp overexpressed cell lines, LLC-GA5-COL 150 cells was to investigate whether transepithelial transport of cadmium (Cd) is mediated by P-glycoprotein (P-gp). These cell monolayers cultured on permeable membranes were incubated at 37C for 60 min with 1_{μ}M $CdCl_2$ from either the apical or basolateral side. The transepithelial transport of Cd from the basolateral side was 1.7 times higher than that from the apical side in LLC-GA5-COL 150 cells, while that of Cd from the basolateral side was almost similar to that from the apical side in LLC-PK_1 cells at 60 min. Pretreatment with the P-gp antibody, UIC2, increased the apical-to-basolateral transport of Cd in LLC-GA5-COL 150 cells by 20%, while it decreased the basolateral-to-apical transport of Cd by 20%. On the other hand, the pretreatment increased the apical-to-basolateral transport of Cd in LLC-PK_1 cells by 20%, but it

did not decrease the basolateral-to-apical transport of Cd. The typical P-gp inhibitors, such as cyclosporin A, verapamil, nifedipine and quinidine, markedly decreased basolateral-to-apical transport of Cd in LLC-PK_1 and LLC-GA5-COL 150 cells. These results suggest that Cd is extruded from apical membrane of renal cultured epithelial cell *via* P-gp.

1949 EFFECT OF DMT1 KNOCKDOWN ON IRON, CADMIUM, AND LEAD UPTAKE IN CACO-2 CELLS.

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DMT1 (divalent metal transporter) is a hydrogen-coupled divalent-metal transporter with a substrate preference for iron, which also transports a broad range of metals, including the toxic metals cadmium and lead. The intestinal cell line, Caco-2, which has been shown to express DMT1 at its apical membrane, was used to study the transport of iron, cadmium and lead. Wild-type Caco-2 cells displayed saturable transport of lead and iron that was acid-stimulated, a property of DMT1. Cadmium and manganese inhibited transport of iron whereas zinc and lead did not. The involvement of DMT1 in the transport of toxic metals was further examined by establishing clonal DMT1 knockdown and control Caco-2 cell lines. Knockdown cell lines displayed lower levels of DMT1 mRNA and a lower Vmax for iron uptake compared to control cell lines. The functional consequence of DMT1 knockdown was a 50% reduction in uptake of iron across a pH range from 5.5 to 7.4. Uptake for cadmium also decreased 50% across the same pH range. Lead was unaffected by DMT1 knockdown, even though uptake was acid-stimulated in wild type cells. These results show that DMT1 is important in iron and cadmium transport in Caco-2 cells, but that lead enters these cells through an independent hydrogen-driven mechanism.

1950 DIETARY IRON REGULATES INTESTINAL CADMIUM ABSORPTION THROUGH DMT1 IN RATS.

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The main exposure source of Cd is oral ingestion with food in general population. In previous study, the depletion of iron(Fe) up-regulated the expression of Divalent Metal Transporter 1(DMT1) and increased Cd absorption in intestine. However, it is not clear whether the dietary Fe plays a role of regulation Cd absorption in intestine through DMT1. This study has done to clarify the role of Fe in intestinal Cd absorption by control body Fe stores with diet. In first model, rats were fed with a Fe-deficient (FeD-, 2-6mg Fe/kg) or Fe-supplemented (FeS-, 120mg Fe/kg) diet for 4 wk, and followed single administration of 109CdCl2 orally. In the other model, rats were fed FeD- or FeS- diet for 4 wk, and replaced FeD-diet with FeS-diet (FeR-diet group) for 4 wk but kept FeS diet for 8wk, and followed single oral administraion of 109CdCl2. Animals of each model were killed at 24 hr after Cd administration. Body Fe status was evaluated and tissue Cd concentration was determined. The level of DMT1 mRNA in duodenum was analyzed by RT-PCR method. Feeding the FeD-diet for 4 wk produced Fe deficiency anemia in animals with findings of decreased serum total Fe, increased UIBC and hypochromic microcytic RBC. Tissue Cd concentration was significantly higher with 2-6 folds in FeD-diet rats than FeS-diet. Body burden of Cd in FeD-diet rats was 3 folds higher. Also, the level of DMT1 mRNA in duodenum was highly expressed in FeD-diet group than FeS-diet. In FeR-diet group, body Fe status returned to the control level. Tissue Cd concentration and body burden of Cd was not significant with FeS-diet fed rats for 8 wk. The level of DMT1 mRNA expression in FeR-diet rats was similar with control group. These findings support to understand more clearly the role of dietary Fe in the intestinal Cd absorption. Dietary Fe effects on the body Fe stores directly, follows the up- or down- regulation of DMT1 expression at transcription level, and results in the regulation of intestinal Cd absorption in mammalian.

1951 ZINC SUPPLEMENTATION MAY DECREASE HEPATIC COPPER ACCUMULATION IN LEC RAT: A MODEL OF WILSON'S DISEASE.

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The Long-Evans Cinnamon (LEC) rat is an animal model for Wilson's disease where copper (Cu) accumulates in the liver with age. The condition in LEC rats is characterized by development of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. The defective Cu-transporting ATP7B gene is homologous to the gene responsible for Wilson's disease. The objective of this study was to evaluate the effect of Zinc (Zn) supplementation in the development of Cu-associated liver damage in LEC rats. Female, 4 weeks-old, LEC (N=48) and LE rats were divided in two groups. One group was fed a Zn-supplemented diet, and the other group was given a normal rodent diet. Four LEC rats in each group were sequentially sacrificed at 6, 8, 10, 12, 18, and 20 weeks of age. Controls were sampled at 6, 12, 18 and 20 weeks of age. Hepatocyte apoptosis was detected in LEC rats fed with Zinc-supplemented diet much later (20 weeks) than in LEC rats fed with normal diet (10 weeks). Metallothionein (MT) concentration in livers of LEC rats with Zn-supplemented diet increased after 12 weeks of age up to 20 weeks, while hepatic MT concentration in LEC rats fed a normal diet decreased after 12 weeks. Between 6-18 weeks of age, Cu concentration in the liver was higher in LEC rats fed a normal diet compared to that detected in rats fed a Zn-supplemented diet. After 8 weeks, serum levels of alkaline phosphatase (ALP) were increased only in LEC rats fed a normal diet. Hepatocyte Growth Factor (HGF) was detected by immunohistochemical staining in livers of all LEC rats with strong positive HGF staining in LEC rats over 12 weeks of age and fed a normal diet. The results suggest that Zn supplementation can reduce the hepatic Cu concentration and delay the onset of clinical and pathological changes of Cu toxicity in LEC rats. Although the actual mechanism of the protection in not known, it could be to sequestration of dietary Cu by intestinal MT, induced by high Zn diet in LEC rats. (Supported by a Grant from CIHR).

1952 EFFECT OF SELENITE ON THE DISPOSITION OF ARSENATE AND ARSENITE IN RATS.

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Selenite (SeIV) and inorganic arsenicals counter the toxicity of each other. SeIV inhibits arsenic methylation in hepatocytes, however, it is unknown whether it decreases the formation of the highly toxic monomethylarsonous acid (MMAsIII). Therefore, we examined, in comparison with the methylation inhibitor periodate-oxidized adenosine (PAD), the effect of SeIV (10 μmol/kg, i.v.) on the appearance of arsenic metabolites in blood, bile and urine as well as the distribution of arsenic metabolites in the liver and kidneys in rats injected i.v. with 50 μmol/kg arsenite (AsIII) or arsenate (AsV). Arsenic metabolites were analyzed by HPLC-hydride generation-atomic fluorescence spectrometry. In rats given either arsenical, PAD decreased the excretion and tissue concentrations of methylated arsenic metabolites (MMAsIII, monomethylarsonic acid [MMAsV], and dimethylarsinic acid [DMAsV]), while increasing the tissue retention of AsV and AsIII. The effect of SeIV on arsenic disposition differed significantly from that of PAD. For example, both in AsIII- and AsV-injected animals, SeIV lowered the tissue levels of MMAsIII and MMAsV, but increased the levels of DMAsV. SeIV almost abolished the biliary excretion of MMAsIII in AsV-exposed rats, but barely influenced it in AsIII-dosed rats. The SeIV-induced changes in arsenic disposition may largely be ascribable to formation of the known complex containing trivalent arsenic and selenide (SeII), which not only depends on but also influences the availability and effects of these metalloid species in tissues. By such complexation SeII compromises monomethylation of arsenic when trivalent arsenic availability is limited (e.g. in AsV-exposed rats), but affects it less when the presence of AsIII is overwhelming (e.g. in AsIII-dosed rats). As an auxiliary finding, it is shown that DMAsV occurs in the blood of rats not injected with arsenic and that DMAsV formation in rats can be followed by measuring the build-up of blood-borne DMAsV. (Supported by OTKA and the Hungarian Ministry of Health)

1953 RELATION OF URINARY TRIVALENT METABOLITES OF INORGANIC ARSENIC WITH ARSENIC-SKIN LESIONS IN HUMANS.

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Chronic ingestion of arsenic (As) from drinking water is associated with the occurrence of skin cancer. Exposure of humans to inorganic As (iAs) results in the urinary excretion of iAs and two major methylated metabolites MAs and DMA. In experimental models, methylated and dimethylated arsenicals that contain As in the trivalent oxidation state (MAsIII and DMAIII) are more potent toxins than iAs. The aim of this study was to evaluate the pattern of urinary trivalent As species in people chronically exposed to iAs and the association of trivalent As species with the development of arsenic-skin lesions. A cross-sectional study was conducted in the central part of Mexico, in which 24 participants chronically exposed to water naturally contaminated with high arsenic content (363 micrograms/l) were evaluated by questionnaire and clinical examination. Participants provided drinking water and spot urine samples. Trivalent As compounds were quantified within the

first 6 hours of collection. Trivalent forms of As were the predominant species in urine (59.3 %). iAsIII accounted for 7.2 %, MAsIII for 9.1 % and DMAIII for 43% of the sum of all arsenic in urine. Content of urinary MAsIII and DMAIII were directly correlated with the iAs concentration in water. Individuals exposed with skin-lesions presented higher concentration of MAsIII and DMAsIII in urine than those without skin lesions. Characterization of the urinary excretion of MAsIII and DMAsIII may provide a new biomarker of the effects of chronic exposure to As. This study was supported by Conacyt-Mexico (grant 3871-M).

1954 DOSE-RESPONSE ALTERATION IN THE URINARY PATTERN OF TRIVALENT ARSENIC SPECIES IN MICE EXPOSED TO ARSENITE.

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Biomethylation of arsenic (As) is the major process of metabolism of As. This procedure involves a stepwise reduction of pentavalent to trivalent arsenicals followed by oxidative addition of a methyl group to the trivalent As. Because methylated metabolites that contain trivalent As (MAsIII and DMAIII) are toxic intermediates in the metabolism of As, there is increasing interest in the determination of MAsIII and DMAIII in studies of As biotransformation. The purpose of this study was to determine As species, including the putative more toxic trivalent methylated As species, MAsIII and DMAIII, in mice exposed to inorganic As and its relation with toxic effects. Female C57BL/6 mice were orally administered with 3 or 6 mg arsenite/kg, daily for nine days. Because trivalent methylated species are unstable in urine, this was recollected during three periods, 0-4 h, 4-8 h and 8-24 hr after the last arsenite dose; during this process the urine was preserved on dry ice. After collection, the urine was immediately analyzed for As species. Hepatic lipoperoxidation was increased in dose-depend fashion. Concentration of urinary iAsIII, MAsIII and DMAIII were directly correlated with dose of arsenite. However, a significant decrease in the relative proportion of DMAIII and increase in the MAsIII and iAsIII were associated with magnitude of As exposure. These data showed a negative relationship between relative urinary proportion of DMAIII and hepatic lipoperoxidation.

1955 COMPARISON OF THIMEROSAL AND METHYL MERCURY DISTRIBUTION IN NEONATAL MICE.

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Thimerosal, which contains the ethyl mercury radical as the active species, has been used as a preservative in many currently marketed vaccines throughout the world. Because of the assumption that its toxicity is similar to that of methyl mercury, it is no longer incorporated in many vaccines in the United States. There are reasons to believe, however, that this assumption may not be correct; that is, the disposition and toxicity of ethyl mercury compounds, including thimerosal, may differ substantially from those of the methyl form. To further explore this question, the current study sought to compare, in neonatal mice, the tissue disposition of thimerosal with that of methyl mercury. Female ICR mice were given single intramuscular injections of thimerosal or methyl mercury (1.4 mg Hg/kg) on postnatal day 10 (PND 10). Tissue samples were collected daily on PND 11-14. Absorption from the application site was similar in both groups. In our study it has been documented that ethyl mercury accumulates in growing hair similarly to methyl mercury. Mean total mercury blood and brain levels were approximately two times lower in the mice treated with thimerosal than in the methyl mercury-treated group. In the brain, thimerosal-exposed mice showed a steady decrease of mercury levels following the initial peak, whereas, in the methyl mercury-exposed mice, concentrations peaked the second day after exposure. In the kidneys, thimerosal-exposed mice retained higher total mercury levels than methyl mercury-treated mice 4 days after exposure. These preliminary data on the toxicokinetic behavior of the chemicals challenge the assumption that ethyl mercury is toxicologically identical to methyl mercury. (Funded by Grant H75/ATH270795 from ATSDR.)

1956 (1795a) CYP1A2 IS NOT REQUIRED FOR 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN-INDUCED IMMUNOSUPPRESSION

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One of the most sensitive and reproducible immunotoxic endpoints of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure is suppression of the antibody response to sheep red blood cells (SRBCs) in mice. Immunosuppression occurs in concert with hepatomegaly and associated induction of several hepatic cytochrome P450 enzymes, including CYP1A2 which is responsible for the hepatic sequestration of TCDD. In this study, TCDD-induced immunosuppression was evaluated in CYP1A2 (-/-) knockout mice and compared with that of age-matched female C57/BL/6N CYP1A2 (+/+) wild-type, and CYP1A2 (+/-) heterozygote mice. Groups of mice were given a single gavage dose of 0, 0.03, 0.1, 0.3, 1.0, 3.0 or 10.0 μg TCDD/kg, followed seven days later by immunization with SRBCs. Serum was obtained five days after immunization and body, spleen, thymus and liver weights were measured. SRBC antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA). Anti-SRBC titers were suppressed at 1.0, 1.0 and 0.3 μg TCDD/kg for CYP1A2 (+/+), CYP1A2 (+/-), and CYP1A2 (-/-) mice, respectively, which indicated a three fold increase in TCDD immuno-sensitivity for the CYP1A2 (-/-) mice. This increase in TCDD-induced immunosuppression may be due to the inability of CYP1A2 (-/-) mice to sequester TCDD in the liver, leading to a higher dose to the immune system. In CYP1A2(+/+) mice a dose of 0.3 μg TCDD/kg was sufficient to increase the liver-to-body weight ratio, while in CYP1A2 (-/-) mice a dose of 10 μg TCDD/kg was required. Nevertheless, calculated TCDD ED50 values of immunosuppression were no different across the three groups of mice examined (i.e., mean ±SEM of 0.98 ±0.32, 1.02 ±0.89 and 0.58 ±0.71 μg TCDD/kg for CYP1A2 (+/+), (+/-), and (-/-) mice, respectively). Thus CYP1A2 is not required for TCDD-induced immunosuppression. (This abstract does not reflect EPA policy.)

NOTE: This abstract will be presented as #1795a.

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